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More Evidence that Fish Feeds the Brain

Ah, December. The posting of 2004’s fourth *PUFA Newsletter* coincides with winter, heir of summer, season of discontent, spring of genius, celebration of solstice. Let’s endorse sage Samuel Johnson’s declaration that winter is the proper season for domestic merriment and gaiety. Here in Colorado, winter brings snow, skiing, and the silly season. We watch for Orion to stride across the night sky while readers “down under” herald the southern cross.

This issue of the *PUFA Newsletter* is pleased to present a guest commentary on omega-3 fatty acids and the brain by Andrew J. Sinclair, professor of food science at RMIT University, Melbourne Australia. In this overview, he shows that docosahexaenoic acid (DHA) is where the action is in cell membranes, intracellular metabolism, and the regulation of gene expression. His article also draws dotted lines between DHA involvement in brain metabolism and neuropsychiatric conditions.

Extending the brain connection, this issue presents two articles on the function of docosahexaenoic acid (DHA) in the brain. In Clinical Conditions Dr. Eliot Berson’s studies in patients with retinitis pigmentosa, a condition that inevitably leads to blindness, showed that patients who had consumed fish regularly and took vitamin A had significantly slower deterioration in their visual function. Under some circumstances, this slowing of visual loss translated to many additional years of eyesight.

In Frontiers, Dr. Norman Salem’s team described a series of studies showing how DHA functions in retinal cell signaling. When DHA is insufficient, cell communication is impaired, enzyme activity reduced and visual function reduced. These studies may be linked to some of the deficits in neural function observed in infants with insufficient DHA early in life.

This issue also includes studies on alpha-linolenic acid in cardiovascular health and more intriguing observations from Trevor Mori’s group on fish consumption during weight loss. In the latter study, both leptin and blood pressure fell–Mori wonders about a connection between the two.

Also reported in Frontiers is another advance in the application of biotechnology for the synthesis of long-chain polyunsaturated fatty acids in yeast and linseed. Ernst Heinz’ team at the University of Hamburg, Germany, cloned new enzymes and tracked metabolic pathways yielding 20-carbon PUFAs and encountering a bottleneck or two.

Best wishes to all for peace, wisdom, and a giving spirit this holiday season.

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*Let us love winter, for it is the spring of genius.* - Pietro Aretino, 1492-1556
**Guest Article**

**BY ANDREW J. SINCLAIR, PROFESSOR OF FOOD SCIENCE, RMIT UNIVERSITY, MELBOURNE, AUSTRALIA**

**Omega-3 Fatty Acids and the Brain**

The brain contains the second highest concentration of lipids in the body, after adipose tissue, with 36-60% of nervous tissue being lipids. Most brain lipids are complex structures, such as glycerophospholipids and sphingolipids, with little or no triglycerides and cholesterol esters. The glycerophospholipids contain a high proportion of long-chain (LC) omega-3 (n-3) and omega-6 (n-6) polyunsaturated fatty acids (PUFAs), mainly docosahexaenoic acid (DHA) and arachidonic acid, with trace amounts of their 18-carbon precursors. The highest proportion of DHA in membrane lipids—up to 50 mol percent—is found in the disk membranes of the photoreceptor cell rods in the retina (Fliesler and Anderson 1983). DHA acts as a molecular spring when light activates rhodopsin in the photoreceptor cells.

**What is the role of DHA in the brain and retina?**

Classical studies of feeding n-3-deficient diets to laboratory animals have shown reductions in brain lipid DHA, accompanied by dramatic changes in brain function. These alterations include changes in the size of neurons, learning and memory, auditory and olfactory responses to stimuli, and levels of nerve growth factor and membrane receptors. These responses appear to be related to changes in membrane function and altered gene expression in the brain (Figure). This article summarizes the ways DHA may be involved in brain function.

**Membrane-related events:** (a) membrane order (fluidity) which can influence the function of membrane receptors such as rhodopsin; (b) regulation of neurotransmission involving dopamine and serotonin; (c) regulation of membrane-bound enzymes such as Na/K-dependent ATPase; (d) signal transduction via effects on inositol phosphates, diacylglyceride, and protein kinase C; and (e) alteration of ion flux through voltage-gated K+ and Na+ channels.

**Metabolic events:** (a) regulation of the synthesis of eicosanoids derived from arachidonic acid, and (b) precursor of docosatrienes and 17S resolvins, novel anti-inflammatory mediators.

**Gene expression:** regulation of the expression of many different genes in rat brain in short- and long-term studies. In a study by Kitajka et al. (1999), rats were fed throughout life with either vegetable oil (rich in alpha-linolenic acid) or fish oil, rich in eicosapentaenoic acid (EPA) and DHA. Control rats were fed a diet rich in linoleic acid (n-6). There was an increase in the brain DHA level in both test groups. Cloned DNA microarray analysis showed highly significant alterations in the expression of more than 100 genes in the brain, with approximately equal numbers over- and under-expressed. Of interest was the fact that the ATP-generating machinery of the brain, which releases energy, responded to the dietary n-3 PUFAs most intensively.

The brain is known to exhibit a high metabolic rate. A high proportion of this energy is used to maintain Na/K ATPase activity, which regulates ion flow resulting from nerve transmission. Genes participating in signal transduction were also overexpressed, almost to same extent, by both the alpha-linolenic acid and EPA plus DHA diets. Also of interest is that genes encoding for the proteins synuclein alpha and gamma were overexpressed. Synucleins play a role in neural plasticity and Parkinson’s disease. They are associated with synaptosomes (nerve terminals) and learning in the brains of songbirds.

**Cellular events:** (a) regulation of phosphatidyl-serine levels which appear to be involved in the protection of neural cells from apoptotic death, (b) stimulation of neurite outgrowth in cultured PC-12 brain or neuron cells, (c) selective accumulation of DHA by synaptic growth cones during neuronal development, (d) regulation of neuron size (e) regulation of nerve growth factor,
and (f) precursor of neuroprostanes (DHA oxidation products) (Figure 1).

Recently, DHA has been established as the precursor of a novel series of endogenous mediators in human blood, leukocytes, murine brain, and human glial cells. These mediators, called docosatrienes (17S-hydroxy-containing docosanoids) and 17S series resolvins, are biosynthesized via enzymatic oxygenation, and are potent regulators of both leukocytes (reducing infiltration in vivo) and glial cells (blocking their cytokine production). It is not known whether these bioactive substances are responsible for some of the beneficial actions reported following dietary DHA supplementation.

**Figure 1. DHA in the brain.**

DHA can also undergo non-enzymatic free-radical catalyzed oxidation in the brain to form F₂-isoprostane-like compounds known as F₄-neuroprostanes and highly reactive A/J-ring neuroprostanes. These neuroprostanes may provide a marker of oxidative injury in the brain, and suggest that oxidative stress is ongoing in the central nervous system. Preliminary studies show significantly increased levels of neuroprostanes in brain regions of Alzheimer’s patients.

**EPA and DHA in neuropsychiatric disorders**

Manic-depressive illness (bipolar disorder), depression, and schizophrenia are common neuropsychiatric disorders. Results from case-control studies, clinical trials, and case studies have shown that n-3 LC-PUFAs can play a beneficial role in these neuropsychiatric conditions.

There is no widely accepted mechanism of action of n-3 LC-PUFAs in these conditions; however, the biophysical properties of synaptic membranes directly affect neurotransmitter biosynthesis, signal transduction, uptake of serotonin, binding of alpha-adrenergic and serotonergic receptors, and monoamine oxidase activity. The proposed mechanisms of action of the n-3 LC-PUFAs in neuropsychiatric disorders include effects on neurotransmitter receptors and G-proteins via effects on biophysical properties of the membrane, effects on secondary messengers, protein kinases and the inflammatory response of eicosanoids derived from arachidonic acid.
Recent data from rats treated with lithium, a therapeutic used in treating bipolar disorder, demonstrated a reduced turnover of arachidonic acid in brain phospholipids, and decreased mRNA, protein levels, and enzyme activity of a cytosolic phospholipase A2, which is arachidonic acid-specific. This treatment also reduced the level and activity of cyclooxygenase and its product prostaglandin E2, and the turnover of arachidonic acid, but not DHA in the brain. It is possible that EPA is effective in depression through its ability to inhibit cyclooxygenase activity, although the levels of EPA in the brain phospholipids are very low.

It may also be possible that n-3 PUFA are operating through the neuromodulatory actions of the endocannabinoids, substances that bind to cannabinoid receptors in the brain. In piglets, brain levels of the endocannabinoid NAE increased 4-fold for arachidonic acid, 5-fold for EPA, 9-fold for 22:5n-3 and 10-fold for DHA after they were fed a diet with arachidonic acid and DHA for 18 days, compared with a diet without these fatty acids.

More than 25 years ago, two different investigators proposed an alternative hypothesis for schizophrenia that involved abnormalities in brain phospholipid metabolism. Patterns of decreased LC-PUFAs and increased phospholipid turnover have been reported in patients with schizophrenia. Skosnik and Yao have proposed that arachidonic acid may play a crucial role in the pathophysiology of schizophrenia. Reportedly, EPA is able to reverse the phospholipid abnormalities in this condition. One mechanism may be via inhibition of PUFA-specific phospholipase A2, an enzyme that removes LC-PUFAs from the sn-2 position of membrane phospholipids. However, treatment with n-3 and n-6 LC-PUFAs and antioxidants is yet to be fully explored in schizophrenia.

Peet has recently suggested that there might be a common mechanism whereby diets rich in saturated fat, with a high glycemic load and low n-3 PUFA content, influence schizophrenia, namely through brain-derived neurotrophic factor. This factor might have a role in stabilizing the survival of circuitry of the brain in critical periods, by maintaining cortical neuron size and dendrite structure.

**What is the future?**

More research is urgently required, with the emphasis on collaboration between nutritionists, psychiatrists, and neurochemists. Of the n-3 PUFAs, EPA appears to be more effective than DHA in neuropsychiatric conditions in clinical studies.

* A copy of the fully referenced version of this article is available from the editor.

**REFERENCES**

Consumption of alpha-linolenic acid, the plant-based 18-carbon precursor of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) has been associated with reduced risk factors and lower cardiovascular disease, particularly in observational studies. Intake of alpha-linolenic acid has also been linked to lower levels of plasma inflammatory markers generally considered protective of cardiovascular health. However, data are not consistent and few intervention studies have been reported.

Updated dietary guidelines for western-style countries advise increased consumption of n-3 PUFAs and limited consumption of saturated fats. With saturated fat intake held low, increased n-3 PUFA consumption can be achieved at the expense of n-6 PUFA or monounsaturated fat, with some experts favoring a reduction in n-6 PUFA intake. To explore the effects on blood lipids and inflammatory markers of increased consumption of alpha-linolenic acid or linoleic acid, Zhao et al. at Pennsylvania State University, USA, fed 23 moderately hypercholesterolemic subjects (36-65 yrs) diets containing 35% energy from fat with relatively high levels of alpha-linolenic acid (6.5 or 3.6%) or linoleic acid (12.6 or 10.5%). These PUFA diets were low in saturated fat (8% energy) with monounsaturated fat held constant (13% energy). The reference diet resembled the average American diet, having 13% energy each from saturated and monounsaturated fatty acids, 8% energy from linoleic acid and 1% energy from alpha-linolenic acid. The study was a controlled crossover design in which subjects were randomized to receive the linoleic acid, alpha-linolenic acid, or average American diet for six weeks, followed by a three-week washout and the next diet until subjects had consumed all diets. Alpha-linolenic acid was supplied in both PUFA diets from walnuts, walnut oil, and flaxseed oil.

As expected, consumption of the PUFA diets increased serum PUFA levels in both diet groups compared with the reference diet. Levels of n-3 LC-PUFAs mainly eicosapentaenoic acid (EPA) but not docosahexaenoic acid (DHA) were significantly increased in both PUFA groups compared with the reference diet, with EPA values significantly greater in the alpha-linolenic acid group compared with the linoleic acid group. These findings appear to confirm the conversion of alpha-linolenic acid to EPA, but not DHA, in adults.

Both PUFA diets decreased C-reactive protein levels, by 75% for the alpha-linolenic acid diet and 45% for the linoleic acid group compared with the control diet. Although the C-reactive protein levels did not differ between the two PUFA groups, both PUFA-induced reductions differed significantly. Elevated C-reactive protein is an independent risk factor for cardiovascular disease and a sign of subclinical inflammation. Changes in C-reactive protein were inversely related to the changes in serum EPA. Nevertheless, there was substantial variation in C-reactive protein responses among individuals on each diet, with some exhibiting increased, decreased, or unaltered levels. Such variation may reflect genetic variability among subjects.

Both PUFA diets significantly reduced the serum concentration of three different cellular adhesion molecules compared with the control diet. Vascular and intercellular adhesion molecules are involved in inflammatory responses, facilitating the interaction between leukocytes and activated endothelial cells lining the blood vessels. Consumption of the alpha-linolenic acid-rich diet was associated with significantly greater reductions in vascular cell adhesion molecules and E-selectin, compared with the linoleic acid-rich diet (data not available). These observations, along with the reduced C-reactive protein levels, suggest that alpha-linolenic acid may exert its cardioprotective effects mainly by diminishing endothelial activation and inflammatory responses. The linoleic acid-rich diet was associated with more modest effects on C-reactive protein and cellular adhesion molecules. Both PUFA diets reduced serum triglycerides and all lipoprotein fractions to a similar degree.

This study sheds light on the differences between the 18-carbon n-3 and n-6 PUFAs in reducing risk of cardiovascular disease. It suggests that alpha-linolenic acid is more likely to exert anti-inflammatory effects in cardiovascular disease than linoleic acid, although
it could not distinguish whether the observed effects were directly related to alpha-linolenic acid itself, or to the increased production of EPA associated with its consumption. Although its effects were more modest, linoleic acid was associated with reduced levels of some inflammatory markers, suggesting that diets high in linoleic acid may not be proinflammatory.


### Short Takes

**Alpha-Linolenic Acid Lowers C-Reactive Protein Without Reducing Arterial Wall Thickness**

There are many clinical conditions that increase the chance of heart attack and stroke. One of the most easily measured and predictive factors is the thickness of the carotid artery intima-media, the first two inner cellular layers of the artery (Figure 1). These layers accumulate plaque, which narrows the diameter of the artery and impedes blood flow. The consumption of fish was reported to slow the rate of arterial narrowing in diabetic women. It is not known whether the consumption of alpha-linolenic acid, the 18-carbon omega-3 polyunsaturated fatty acid (n-3 PUFA) found in plants, affects the rate of intima-media thickening. In addition, because arteries become inflamed in cardiovascular disease, some evidence suggests that alpha-linolenic acid may reduce inflammation as well.

Consumption of alpha-linolenic acid has been associated with heart health benefits including reduced serum triglycerides and inflammatory markers, but the effects of alpha-linolenic acid have been less potent and extensive as those of the long-chain n-3 PUFAs, eicosapentaenoic acid and docosahexaenoic acid.

In this randomized, double-blind, controlled study, Bemelmans and colleagues at the University of Groningen, The Netherlands, fed 103 moderately hypercholesterolemic subjects margarine enriched with either alpha-linolenic acid or linoleic acid for two years. Consumption of the alpha linolenic acid-enriched margarine increased the total intake of acid by 4.5 g/day and linoleic acid by 3.0 g/day. Consumption of the linoleic-acid rich margarine decreased total alpha-linoleic acid intake by 0.3 g/day and increased linoleic acid intake by 4.6 g/day. Fish intake increased unintentionally in both groups by 14 g/day and 20 g/day, respectively.

After two years, there was a significant increase in carotid intima-media thickening of 0.05 mm (6%) in both groups compared with baseline values. However, C-reactive protein values were significantly reduced by 0.56 mg/l \( (P<0.05) \) in the alpha-linolenic acid group after two years, but not in the linoleic acid group. Differences between both groups in C-reactive protein reductions between the two groups were apparent after one year already. Values were adjusted for changes in fish intake, use of lipid-lowering medication, and reduced saturated fat intake, which decreased in both groups.

The study confirms previous reports that alpha-linolenic acid and n-3 LC-PUFAs reduce C-reactive protein levels, but in contrast to some reports, alpha-linolenic acid consumption had no effect on other indices of heart disease risk, such as serum triglyceride levels.

Increased intake of alpha-linolenic acid did not affect the progression of intima-media thickening in subjects with cardiovascular disease.

Arachidonic Acid in Adipose Tissue Linked to Higher Risk of Second Heart Attack

Arachidonic acid, the long-chain omega-6 polyunsaturated fatty acid (n-6 LC-PUFA) derived from linoleic acid, is the precursor of pro-inflammatory eicosanoids thought to exacerbate cardiovascular disease. The formation of certain atherogenic eicosanoids produced from arachidonic acid can be inhibited by omega-3 LC-PUFAs. Increased concentration of arachidonic acid, but not linoleic acid, in adipose tissue has been associated with increased risk of myocardial infarction in a population consuming 10% of dietary energy from PUFAs. Other studies have not observed such an association. In contrast, adipose tissue alpha-linolenic acid, an n-3 PUFA, is reportedly cardioprotective. Overall, data linking adipose fatty acid concentrations with risk of cardiovascular disease are limited.

In a series of studies on the associations between different adipose tissue fatty acid concentrations and cardiovascular disease, Drs. Ana Baylin and Hannia Campos at the Harvard School of Public Health and the University of Costa Rica measured adipose tissue fatty acids in 466 patients who had survived a myocardial infarction and 466 matched control subjects whose age averaged 57 years. Subcutaneous adipose tissue biopsies were obtained from the upper buttock approximately three weeks after the acute myocardial infarction.

Adipose tissue fatty acid concentrations were divided into quintiles ranging from the lowest median arachidonic acid content of 0.29% to the highest of 0.64%. There was a significant trend between adipose arachidonic acid concentrations and risk of myocardial infarction (Figure 1), with risk in the highest quintile nearly doubling according to multivariate risk analysis (OR-1.9, CI=1.07 to 3.53). Arachidonic acid is the precursor of thromboxane A2, a procoagulant produced by platelets. Dietary consumption of linoleic and arachidonic acids was unrelated to adipose tissue arachidonic acid concentration, suggesting tight metabolic control of arachidonic acid production or tissue saturation.

These observations leave open the question of what determines adipose tissue concentrations of arachidonic acid and to what extent they can or should be modified by diet. Further, adipose tissue concentrations of linoleic acid were unrelated to risk of myocardial infarction, but content of alpha-linolenic acid was associated with reduced risk. Questions about the interpretation of adipose tissue fatty acid concentrations can be debated, but regulation of arachidonic acid metabolism and storage remains unclear.


The ability of moderate amounts of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) to lower circulating triglyceride levels by approximately 25% has made these fatty acids the most effective triglyceride-lowering agents available. Elevated triglycerides are characteristic of type 2 diabetes and other forms of dyslipidemia and independently increase the risk of cardiovascular disease. Relatively few studies have compared the separate triglyceride-lowering ability of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the principal n-3 LC-PUFAs present in fish oils. Those studies indicated that DHA may be more effective than EPA in reducing triglyceride levels in mildly hypercholesterolemic or hypertriglyceridemic subjects. However, some studies have reported a greater triglyceride-lowering effect with EPA, and others have found no difference between the two.

A study by Buckley and colleagues examined the effect of providing approximately 5 g/day of EPA or DHA to 42 healthy subjects with normal blood lipid levels, aged 20-70 years (mean age 46 yrs), for four weeks. Control subjects consumed olive oil placebo capsules. The EPA capsules contained 8% DHA and the DHA capsules contained 9% EPA, present as unavoidable carryover during the purification of fish oil.

Following EPA supplementation, there was a significant increase in plasma phospholipid EPA and n-3 docosapentaenoic acid, and a significant decrease in linoleic acid. Consumption of DHA led to significant increases in EPA and DHA in plasma phospholipids compared with placebo. Consumption of EPA was accompanied by a 4-fold increase in EPA and a doubling of n-3 docosapentaenoic acid, while DHA supplementation was followed by a more than doubling of both the DHA and EPA levels. The increase in EPA suggests retroconversion of DHA to EPA. Within each treatment, EPA or DHA led to significant displacement of linoleic, arachidonic, and oleic acids from plasma phospholipids compared with baseline values.

Consumption of either n-3 LC-PUFA had no effect on total, low-density or high-density lipoprotein levels, but both EPA and DHA significantly reduced triglyceride concentrations within each treatment group (Figure 1). Compared with the placebo response (-7%), however, triglyceride reduction by EPA (15%) was not significantly different, whereas the 22% reduction in triglycerides with DHA was (P=0.03). However, the EPA and DHA responses did not differ significantly between each other. There were no overall effects of treatment on circulating apo protein E levels.

These findings again suggest superior effectiveness of DHA compared with EPA in lowering circulating triglyceride levels in healthy subjects (22% for DHA vs 15% for EPA), although this difference was not statistically significant. In the present study, the reductions were achieved with consumption of highly purified fatty acids at 5 g/day. The magnitude of the reductions reported here was less than those observed in some hypertriglyceridemic subjects, but similar to those achieved with fish oil. Therefore, there does not appear to be a significant clinical advantage in using purified DHA compared with fish oil containing both EPA and DHA.

Fish and Omega-3s Linked to Lower Birthweight in US Study

There is growing professional and public awareness of the importance of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) during pregnancy, nursing, and infancy. Because docosahexaenoic acid (DHA), an n-3 LC-PUFA, concentrates in the brain during fetal and infant development, and is essential for optimum retinal function and neurodevelopment, its availability during brain development is of paramount importance. The potential effects of n-3 LC-PUFAs on fetal growth and gestation time have received less attention outside the research community, in part because findings are mixed and modest. For example, a meta-analysis of studies in term and preterm infants fed cow’s milk formula with or without additional LC-PUFAs, reported no significant differences in growth between supplemented and unsupplemented infants in either group. However, provision of n-3 LC-PUFAs or LC-PUFAs or fish consumption during pregnancy was associated with reduced intrauterine growth retardation and increased gestation time. In addition, there are some reports of lower birthweight with n-3 LC-PUFA consumption. Is there an association between n-3 LC-PUFAs and birthweight or gestational age, and if so, in which direction?

A recent report from Dr. Emily Oken and colleagues at Harvard Medical School in Boston, Mass., USA, provides one answer. Oken’s team conducted a prospective cohort study of 2,109 mothers living in eastern Massachusetts, aged 14 to 44 yrs, and their infants which examined maternal diet, pregnancy outcomes, and offspring health. Dietary information was obtained by a semiquantitative food frequency questionnaire administered upon enrolment and at 26 to 28 weeks’ gestation, as well as by a nine-question form shortly after delivery. Types of seafood consumed were grouped in four categories: canned tuna, shellfish, dark meat fish, and other fish, from which the intake of n-3 LC-PUFAs was estimated on the basis of earlier validation studies. Associations between birth weight, fetal growth (i.e., birthweight corrected for gestational age), and length of gestation with seafood or n-3 LC-PUFA consumption were calculated by multivariable linear or median regression.

The study population was multi-ethnic (29% Black, Hispanic-American, or Asian-American), and relatively well educated (12% high school or less, 64% college or graduate degree). Ten percent smoked. Mean birthweight was 3,466 g (95% CI: 3,441-3,491) and mean length of gestation 278 days. About 7% of infants were born before 37 weeks of gestation, and 6% of infants had birthweights below the 10th percentile for gestational age.

Women who consumed more seafood, and thus more EPA and DHA, were more likely to be older (by 2 yrs on average), non-white, more educated, and have had a previous birth. However, infants of mothers in the highest quartile of EPA and DHA intake as assessed upon enrolment (mean 0.36 g/day, range 0.24 to 2.53 g/day) weighed an average of 100 g less (P=0.01) than infants of mothers in the lowest quartile of intake (0.02 g/day, range 0 to 0.05 g/day). Similarly, fetal growth was significantly lower in infants of mothers consuming the most EPA and DHA compared with infants born to mothers consuming the least amount (z value 0.10 vs 0.28, P=0.003).

Length of gestation and risk of preterm birth were not associated with EPA and DHA consumption. The observations were similar for dietary intake assessed in the second trimester and immediately post partum, except for a difference in birthweight of about 60 g.

Curiously, when birthweight and fetal growth were examined in relation to the frequency of total seafood consumption, the trend for reduced birthweight with increased consumption was less pronounced (P=0.05) than for EPA and DHA consumption. Using dietary data for the second trimester, the trend did not reach statistical significance. The pattern for fetal growth was similar and fish consumption was not associated with gestational age in any analysis.

These findings contrast with the majority of observational studies, reporting increased birthweight and gestational
age with higher consumption of EPA and DHA. What these findings mean, however, is unclear. The study population was well educated and likely accustomed to eating seafood, judging from the eastern Massachusetts location and the proportionately low numbers of subjects not eating any fish (about 13%). Moreover, the occurrence of premature and low birthweight deliveries (7% and 5%, respectively) was substantially below US national rates (12.3% premature births for whites and 20.0% for blacks). In a healthy, well-nourished, and cared-for population, most of whom were consuming seafood at least three times/mo, fish consumption might be expected to have little effect on birthweight gestation time or fetal growth. The advantages in fetal and infant neurodevelopment of adequate maternal and infant exposure to n-3 LC-PUFAs, especially DHA, during pregnancy and lactation remain compelling.


**Clinical Conditions**

**Retinitis Pigmentosa**

**The Eyes Have It: Blindness Deterred with DHA and Vitamin A in Retinitis Pigmentosa**

To be censured for “tunnel vision” is to be accused of narrow-mindedness. But if you are among the one in 4,000 who, because of family genetics, develops this progressive degenerative disease of the retina, loss of peripheral vision is no verbal slur. The hallmark of retinitis pigmentosa is poor vision in reduced light and constricted visual fields. The condition results from degeneration of the photoreceptors in the rods and cones of the retina.

Readers of the *PUFA Newsletter* are aware of the importance of docosahexaenoic acid (DHA) in the development and function of the retina in early infancy. Fish consumption, which implies intake of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) including DHA, has also been linked to lower risk of age-related macular degeneration, another retinal disease. And, as described elsewhere in this issue, DHA is critical for cell signaling in the retinal rods. Thus, it is reasonable to ask whether DHA might be involved in retinitis pigmentosa. Dr. Eliot Berson, director of the Berman-Gund Laboratory for the Study of Retinal Degenerations at the Massachusetts Eye and Ear Infirmary, Boston, Mass., USA, wondered if patients with retinitis pigmentosa would benefit from increased consumption of DHA.

To find out, Berson and colleagues conducted a 4-year, randomized, double-blind, placebo-controlled trial in 208 patients with retinitis pigmentosa, aged 18 to 55 years, who were supplemented with 15,000 International Units of vitamin A. They had previously shown that vitamin A supplementation significantly slowed the rate of loss of visual function in patients with this condition. Study patients were randomly assigned to consume either 1200 mg/d DHA in vegetable oil capsules, or vegetable oil capsules without DHA, in addition to vitamin A, for four years. Visual assessment was conducted at baseline and annually over the duration of the study using the Humphrey Field Analyzer to measure central visual field sensitivity. Measurements of total visual field sensitivity, electroretinogram amplitude, and visual acuity were also performed each year.

As expected, consumption of DHA increased the DHA content of total plasma fatty acids three-fold above baseline ($P<0.001$), and of red blood cell phospholipids (phosphatidylethanolamine, PE) by 67% ($P<0.001$). Increases were detected at one year and remained at that level throughout the study. Although the patients assigned to consume DHA supplements had a significantly higher percentage of DHA in their red cell phospholipids at baseline than patients in the control group, the groups were significantly different from each other and from baseline values at the end of the study, when the average DHA values after one and four years were compared.

When visual function was compared between the two groups, there were no significant differences in the rate of visual deterioration. However, the investigators noted a highly significant interaction between the effect of DHA supplementation on visual field sensitivity...
and vitamin A status upon entry to the study. To explore this observation further, they performed subgroup analysis of the data in each group, according to vitamin A status prior to entry to the study.

Patients were divided into two groups according to whether they had taken vitamin A before enrolling in the study. The effect of DHA supplementation on the decline in total and central visual field sensitivity over the four-year period was compared in both groups. As shown in Table 1, those supplemented with DHA, who had not taken vitamin A prior to the study, had significantly lower rates of decline than controls (30.7 ± 6.5 dB/y vs 52.5 ± 6.0 dB/y, central visual field, \(P=0.01\)). Rates of decline in those already taking vitamin A before the study were not significantly affected by DHA supplementation, although rates of loss of visual function were greater than in the control patients (39.4 ± 3.8 dB/y in DHA group vs 30.3 ± 3.9 dB/y in controls, NS). Differences in total visual field sensitivity and ERG showed similar significant differences as those observed for central visual field sensitivity. When the decline in visual function was examined on a year-by-year basis according to prior vitamin A consumption, the investigators observed that the rate of decline was significantly slower in the DHA supplemented group (mean loss 25 dB/2 yr or about 2%) compared with control patients (mean loss 141 dB/yr or about 10%) during the first two years (\(P=0.06\)).

This saving amounted to about 8% over two years. Rate of visual decline was not affected by DHA supplementation in the last two years of the study (\(P=0.57\)). The significant interaction indicated that the benefit of DHA supplementation depended on whether patients were consuming vitamin A before entering the study.

More important, higher DHA status and dietary n-3 PUFA intake prior to entering the study was associated with slower visual loss. Patients not consuming vitamin A prior to the study whose red cell phospholipid DHA levels below 5% of total fatty acids had a significantly faster rate of loss of total field sensitivity compared with those having a level of at least 5% DHA (Figure 1). This effect was statistically significant only during the first two years of the study, but the trend was similar during the last two years as well.

The effect of dietary n-3 PUFA consumption was examined in 68 control patients who were consuming vitamin A prior to the study. These patients were divided into two groups according to whether dietary n-3 PUFA consumption was <0.2 g/d or 0.2 g/d or more. For the entire 4-yr study period, patients consuming 0.2 g/d or more had a 40% to 50% slower rate of visual function decline than those who consumed less than this amount (20.8 ± 5.7 dB/yr vs 39.2 ± 5.5 dB/yr, \(P=0.02\)). These findings indicate that dietary n-3 LC-PUFA intake supports the protective effect observed in relation to red cell DHA status. It was also noted that the longer patients had been taking vitamin A supplements, the slower their rate of visual decline.
With these long-term studies, Berson and colleagues suggested a protective effect of DHA status on the rate of visual decline in patients with retinitis pigmentosa. Those whose DHA levels were below 5% of their red cell phospholipid PE had a significantly greater rate of visual deterioration compared with those having DHA levels above 5%. For those not receiving the DHA supplement, dietary DHA was also shown to be protective. The greatest protection against visual loss, as much as 40% to 50% per year, was afforded by the consumption of 15,000 IU of vitamin A with a diet providing at least 200 mg/day n-3 LC-PUFAs. The authors estimated that following this recommendation a 37-year old patient could gain an additional 19 years of vision, compared with a patient who consumed less than 200 mg/day n-3 LC-PUFAs. The authors cautioned that for patients just beginning to take vitamin A supplements, the consumption of 1200 mg/day of DHA capsules longer than two years was associated with a slightly greater rate of visual decline. However, such patients were advised to consume one to two servings of fish rich in n-3 LC-PUFAs per week to ensure adequate DHA, but in amounts much lower than used in these studies.

The good news is that a diet rich in n-3 LC-PUFAs with vitamin A supplementation may be able to prevent premature visual loss in retinitis pigmentosa. Further, the additional n-3 LC-PUFAs prevented the increase in triglycerides observed with vitamin A. The implications of this work suggest that measuring red cell phospholipid DHA (target: 5% DHA in red cell phosphatidylethanolamine) in patients with retinitis pigmentosa could be a sensitive way to identify those who have reached a warning level for being at greatest risk of rapid visual loss. The Berson team also showed that in patients who have not been taking vitamin A supplements, well known to slow visual decline, the addition of DHA capsules to the vitamin supplementation protocol for the first two years, enhanced the effectiveness of the vitamin in slowing the rate of visual loss. Whether DHA is necessary for the release of the active form of vitamin A that is critical to photoreceptor survival, or vitamin A facilitates incorporation of DHA into the retina is not known. Ideally, a prospective trial among patients grouped by vitamin A status would provide a stronger experimental design, but is arguably unethical to do. One would also like to know whether boosting the consumption of DHA before adulthood might affect the manifestation and course of this condition.


**Childhood Asthma**

**Children Prone to Allergies Benefit from Long-Chain Omega-3 Fatty Acids**

Prevalence of childhood asthma has been increasing sharply in "western-style" countries and urban areas around the world. Prevention strategies aimed at avoiding tobacco smoke, extending breast-feeding, and avoiding allergens have had only small effects. Observational studies suggest that children who consume fatty fish are less likely to develop symptoms of asthma, but data from clinical trials are sparse. Evidence from the effects of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) on respiratory conditions in adults makes it reasonable to explore whether consumption of these fatty acids early in life might be beneficial in childhood asthma. Could consumption of n-3 LC-PUFAs be an effective primary prevention strategy?

The Childhood Asthma Prevention Study in Australia was designed to measure the separate and combined effects of dietary supplementation with n-3 LC-PUFAs and avoidance of house dust mite allergen in children with a family history of asthma. Sixty percent of these children would be expected to develop asthma. The study team designed a randomized controlled trial with a factorial design to assess the effect of the primary variables on asthma and cough, defined according to International Consensus Guidelines. Sensitivity to allergens was determined by skin prick tests using various ingested and inhaled allergens.

From the age of six months, half of the children consumed n-3 PUFAs from one tuna oil capsule/day containing 184 mg of n-3 PUFAs. Their families also received canola-based oil and spread. The other children received placebo supplements containing 80% monounsaturated oils and their families were given soybean-based oil and spread. In half of both groups of families, exposure to dust mite allergen was minimized by the use of allergen-impermeable bed coverings, latex-free playmats, and restricted use of soft toys in the child’s bed. Acaricidal washing detergent was also provided to intervention families. The other families received no interventions to reduce dust mites. Clinical assessment of the 526 children occurred when they were 3 years old.

Comparisons between groups on the basis of dietary intervention (Table 1) revealed that among children with at least one positive skin prick test (atopic subjects) significantly more who consumed n-3 PUFAs had no cough (51%) compared with those consuming the placebo oil (39%). Similarly, for nonatopic children, significantly more in the tuna oil group had no cough compared with placebo subjects. Consumption of n-3 PUFAs had no significant effect on any other measure of asthma or sensitivity to allergens.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Number of subjects</th>
<th>Placebo (Soybean) (%)</th>
<th>Tuna oil (%)</th>
<th>Difference</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atopic subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cough</td>
<td>237</td>
<td>102 (39.4%)</td>
<td>135 (50.6%)</td>
<td>-11.2%</td>
<td>0.003</td>
</tr>
<tr>
<td>Nonatopic cough</td>
<td>200</td>
<td>100 (38.6%)</td>
<td>100 (37.5%)</td>
<td>1.1%</td>
<td></td>
</tr>
<tr>
<td>Atopic cough</td>
<td>89</td>
<td>57 (22.0%)</td>
<td>32 (12.0%)</td>
<td>10.0%</td>
<td></td>
</tr>
<tr>
<td><strong>Nonatopic subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cough</td>
<td>237</td>
<td>102 (39.4%)</td>
<td>135 (50.6%)</td>
<td>-11.2%</td>
<td>0.03</td>
</tr>
<tr>
<td>Mild cough</td>
<td>239</td>
<td>127 (49.0%)</td>
<td>112 (41.9%)</td>
<td>7.1%</td>
<td></td>
</tr>
<tr>
<td>Moderate cough</td>
<td>50</td>
<td>30 (11.6%)</td>
<td>20 (7.5%)</td>
<td>4.1%</td>
<td></td>
</tr>
</tbody>
</table>

*At least one positive skin prick test

The Childhood Asthma Prevention Study in Australia was designed to measure the separate and combined effects of dietary supplementation with n-3 LC-PUFAs and avoidance of house dust mite allergen in children with a family history of asthma. Sixty percent of these children would be expected to develop asthma. The study team designed a randomized controlled trial with a factorial design to assess the effect of the primary variables on asthma and cough, defined according to International Consensus Guidelines. Sensitivity to allergens was determined by skin prick tests using various ingested and inhaled allergens.
7%, from 26% in the control children to 18% in those receiving the intervention ($P = 0.05$).

The authors interpreted the reduction in atopic cough associated with the consumption of n-3 PUFAs as consistent with reduced inflammation in the airways that has been observed in adults who consume fish. The findings are consistent with observational reports of lower prevalence of asthma in children who eat fatty fish and in reduced bronchitis in US children with high fish consumption. The authors pointed out that cough differs from the wheeze characteristic of asthma, which was not affected by dietary treatment, and suggested that n-3 PUFAs affect allergic inflammation in airways rather than cough receptors. Even though the effects of consuming n-3 PUFAs, especially those in fish, were modest, they may be important in reducing the severity of the disease and improving quality of life in children at high risk of developing asthma and atopy. The investigators anticipate monitoring these children for long-term effects.


### Hypertension

#### Fish, Hypertension and Weight Control: The Leptin Connection

Consumption of fish in a calorie-restricted diet among overweight subjects with hypertension enhances the beneficial effects of weight loss. With both energy restriction and daily fish consumption, reductions in blood pressure and improvements in blood lipids, heart rate, and glucose metabolism are greater compared with weight loss alone. Whether fish consumption itself facilitates or improves weight loss is not known. Animal studies suggest that dietary omega-3 polyunsaturated fatty acids (n-3 PUFAs) may affect adipose tissue metabolism, possibly by affecting the disposition of dietary fatty acids, or the production of leptin, in ways that favor weight loss. Leptin is a hormone produced by adipocytes that is involved in the complex regulation of weight control and appetite. It acts on the brain to regulate food intake and fat storage and affects the nervous and immune systems.

Little is known about fatty acid regulation of adipose tissue fat storage and mobilization. Some animal studies have reported, reduced leptin levels and increased fatty acid oxidation with the consumption of n-3 LC-PUFAs. In healthy nonobese subjects, a diet high in canola oil was associated with significantly lower leptin levels in women, but not men in one study. Data on the effects of n-3 PUFAs on leptin in human subjects are almost non-existent.

To explore the potential effects of fish consumption on weight loss and leptin in human subjects, Mori and colleagues conducted a randomized, controlled, factorial design clinical trial in 63 overweight, mildly hypertensive subjects, aged 40 to 70 yr (mean age 54 yr), who were being treated for hypertension. These investigators had previously shown that daily fish consumption enhanced the effects of weight loss in reducing blood pressure. After stratification by gender, age, and body mass index, and a 4-week familiarization period, subjects were randomly assigned to consume either a weight-maintaining diet with or without a daily fish meal, or an energy-restricted diet with or without a daily fish meal for 12 weeks. Subjects then consumed a weight maintenance diet for 4 weeks for a total study period of 16 weeks. Canned and filleted fish providing an average of 3.6 g n-3 PUFAs/day was provided to the participants. Plasma phospholipids, fasting serum glucose, insulin, lipids, and leptin were measured at baseline and 16 weeks.

After 16 weeks, those in the weight loss groups lost an average of 5.6 ± 0.8 kg (12.3 ± 1.8 lb), while weight remained unchanged in the weight maintenance groups. Weight loss among those consuming fish was not significantly different from those not consuming fish. Thus, fish consumption did not affect the amount of weight lost. However, it significantly increased total plasma phospholipid n-3 LC-PUFA levels and decreased n-6 LC-PUFAs compared with the control group.

Dietary fish consumption and weight loss were significantly inter-related in reducing serum leptin levels in overweight hypertensive subjects. Leptin levels fell only when the intervention included both fish and weight loss.
The reduction in leptin was significantly associated with reductions in serum insulin, blood pressure, and heart rate. In the fish-weight loss group, the reduction in leptin was significant compared with the control and fish only groups, but not compared with the weight loss only group.

The study also showed a significant reduction in fasting insulin in those losing weight (−4.05 pmol/L, \( P = 0.003 \)), with the reduction being greatest in fish-weight loss group (−4.28 pmol/L, \( P = 0.03 \)). With fish alone, insulin rose slightly. These findings confirm the animal studies that showed a relationship between serum leptin and insulin levels. In human obesity, both serum insulin and leptin levels are elevated.

These findings, in conjunction with previous observations from the same subjects of reduced blood pressure with weight loss and fish consumption, suggest that leptin may be involved in the regulation of blood pressure in overweight hypertensive subjects, possibly through its effects on the sympathetic nervous system. The authors speculated that leptin may be critically involved in the development of insulin resistance, and that the combination of weight loss and fish consumption may be effective synergistically in reducing the risk of stroke, cardiovascular disease and myocardial infarction. For fish lovers, that may be just the necessary hook to let more of those calories get away.


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**Frontiers**

**DHA Structure Holds the Key to Retinal Cell Communication and Visual Function**

Cells respond to changes in their surroundings with a variety of communications tools, many of which are activated at the cell membrane surface. For example, a hormone outside the cell may attach to a receptor protein in the cell membrane that will bind only that hormone. The binding of the hormone sets off signals inside the cell that call other membrane-bound proteins to action. G proteins, located on the inside surface of the cell membrane are one class of these proteins that are linked or “coupled” to specific membrane receptors. When activated by receptor-hormone binding, or other stimulus at the membrane, G proteins bind to their own target enzymes, which in turn trigger additional responses. In this way, the signal from the original hormone, or other receptor-binding substance, becomes amplified. This cascade of events, triggered by receptor binding, is called cell signaling.

Changes in membrane composition can affect cell signaling pathways. In particular, docosahexaenoic acid (DHA) a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) that is especially abundant in retinal membranes, appears to be a critical factor in visual signaling. When DHA is insufficient, a substitute is synthesized from n-6 fatty acids,
called docosapentaenoic acid (DPAn-6). This substitution appears to reduce G-protein cell signaling in the retina, which is linked to the suboptimal visual responses observed when diets deficient in n-3 PUFAs are consumed.

Dr. Niu and colleagues at the National Institutes of Health, Rockville, MD, sought to explain how DHA facilitates cell signaling in the retina, and thus why it appears essential for optimal visual function. To do so, they examined parameters of the G-protein signaling system from retinas of rats fed diets adequate or deficient in n-3 PUFAs. A simplified description of the retinal signaling system begins with light energy activating the photoreceptor protein, rhodopsin, located in the rod cells in the retina (Figure 1). This event triggers the formation of the active receptor, metarhodopsin II, which then binds to the visual G protein. The activated G protein in turn activates the enzyme phosphodiesterase whose activity leads to the closure of calcium/sodium channels in the rod outer segment membrane. Changes in ion flow across the membrane initiate the visual response that is measured in electroretinograms.

The effect of n-3 LC-PUFAs was examined by comparing the activity of various steps in the visual G protein signaling pathway in retinas from deficient and adequately fed animals. Deficient animals were bred from females raised on n-3 PUFA deficient diets, so they were severely depleted in DHA. As expected, the depleted animals had greatly reduced levels of DHA in the phospholipids of their retinal rod outer segments (Figure 2). While the DHA content in the phospholipids of rats fed n-3 LC-PUFAs was abundant, about 45 and 50% of total fatty acids in PS and PE, respectively, the docosapentaenoic acid level (DPAn-6) was very low, 2.4% and 1.8%, in PS and PE respectively. In contrast, n-3 PUFA-deficient animals had the reverse fatty acid pattern, low levels of DHA and high levels of DPAn-6 (Figure 2). The levels of DPAn-6 in phospholipids of n-3 PUFA-deficient animals were 80% and 63%, respectively, of the DHA in PS and PE of the adequately fed animals.

The investigators also compared the phosphodiesterase enzyme activity in rod outer segments of the n-3 PUFA adequate and deficient animals. This enzyme triggers the visual neuronal response, which results in closure of the calcium/sodium channels. At physiological levels of light activation, upon which the enzyme activity depends, there was a three-fold reduction in enzyme activity in deficient animals compared with adequately fed ones. The researchers also showed that the number of rhodopsin molecules activated by light was diminished by approximately 12% and this resulted in reduced activation of G proteins with consequent reduction in phosphodiesterase enzyme activity. These results would explain the reduction in amplitude of the electroretinograms observed in n-3-PUFA-deficient laboratory animals.

This paper presents direct evidence that the 80% loss of DHA in rod outer segments that occurred in the n-3 LC-PUFA deficient animals, and its replacement by DPAn-6, was sufficient to affect the individual steps in the G-protein signaling pathway. These changes resulted in functional deficits in cell signaling that was evident in reduced phosphodiesterase enzyme activity. The authors noted the “exquisite sensitivity and fine tuning” of the membrane properties in rod outer segments and suggested that G-protein signaling systems may be affected by n-3 LC-PUFA insufficiency.
in a similar manner in brain. This study has revealed more precisely why DHA is critical to optimum visual function.


**DHA Synthesis Achieved in Transgenic Yeast**

Somewhere along the evolutionary path from algae to terrestrial plants, the ability to make polyunsaturated fatty acids with 20 or more carbons vanished. Algae, and some fungi and lower plants happily synthesize complex long-chain polyunsaturated fatty acids (LC-PUFAs) having up to 22 carbons and six double bonds. Most terrestrial plants call it quits at 18 carbons and two or three double bonds. Lacking the necessary enzymes for further desaturation and chain elongation, plants cannot synthesize LC-PUFAs. What are the stumbling blocks to the synthesis of LC-PUFAs in plants, especially those of the n-3 family?

Synthesis of LC-PUFAs from their 18-carbon omega-6 (n-6) and omega-3 (n-3) precursors, linoleic and alpha-linolenic acids, respectively, requires alternating steps of desaturation to add a double bond and elongation to add two carbons. After three such steps, linoleic acid is converted to arachidonic acid (20:4n-6*) and alpha-linolenic acid to eicosapentaenoic acid (EPA, 20:5n-3). Qi and colleagues were the first to develop transgenic plants able to synthesize these 20-carbon fatty acids. Those investigators incorporated three transgenes in *Arabidopsis thaliana* to provide the necessary desaturase and elongase enzymes for LC-PUFA synthesis. However, conversion of EPA to docosahexaenoic acid (DHA, 22:6n-3) was blocked. It is this hurdle that Dr. Ernst Heinz’ team at the University of Hamburg and in France has just cleared.

Finding the specific enzymes to perform the additional elongation and desaturation steps to convert EPA to DHA was the first task, as none specific for 20-carbon PUFAs had been identified and cloned. The elongase that converts EPA to DHA in mammals acts on fatty acids of various lengths and therefore would have low specificity for EPA. After searching different gene banks and species, the Heinz group identified possibilities in fish, frog, sea squirt, and two species of algae that make DHA. First they isolated and cloned the potential genes and then incorporated them into yeast. The expressed enzymes were evaluated for activity and specificity by growing the yeast cultures with either stearidonic acid (18:4n-3) or EPA, and analyzing the fatty acid products by gas chromatography and mass spectrometry. From these detailed procedures, they identified and cloned two elongases from the green alga *Ostreococcus tauri* and the diatom *Thalassiosira pseudonana* specific for the elongation of 20-carbon PUFAs at the delta-5 carbon. The enzyme from *O. tauri* elongated 11% of arachidonic acid and 56% of EPA, the latter being converted to docosapentaenoic acid (22:5n-3).

To obtain DHA, the investigators transformed yeast to co-express the four enzymes needed to convert
stearidonic acid to DHA. One enzyme was a new delta-6 elongase needed to convert stearidonic acid (18:4n-3) to the 20-carbon precursor (20:4n-3) of EPA (Figure 1). The second enzyme was a delta-5 desaturase, needed to convert 20:4n-3 to EPA. The third enzyme was the new delta-5 elongase that would take EPA to 22:5n-3. Finally, the delta-4 desaturase, cloned from the single-celled aquatic organism *Euglena gracilis*, would convert 22:5n-3 to 22:6n-3 or DHA. By using different combinations of the elongase and desaturase enzymes they had cloned, the researchers were able to study the specificity of the different enzymes for various fatty acids.

The fatty acid profiles produced in the transformed yeast, identified by mass spectrometry, confirmed the synthesis of DHA. Although the amount produced was relatively low, up to 5%, this may have been related to the specificity of the delta-5 desaturase enzyme for fatty acids in the 2-position of phospholipids.

These researchers also developed transgenic flax (linseed), *Linum usitatissiumum*, incorporating genes for a delta-6 desaturase enzyme from *P. tricornutum*, a delta-5 desaturase from *P. tricomutum*, both algae, and a delta-6 elongase from the moss, *P. patens* (Figure 2). These genes were expressed in transgenic lines and resulted in the synthesis of 20-carbon n-6 and n-3 PUFAs. However, it appeared that the C-18 fatty acid products produced from the first delta-6 desaturase activity were channeled from phospholipids to triglycerides where the requirements for elongase activity could not be met sufficiently. Thus, production of 20-carbon PUFAs was greatly limited in linseed. The researchers showed that the enzymes used in these transformations specifically required the fatty acids to be present in phosphatidylecholine. Enzymes derived from mammals do not have this requirement.

The authors proposed two alternate routes of LC-PUFA biosynthesis to get around the bottleneck created by the accumulation of 18-carbon PUFAs in triglyceride pools. In one model, they suggested using desaturase and elongase enzymes from mammals to convert the precursor fatty acids in the acyl-CoA pools to 20- and 22-carbon derivatives. Another alternative using different desaturase and elongase enzymes would return the fatty acids to their phospholipid environment for desaturation after an initial elongation. This strategy was already demonstrated successfully in the leaves of transgenic *Arabidoposis thaliana* by Qi and colleagues. Whether this approach will work in seeds remains to be shown.

In these elegant and extensive studies, the Heinz team has demonstrated the synthesis of 20-carbon PUFAs in yeast and linseed and identified the biosynthetic bottlenecks to boosting the amounts produced. By using enzymes for desaturation and elongation in transgenic yeast that were specific for the location of the fatty acid in the 2-position of the phospholipid, the investigators accomplished DHA synthesis with only low levels of unwanted by-products. Their biosynthetic pathway
also avoided the additional steps for DHA synthesis that occur in mammalian systems. Moreover, the use of enzymes cloned from algae avoided contentious issues associated with using mammalian genes. In transgenic linseed, the investigators identified the highly specific requirements for the activity of several desaturase and elongase enzymes for the production of 20-carbon PUFAs. These studies have taken the quest for LC-PUFA synthesis in oilseed plants, especially for DHA, another step forward, with the identification and expression of unique enzymes from algae and moss, and the demonstration of their functionality in transgenic yeast and linseed.

*The first number is the number of carbons, the second the number of double bonds*
