Bioactive forms of omega-3 and omega-6 fatty acids

Monograph #3
Essential Lipids Education Program
**Bioactive Forms of Omega-3 and Omega-6 Fatty Acids**

Stimulated cells can activate cytosolic phospholipase A₂, which hydrolyzes fatty acids from position-2 of membrane phospholipids. This is where many essential n-3 and n-6 highly unsaturated fatty acids (HUFA) are stored. The released HUFA can form CoA esters and be converted to CO₂ and ATP or be re-esterified back into cellular lipids. Alternatively, three HUFA, eicosatrienoic (20:3n-6), arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3), can be oxidized to bioactive derivatives called eicosanoids.

The first recognized bioactive derivatives formed from essential fatty acids were prostaglandins, PGE₁, PGE₂ and PGE₃. They enhance inflammatory events and stimulate smooth muscle contraction. Other closely related derivatives formed were prostaglandins D and F. Study of inflammatory situations led to the discovery that aspirin blocks the enzyme, cyclooxygenase, which catalyzes formation of prostaglandins. Subsequently, the potent stimulation of blood platelet aggregation by the prostaglandin derivative, thromboxane (TXA₂), led to use of aspirin for antithrombotic prophylaxis of CHD deaths. Later, endothelial cells in blood vessels were found to form a potent anti-thrombotic derivative, prostacyclin (PGI₂).

The next recognized bioactive derivatives were leukotrienes that are formed by lipoxygenases. The leukotriene LTB₄ affects immune-inflammatory processes, and LTC₄ and LTD₄ act in pulmonary airway contraction. Differentiated cells contain diverse synthases that convert the oxidized prostaglandin intermediates into diverse eicosanoids that act at specific G-protein coupled receptors. They alter intracellular second messengers and protein kinase actions. Eicosanoid receptors of one type or another occur on nearly all cells and tissues of the human body. Their intracellular signals influence nearly all physiological processes. As a result, many unwanted health conditions can result from unbalanced eicosanoid actions that shift healthy physiology to pathophysiology.

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Structure</th>
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<tbody>
<tr>
<td>Eicosatrienoic (20:3n-6)</td>
<td>CH₃(CH₂)₄(CH=CHCH₂)₃(CH₂)₅COOH</td>
</tr>
<tr>
<td>Arachidonic (20:4n-6)</td>
<td>CH₃(CH₂)₄(CH=CHCH₂)₄(CH₂)₂COOH</td>
</tr>
<tr>
<td>Eicosapentaenoic (20:5n-3)</td>
<td>CH₃CH₂(CH=CHCH₂)₅(CH₂)₂COOH</td>
</tr>
</tbody>
</table>

**Health conditions that are likely made worse by n-6 eicosanoid actions:**

- Depression
- Obesity
- Arthritis
- Back/neck pain
- Anxiety
- Allergy
- Cancer proliferation
- Chronic pain
- Hypertension
- Asthma
- Migraine
- Sleeping problem
- Irritable bowel
- Fatigue
- Headache
- Diabetes
- Coronary heart disease
- Osteoporosis
- Bronchitis
- Emphysema
- Congestive heart failure
- Chronic Obstructive Pulmonary Disorder

Health disorders with names ending in "-itis" have inflammatory processes linked to excessive actions of n-6 eicosanoids. There are many.
**Actions of non-steroidal anti-inflammatory drugs (NSAID)**

Concern about NSAID use is that it may lower a beneficial eicosanoid action when lowering a harmful one. Balancing the benefit and harm requires using the lowest effective dose of drug and checking carefully for adverse events. This was evident when too much aspirin was first used to lower thromboxane-mediated platelet action. High doses lowered endothelial prostacyclin, allowing increased thrombosis. Low-dose aspirin then became widely used to lower risk of heart attacks.

**Different dynamics for n-3 and n-6 eicosanoid actions**

The balance of n-3 and n-6 nutrients in the average daily diet control the balance of n-3 and n-6 HUFA accumulated in membrane phospholipids. Similarly, the balance of n-3 and n-6 HUFA in membrane phospholipids affect the relative amounts released by cytosolic phospholipase A₂. The 5-lipoxygenase is also indiscriminate, whereas cyclooxygenase oxidizes n-6 HUFA much faster than n-3 HUFA. Some eicosanoid synthases and receptors also discriminate between the two structures in essential lipids and some do not. The discriminate actions create conditions altered by food choices (Figure 3-2 below).
Different intensities of action with n-3 derivatives relative to n-6 derivatives are shown in Figure 3-2 adjacent to the enzyme or receptor process. The actions are limited by rapid removal of the active hormone by dehydrogenases and oxidases (prostaglandins), spontaneous loss (thromboxane) and peptidic cleavage (leukotrienes). Degradation quickly dampens the signaling and allows activated systems to return quickly to a basal state once formation of the bioactive lipid stops.

Many actions are less intense with 20:5n-3 than 20:4n-6. Also, 22:6n-3 inhibits both COX and LOX reactions and lowers signaling. With more n-3 than n-6 HUFA in platelets there is less intense formation of thromboxane TXA2, causing less aggregation and less risk of heart attack or stroke. A 50-100 fold lower response of the BLT1 receptor to LTB5 compared to LTB4 means that recruitment of inflammatory cells is very much less when the HUFA balance has more n-3 than n-6 HUFA. Many health conditions reflect the accumulated tissue HUFA balance that came from uninformed food choices with unintended consequences.

For example, traditional Mediterranean food maintained a HUFA balance in blood lipids near 60% n-6 in HUFA and lower rates of heart attacks compared to American food that maintains proportions near 80% (see Essential Lipids Booklets #1). Similarly, people in Greenland and Japan with proportions near 30% and 40% n-6 in HUFA, respectively, have correspondingly lower rates of cardiovascular death. Overall, people with less than 50% n-6 in HUFA have less severe cardiovascular disease than people with more than 50%. It appears that some traditional ethnic food habits have unknowingly developed useful forms of preventive nutrition.

Considering a therapeutic window

In the absence of dietary n-3 nutrients, a very effective conversion of dietary linoleic acid (18:2n-6) into tissue HUFA allows 0.4 percent of food energy (0.4 en%) to maintain more than 50% n-6 in HUFA. Intake of 0.4 en% prevents essential fatty acid (EFA) deficiency. Eating less than 0.4 en% may not be enough, and eating more would give a HUFA balance above 50% n-6 in HUFA that has a higher risk for excessive n-6 actions.

This means that dietary linoleic acid has a very narrow therapeutic window which can be widened by including essential n-3 nutrients in the diet. With both in the diet, the % n-3 in HUFA will be higher and the %n-6 in HUFA lower. As with a narrow window for NSAID drugs, eating linoleic acid at its lowest effective dose and checking carefully for adverse actions is a prudent food choice.

Current average daily intakes of n-6 nutrients in the USA are near 6 en% to 8 en%, much more than the 0.4 en% that prevents EFA deficiency. They create a HUFA balance near 80% n-6 in HUFA.

Adding more n-3 nutrients and lowering n-6 nutrients can shift HUFA balance levels below 50% n-6 in HUFA.
Eicosanoid receptors create different outcomes

A cell with an EP2 receptor responds to PGE₂, opposite from a cell with an EP3 receptor. EP2 signals via Gαs-activated adenylcyclase which increases cAMP and stimulates protein kinase A (PKA) actions. Alternatively, the EP3 receptor signals via Gαi, inhibiting adenyl cyclase and lowering cAMP-activated PKA activity. Because PKA activates PLA₂ to release HUFA and form eicosanoids, the EP2 receptor gives positive feedback and EP3 gives negative feedback. Figure 3.2 indicates that n-3 PGE₂ is formed less vigorously than n-6 PGE₂, and it will act less vigorously than n-6 PGE₂ at EP receptors.

Endocannabinoids
Receptors coupled to Gαq activate PLC and release diacylglycerol (DAG). Hydrolysis of the DAG by a cellular DAG lipase can produce the n-6 2-arachidonoyl glycerol that acts on cannabinoid receptors, CB₁ and CB₂. Different paths can form the n-6 arachidonoyl ethanolamide, which also activates CB₁ and CB₂. Both endocannabinoids have a short duration of action as hydrolytic enzymes quickly inactivate them. CB₁ receptors are in adipose tissue, pancreas, kidney, liver and highly expressed in neurons. They affect appetite, pain-sensation, mood, and memory. Both CB₁ and CB₂ decrease autophagic, reparative activity of removing excess membrane components (see more in Essential Lipids Booklet #4).

Lipoxins and resolution of inflammation
Lipoxin A₄ (LXA₄, lipoxygenase interaction product) is formed by trans-cellular synthesis as two cells with different lipoxygenases act on arachidonic acid. Neutrophils form the pro-inflammatory leukotriene A₄ (LTA₄). Then LTA₄ diffuses to adjacent eosinophils which form the anti-inflammatory LXA₄. LXA₄ acting through a multi-function receptor, FP₂/LAX, promotes apoptosis and clearance of neutrophils by macrophages, resolving inflammation by mechanisms not yet understood.

Resolvins, Maresins, Protectins
These specialized pro-resolving mediators (SPMs) made from n-3 HUFA are, like lipoxins, implicated in resolving inflammatory conditions. Their exact mode of action is not certain, but their actions emphasize the benefit of eating n-3 nutrients to moderate inflammatory events caused by n-6 mediators.

BLT₁ deletion cuts insulin resistance. A recent epidemic of type 2 diabetes (insulin resistance) is driven by increased macrophages in obese adipose tissue. Their recruitment is cut by deleting the BLT₁ receptor, blocking it with drugs or providing a HUFA balance with more n-3 than n-6 LTB₄ (not 50-100-fold less potent than LTB₄ in chemotaxis with BLT-1). This is how eating more omega-3 and less omega-6 nutrients helps health.

Leukotriene receptors, CysLT₁, CysLT₂, signal via Gαq to increase cytosolic Ca²⁺ and PKC action that leads to bronchial smooth muscle contraction. The more potent conversion of the n-6 LTA₄ than the n-3 LTA₄ to cysteinyl leukotrienes creates more intensive signaling in chronic obstructive pulmonary disorder and asthma when the HUFA balance has more n-6 than n-3 in HUFA.

Evidence from gene deletion studies helps discover eicosanoid actions
Selective deletion of each prostanoid receptor gene gave a clearer view of their multiple actions in diverse conditions.
- DP₁ (Gαs) regulates sleep & inhibits angiogenesis.
- DP₂ (Gαi) gives eosinophil infiltration, activates Th2 lymphocytes.
- EP₁ (Gαq) suppresses acoustic startle, social withdrawal, and improves defeat stress-induced social avoidance.
- EP₂ (Gαs) disrupts blood-brain barrier, gives vasodilation & bone remodeling, mediates epilptic state.
- EP₃ (Gαi) acts in neurons, esp. in the sensory ganglia; suppresses eosinophil migration & mast cell activation and edema.
- EP₄ (Gαs) gives cardiac hypertrophy, remodels ductus arteriosis, bone resorption.
- FP (Gαq) gives luteolysis & parturition.
- IP (Gαs) is antithrombotic, mediates arthritic inflammation, enhances somatostatin release and cuts acid secretion in stomach.
- TP (Gαq) increases aggregation of platelets, bronchoconstriction, cancer cell migration, impairs the microcirculation.
- CysLT₁ (Gαq) gives potent chemotactic response by neutrophils and eosinophils to the n-6 LTB₄ (not the n-3 LTB₄).
- CysLT₂ (Gαq) has potent asthmatic bronchoconstrictor responses; potency is LTD₄ > LTE₄ > LTA₄.
Recognizing the range of HUFA balance that occurs

Epidemiological studies that examine populations with small differences in HUFA balance often see little evidence of benefit from n-3 nutrients. A report on prostate cancer patients noted 76% n-6 in HUFA while controls had 77% n-6 in HUFA. The authors suggested that the lower value may indicate n-3 nutrients enhance risk for cancer. A differing report saw extensive intra-prostatic inflammation linked to 74.7% n-6 in HUFA, whereas the men with no inflammation had 72.7% n-6 in HUFA.

The small differences in observed mean values are less than errors for assay of HUFA balance. The HUFA balance observed in humans ranges from 25% to 85% n-6 in HUFA. That is a proper biological range for interpreting health benefits of n-3 nutrients.

Many intervention studies adding n-3 supplements to decrease health disorders created small net changes in tissue HUFA balance, and they reported little benefit. The lack of beneficial outcomes increased public uncertainty of the efficacy of n-3 nutrients to correct n-6 mediated disorders. However, a randomized clinical trial for reducing pain altered food choices using concepts noted in *Essential Lipids Booklet #1*. It was highly successful. Lowering dietary n-6 linoleic acid allowed added competing n-3 nutrients to shift the HUFA balance from 77% to 61% n-6 in HUFA in 3 months. The clinical status steadily improved throughout the study, and predictably would go further if the trial were extended. Clearly, less medication is needed to treat signs and symptoms when the dietary cause of the signs and symptoms is prevented.

Food oils changed in the USA

High intakes of n-6 linoleic acid shift tissue HUFA balance to high proportions of n-6 in HUFA and favor chronic inflammatory and thrombotic disorders.
Much evidence shows that eating n-6 linoleate at levels far above the minimum needed gives harmfully high values for the health risk assessment biomarker, the % n-6 in HUFA. Values for apparently healthy people range from 25% to 85% n-6 in HUFA. However, the risk for health disorders is greater for people with values above 50% than for those below 50% (http://efaeducation.org/?p=58). The USA average is now near 78% n-6 in HUFA. It is prudent to eat more n-3 nutrients whether from fish, fortified foods or supplements to widen the narrow therapeutic window for n-6 nutrients. The large number of “null” reports on health benefits from n-3 nutrients occurred as investigators failed to examine a sufficient range of HUFA balance. Future clinical investigators can use a simple calculator (http://efaeducation.org/?p=124) to design a sufficient shift in HUFA balance and demonstrate benefits from balancing n-3 and n-6 nutrients. Consumers seeking a healthy balanced lifestyle can begin increasing their intake of food items with positive Omega 3-6 Balance Scores and eating less of the foods with high negative scores. The apps noted in Essential Lipids Booklet #1 (http://efaeducation.org/?p=113) can guide voluntary food decisions that begin to lower health conditions due to excessive n-6 eicosanoid actions. Such steps, when maintained in a pattern of continued wellness activity (http://efaeducation.org/?p=29), are likely to reduce the need for medications. There is less need to use a drug to treat signs and symptoms of an imbalance that is prevented by removing the nutrient imbalance causing the signs and symptoms.

**Overview**

The n-3 and n-6 HUFA released from membrane phospholipids form bioactive eicosanoids that signal through receptors and alter physiology. Formation and action of n-6 eicosanoids is more potent than it is for n-3 eicosanoids, creating a tendency for imbalanced n-6 actions to shift healthy physiology toward pathophysiology. Many chronic inflammatory disorders involving innate immune system actions are diminished when daily foods help diminish n-6 eicosanoid actions.

**Mortality for Prostate Cancer, by age group, year of death**

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<th>USA</th>
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*Age-specific rates for males 50+ years of age by year of death for prostate cancer in five countries, rates per 100,000*
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