PUFA NEWSLETTER

Volume 10 • Issue 3

September 2005 Contents

Editorial

Unexpected Findings with Fish Oil Create a Buzz

Results from a US study of patients with implanted defibrillators who fared worse after consuming fish oil generated hundreds of news stories proclaiming doubts about the cardiovascular benefits of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). In contrast, a study from Denmark reporting lower risk of ventricular events in patients with defibrillators whose omega-3 levels were high received little media attention. Findings from two other trials in patients with defibrillators are expected in the coming months. Besides illustrating that reporters favor bad news over good, these studies raised challenging questions about how fish oils affect patients at high risk of sudden death. Reduction of fatal arrhythmias is considered a primary effect of n-3 LC-PUFAs in patients who have had a heart attack. Both reports are reviewed in this issue of the *PUFA Newsletter*. These controversial findings are a timely reminder of the diverse manifestations of cardiovascular disease and the substantial differences among heart patients. Lost in most news coverage was the fact that no single study constitutes proof. Research on omega-3s continues to be lively.

This newsletter also features a report on maternal DHA (n-3 LC-PUFA) supplementation during lactation and infant neurodevelopment, as well as a critical review of the literature assessing the need for DHA in brain development. Authors of the review conclude, in spite of data limitations, that DHA is associated with improved cognition and behavior.

The importance of n-3 LC-PUFAs in the risk or progression of two neurodegenerative brain diseases, Alzheimer's and Parkinson's, underline the involvement of DHA in brain function later in life. Results from a series of studies in a transgenic mouse model of Alzheimer's disease indicated that DHA is involved in the function of synapses, regulation of beta-amyloid synthesis, and gene expression. DHA deficiency hastened the development and severity of Alzheimer's disease in these mice. In another side of brain function, PUFAs from both the omega-6 and omega-3 families were linked to improved learning and behavior in children with developmental coordination disorder.

The Frontiers section features work from an Australian research group that succeeded in synthesizing DHA in *Arabidopsis thaliana* via a pathway not found in mammals. The team cloned several enzymes from zebrafish, a nematode, and a microalga to accomplish the feat in transgenic cress. Their results achieved a greater production efficiency than previously reported by others and offers the potential for further improvement. This work hastens the prospect of transgenic oilseed plants being able to produce the desirable fatty acid DHA.

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Cardiovascular Health

Do Omega-3s Help or Harm Patients with Implanted Defibrillators?

Irregular heart rhythms or arrhythmias indicate a problem with the heart's electrical system. Things can go awry in either the upper (atrial) or lower (ventricular) chambers of the heart, and when they do, they may impair or completely shut down the heart's ability to pump blood. Arrhythmias can be fatal and are the chief cause of sudden death. The American Heart Association estimates 335,000 sudden cardiac deaths occur in the U.S. each year, mostly from ventricular fibrillation, the rapid quivering of the heart's lower chambers that prevents pumping.

Patients at high risk for dangerous ventricular arrhythmias may have their heart rhythms controlled by means of a small device called an implantable cardioverter defibrillator (ICD) implanted in the chest. The device monitors the heart rhythm and when needed, delivers electrical shocks to stop fibrillation. ICDs differ from pacemakers, which prevent dangerously slow heartbeats.

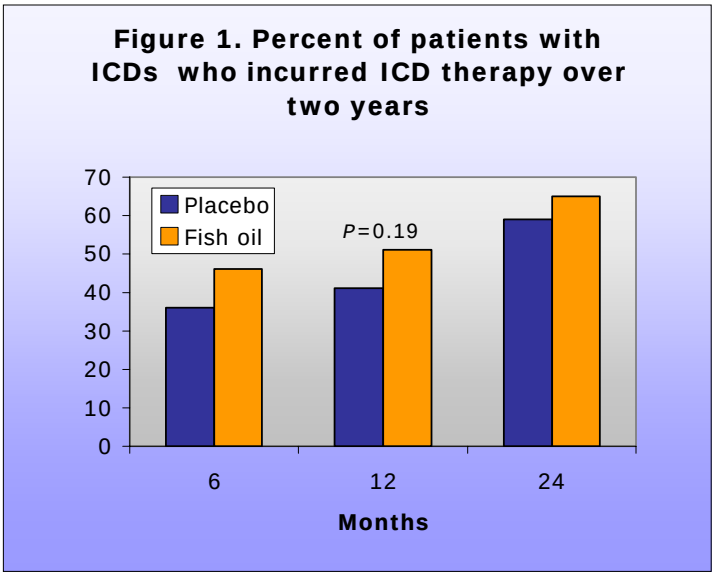
The cause of many arrhythmias is unknown; however, primary heart disease, diabetes, restricted blood flow to the heart (ischemia), certain medications, and supplements, and other stresses can contribute to them. Studies in animals and some in patients with various types of heart disease have reported a reduction in ventricular fibrillation with the administration of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). Other studies have found no effect of these fatty acids on ventricular function. Many reports have described reduced atrial fibrillation with the consumption of n-3 LC-PUFAs and a mechanism of action involving changes in the flow of ions has been described.

Because reduction in overall cardiac arrhythmias is thought responsible for the lion's share of reduced sudden cardiac deaths associated with n-3 LC-PUFAs, it came as a surprise when researchers at the Oregon Health and Science University, USA, reported that consumption of fish oil did not reduce risk of ventricular fibrillation in patients with implanted defibrillators. More surprising still, consuming fish oil was apparently pro-arrhythmic in patients with ventricular tachycardia (rapid heartbeats). How could these findings be explained?

In the report by Merritt Rait and colleagues, 200 patients were recruited from six medical centers in the U.S. Eligible participants were those receiving an ICD for documented sustained ventricular tachycardia (rapid heart beats) or fibrillation that was not the result of a myocardial infarction or a reversible cause of the arrhythmia. Also excluded were patients who had a pre-existing ICD and had received ICD therapy for ventricular arrhythmia within the past three months. Those who consumed more than one meal of fatty fish a week, or consumed flax, fish, or cod liver oil were also ineligible. It should be noted that these patients had significantly reduced function in their left ventricles, the heart's main pump, and were not typical heart disease patients.

Participants were sorted by type of arrhythmia (tachycardia or fibrillation) at entry to the study, and time of ICD implantation. They were assigned randomly to consume either 1.8 g/day of fish oil (providing 1.3 g EPA plus DHA/day) or olive oil. Patients were monitored monthly for the first three months and every three months thereafter for up to two years. ICD memory was checked for episodes of ICD therapy at each visit and classified as tachycardia, fibrillation, or other types of arrhythmia. Only episodes of tachycardia and fibrillation were considered end points. Researchers were interested primarily in the time until the first episode of tachycardia or fibrillation that triggered ICD therapy.

Figure 1. Percent of patients with ICDs who incurred ICD therapy over two years

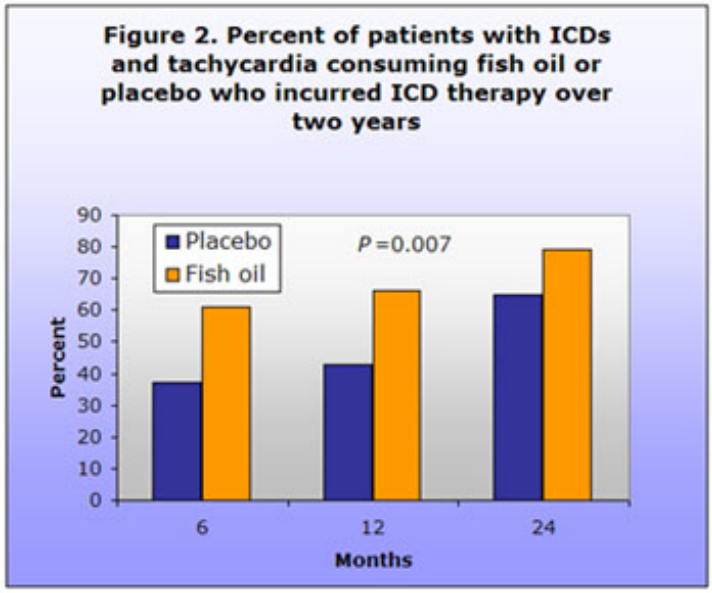


Of the 200 patients enrolled, 192 completed the study, with a median study time of 718 days or nearly two years. Fourteen deaths occurred, 10 in the placebo group and 4 in those taking fish oil (difference not statistically significant).

Throughout the study, patients consuming fish oil had significantly shorter times to first ventricular arrhythmias and higher incidence of these events than those consuming the placebo. This observation is illustrated in Figure 1 showing the percent of patients whose defibrillators were triggered in response to at least one episode of arrhythmia at different intervals during the study.

However, when the patients were grouped according to the type of ventricular arrhythmia at the time of enrollment, times to the first episode of ICD therapy differed according to treatment. Patients presenting with ventricular tachycardia who consumed fish oil had significantly shorter times compared with those on the placebo (Figure 2). Tachycardia episodes were much more frequent than fibrillation both at enrollment (66% of patients) and in events triggering ICD therapy (901 ventricular tachycardia versus 45 fibrillation episodes over two years).

Figure 2. Percent of patients with ICDs and tachycardia consuming fish oil or placebo who incurred ICD therapy over two years



The investigators also reported that among all patients on fish oil, recurrent episodes of ICD therapy—that is, those after the first episode—were significantly greater than for placebo patients, 3.5 vs 2.2 days ($P<0.001$). In these patients, fish oil actually increased the chance of ventricular arrhythmias.

When the findings were expressed in terms of hazard ratios—the chance of a *longer* time to first ICD therapy—for all patients on placebo was nearly 30% greater than for those on fish oil [hazard ratio 1.28 (CI= 0.88-1.85)]. When hazard ratios were calculated according to type of arrhythmia at enrollment, the patients with tachycardia consuming placebo were nearly twice as likely (1.76) to have longer times to first ICD therapy than those on fish oil. Interestingly, patients presenting with fibrillation arrhythmias had a nearly 30% more favorable chance for longer time to first ICD therapy with fish oil compared with placebo.

What does one make of these unanticipated findings? Perhaps the first critical observation is that the participants were not "average" heart disease patients. They had a history of sustained ventricular arrhythmia and seriously impaired ventricular function. These characteristics, highlighted by the need for an implanted defibrillator, put them at very high risk for sudden cardiac death. Second, failure to find reduced risk of ventricular arrhythmia in these patients does not invalidate the many studies indicating reduced risk of cardiac arrhythmias with fish oil consumption, especially in people with ischemic heart disease. The authors of this study suggested that people with ICDs and no history of recent myocardial infarction might have a type of arrhythmia in which interference with sodium ion channels (which lead to muscular contraction) exacerbates arrhythmia. This possibility has been demonstrated with several therapeutic agents.

Finally, fish oils and n-3 LC-PUFAs have been associated with many other benefits to cardiovascular health in people at risk for heart disease and those who have already had a myocardial infarction. However, as this study shows, those with non-ischemic heart disease and a history of sustained ventricular arrhythmias who have implanted defibrillators should avoid fish oil supplementation.

Raitt MH, Connor WE, Morris C, Kron J, Halperin B, Chugh SS, McClelland J, Cook J, MacMurdy K, Swenson R, Connor SL, Gerhard G, Kraemer DF, Oseran D, Marchant C, Calhoun D, Shnider R, McAnulty J. Fish oil supplementation and risk of ventricular tachycardia and ventricular fibrillation in patients with implantable defibrillators: a randomized controlled trial. JAMA *2005;293:2884-2891.*

Improved Prognosis in Patients with ICDs and Higher Blood Omega-3s

In contrast to the worsened outlook for patients with implanted cardiac defibrillators (ICDs) who consumed fish oil reported in the preceding study, some patients with these

devices were reported to fare better when their blood levels contained higher rather than lower levels of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs).

In this observational study conducted by Jeppe Hagstrup Christensen and colleagues at Aalborg Hospital in Denmark, 98 patients who had both ischemic heart disease and an ICD were monitored over the course of one year to determine if there was a relationship between the concentration of n-3 LC-PUFAs in their serum phospholipids and ventricular events. Ninety-two of the study participants, whose average age was 64 years, had had a previous myocardial infarction, but those with known atrial flutter or fibrillation were excluded. Records from patients' defibrillators were analyzed for episodes of ventricular fibrillation and tachycardia throughout the year and blood was sampled at the end of 12 months.

During the study period, 22 patients (25%) incurred a total of 71 ventricular events (39 ventricular tachycardia and 32 fibrillation events). As seen in Table 1, patients who had more than one episode of ventricular arrhythmias had significantly lower total and individual n-3 LC-PUFAs compared with patients who had no ventricular arrhythmias. The association was strongest for the concentration of docosahexaenoic acid (DHA).

tachycardia was similar but did not reach statistical significance. Small numbers of patients in each group would make it difficult to obtain statistically significant results. When the total number of arrhythmic events was compared in the highest and lowest n-3 LC-PUFA groups, six times more events occurred in the lowest versus the highest groups (1.3 vs 0.2).

These findings indicated that in patients who have ischemic heart disease and disturbed ventricular arrhythmias that require an implanted defibrillator, protection against subsequent ventricular arrhythmias was associated with having a higher serum phospholipid n-3 LC-PUFA concentration. The observation of reduced ventricular arrhythmias in high-risk patients who had greater levels of phospholipid n-3 LC-PUFAs supports epidemiological and clinical evidence for the anti-arrhythmic properties of these fatty acids. Only randomized, controlled, clinical trials can provide evidence to validate these observations. Further, as is apparent from the intervention study by Raitt and colleagues, the type of heart disease patient may be critical for determining whether n-3 LC-PUFAs help or harm patients prone to ventricular arrhythmias. Two additional clinical trials nearing completion may provide some answers to the questions raised by these intriguing findings.

Table 1. Occurrence of ventricular arrhythmias (tachycardia and fibrillation) in one year and mean (± SD) serum phospholipid n-3 LC-PUFA levels in 98 patients with implanted defibrillators

	No ventricular arrhythmia	One or more arrhythmic events	More than one arrhythmic event	*P**
No. of patients	76	22	14	
Phospholipid EPA (%)	2.4 ± 1/3	2.0 ± 1.0	1.7 ± 0.9	< 0.04
Phospholipid DHA (%)	5.8 ± 1/6	5.2 ± 1.9	4.5 ± 1.5	< 0.01
Total n-3 LC-PUFAs (%)	9.2 ± 2.7	8.2 ± 2.8	7.1 ± 2.4	< 0.01

*Compared with patients having no arrhythmias

When the number and type of ventricular arrhythmias were examined according to quintiles of total serum phospholipid n-3 LC-PUFAs, patients with the highest concentration of n-3 LC-PUFAs had significantly fewer ventricular fibrillation events compared with those in the lowest concentration group (0.05 vs 0.75, $P < 0.05$). The trend for ventricular

Christensen JH, Riahi S, Schmidt EB, Molgaard H, Pedersen AK, Heath F, Nielsen JC, Toft E. n-3 fatty acids and ventricular arrhythmias in patients with ischaemic heart disease and implantable cardioverter defibrillators. *Europace* 2005;7:338-344.

Electrocardiograms in Patients with Extraventricular Heartbeats Unaffected by Fish oil Consumption

Extra beats or electrical impulses in the heart's main pump, the ventricles, might signal underlying heart disease or increased risk of sudden death. Or they might be benign and without particular significance. Called premature ventricular complexes (PVC) they are detected by electrocardiogram. The dangerous variety of ventricular complexes usually occurs in patients with underlying heart disease. Extra ventricular beats can also result from stimulants such as caffeine and alcohol, metabolic abnormalities such as acidosis, and drugs such as digoxin and antipsychotics. Inappropriate treatment of PVCs may increase mortality. In studying this condition, it is important to know whether the participants have heart disease.

Geelen and colleagues at Wageningen University in The Netherlands reported earlier this year that consumption of fish oil had no effect on PVCs in 84 patients (average age 64 years), a quarter of whom had had a myocardial infarction. However, in this study, consuming fish oil significantly lowered heart rate, which reduces the risk of sudden death.

In a second report from this study, Geelen's group observed no effect of fish oil (1.3 g/day for 14 weeks) compared with placebo on any characteristics of the electrocardiogram, regardless of whether the participants had had a myocardial infarction. The authors noted that possible effects may be too small to be detected in the electrocardiogram, or may be such that they cancel each other out. Failure to detect changes in the electrocardiogram, however, does not rule out effects on heart muscle involving changes that trigger muscle contraction. Use of implantable cardiac defibrillators (see Christensen *et al.* in this issue) may be a more sensitive means of detecting critical arrhythmic events, though the patient population is unique.

Geelen A, Zock PL, Brouwer IA, Katan MB, Kors JA, Ritsema van Eck HJ, Schouten EG. Effect of n-3 *fatty acids from fish on electrocardiographic characteristics in patients with frequent premature ventricular complexes.* Br J Nutr *2005;93:787-790.*

Childhood Fish Intake Related to Adult Risk of Stroke

Atherosclerosis starts early. The foundations of cardiovascular disease are laid in childhood with fatty streaks occurring by 5 years of age and fibrous plaques by age 20. Thus, it would seem obvious that lifelong eating and lifestyle habits contribute to, but may also deter, cardiovascular disease. Parsing out the contributing and protective effects of diet, however, is not as simple as it seems. For one reason, there are few longitudinal studies with sufficient data to permit studying these issues over decades. Food habits, lifestyle, food composition, and many other factors change making it difficult to compare food patterns from decades ago with current information. However, one long-term database from Britain, the Boyd Orr cohort, begun prior to World War II, provides insights for current thinking about diet and chronic disease.

In the report described here, Andrew Ness and colleagues at the University of Bristol estimated childhood dietary exposures to various food constituents and related them to total mortality and cardiovascular deaths. Participants included 4,028 cohort members from 1,234 families who participated in the original survey of diet and health in Britain from 1937 to 1939. Participants were those living in Britain on Jan. 1, 1948 with deaths included up to July 31, 2000. The findings indicated that higher childhood consumption of vegetables and lower intake of fish was associated with lower risk of stroke. Does this make sense?

Dietary data were derived from two 7-day weighed household inventories taken at the beginning and end of the survey period. Original food records were reanalyzed using more advanced analytical techniques. Data were expressed in terms of families rather than individuals. However, each individual's food and nutrient intake was calculated by dividing the daily total household intake by the number of family members, weighted according to their food requirements relative to an adult man. The investigators adjusted for meals missed by family members and those consumed by visitors. Primary outcomes were total mortality, death from coronary heart disease, and stroke. Intakes of foods and nutrients were divided into quartiles and rate ratios between quartiles were calculated. The analysis used Poisson regression to account for the longitudinal nature of the data and reductions in numbers of people at risk over time. Rate

ratios were adjusted for clustering and the analysis controlled for age, sex, and energy intake. Other potentially confounding variables, such as childhood family food expenditure and social class, were included in multivariate analysis.

Rate ratios for total mortality from all causes were significantly lower in the highest quartiles of fruit consumption, but the association did not reach statistical significance when the analysis included multiple variables. For coronary heart disease deaths, none of the childhood dietary components, including fish consumption, was associated with adult mortality. However, for deaths attributed to stroke, rate ratios in multivariate analysis were significantly lower with higher vegetable consumption. In contrast, the risk of stroke mortality increased with greater fish intake in childhood. When the data were stratified according to the consumption of fatty fish versus not eating fatty fish, there was no association with stroke, coronary, or all cause mortality. The analysis also revealed an inverse relationship between the consumption of total fat and saturated fat in childhood and total mortality and deaths from heart disease. At face value, these findings appear to fly in the face of conventional thinking about dietary fat and mortality from heart disease, at least in adults.

The nature of these observations requires cautious interpretation as the authors recognized. Chance may have played a part and the imprecise measurement of dietary exposure might obscure real diet-related associations. However, the authors pointed out that such errors would not explain the observed associations. Possible confounding with other variables is a consideration, but would be an inadequate explanation for the increased risk of stroke with fish consumption, which was higher in those of higher social class. Another consideration is the likelihood of lean fish having been prepared and consumed mainly as battered and fried fish. Some evidence suggests that such fish preparation do not have cardioprotective or stroke benefits. The investigators suggest that higher fish consumption in early life might unfavorably reduce the concentration of arachidonic acid in neural tissue membranes, possibly laying the groundwork for hemorrhagic stroke later in life. Arachidonic acid was reported beneficial in a case report of the treatment of hemorrhagic stroke. Other epidemiological studies in adults have reported no association between fish consumption and hemorrhagic stroke in men and women.

This study raises good questions about the types of dietary fat that are most appropriate throughout the life cycle. Given the known benefits of fish oil polyunsaturated fatty acids in neurodevelopment, this report would not justify eliminating fish from children's diets. It also suggests that total and saturated fat in childhood appear to be beneficial later in life, indicating that moderate intake of all fatty acid classes is prudent.

Ness AR, Maynard M, Frankel S, Smith GD, Frobisher C, Leary SD, Emmett PM, Gunnell D. Diet in childhood and adult cardiovascular and all cause mortality: the Boyd Orr cohort. Heart *2005;91:894-898.*

Maternal & Infant Health

Maternal DHA Supplementation in Lactation Boosts Infant Psychomotor Scores, But Not Visual Acuity

The effects of supplementing mothers with omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) during pregnancy on infant growth and development have been well studied. Across a range of intakes, mothers whose intakes are highest give birth to infants with higher plasma n-3 LC-PUFA concentrations compared with infants of mothers with low n-3 LC-PUFA intakes. Higher n-3 LC-PUFA consumption also reduces arachidonic acid levels. Developmental outcomes in infants receiving these fatty acids from infant formula after birth have been compared with those fed unsupplemented formula. Generally speaking, infants with higher intakes of n-3 LC-PUFAs have improved visual function and cognitive development compared with those having the lowest levels. These findings apply to premature and term infants but have been most consistent for premature infants.

The gold standard for infant nutrition remains breast milk, but even mother's milk exhibits variable nutrient content as a reflection of maternal diet. In particular, docosahexaenoic acid (DHA), an n-3 LC-PUFA, can vary as much as 10-fold depending on maternal fatty fish consumption. Do breast-fed infants of mothers whose milk contains low concentrations of DHA develop similarly to infants of mothers with higher DHA breast milk concentrations? Craig Jensen's group at the Baylor College of Medicine in Houston, Texas, USA, tackled this question.

Two hundred and twenty-seven pregnant women between the ages of 18 and 40, who planned to breastfeed their infants for at least 4 months, were recruited for the study. Women were included if their infants had 37 or more weeks of gestation, weighed between 2,500 and 4,200 grams, and were free of major clinical disorders. Women were randomly assigned to consume one capsule daily containing 200 mg DHA in algal oil or a 50/50 mixture of corn and soy oils containing 3.9% alpha-linolenic acid for 4 months. Consumption of capsules began within 5 days of delivery.

At 4 months postpartum, breast milk was collected for a 24-hour period and blood sampled from both mothers and infants. Infant performance was assessed at 30 months of age using the Bayley Scales of Infant Development. Infant visual function was measured at 4 and 8 months of age using the Teller Acuity Card procedure and visual evoked potentials (VEPs). At the end of 30 months, 83 children of mothers consuming DHA and 77 of mothers consuming corn-soy oil remained in the study.

Infants in both groups of mothers did not differ in gestational age, birthweight, Apgar score, sex, ethnicity, or anthropometric measures. There were no statistically significant differences in motor skills or Bayley Mental Development Index scores between the two groups. However, the Bayley Psychomotor Development Index, which assesses both fine and gross motor development, was significantly higher by 8.4 points in the infants of mothers fed DHA (P=0.005). This score remained significantly higher after controlling for 9 potentially confounding variables. However, the infants' plasma phospholipid DHA content was unrelated to any measure of neurodevelopment or visual function at either 4 or 8 months of age.

As can been seen in Table 1, maternal supplementation with DHA was associated with a 75% increase in DHA breast milk content compared with unsupplemented mothers, 0.35% vs 0.2% total fatty acids, respectively. Arachidonic acid content was significantly lower in the breast milk of DHA-supplemented women compared with the controls.

Fatty acids in infant plasma phospholipids followed a similar pattern as in maternal milk. DHA concentration was significantly higher in infants of DHA-supplemented mothers, while concentrations of arachidonic acid (22:4n-6) and 22:5n-6 were significantly lower. By 8 months of age there were no significant differences in infant plasma phospholipid fatty acid patterns between the groups.

Table 1. Fatty acid composition of breast milk lipids at 4 months postpartum

Fatty acid	Control	DHA supplementation
n-3		
18:3 (ALA)	1.07 ± 0.35	1.2 ± 0.90
20:5 (EPA)	0.07 ± 0.08	0.07 ± 0.04
22:5	0.14 ± 0.08	0.12 ± 0.04
22:6 (DHA)	0.20 ± 0.24[a]	0.35 ± 0.14
n-6		
18:2	15.9 ± 3.6	16.3 ± 2.8
20:4 (AA)	0.44 ± 0.08[b]	0.40 ± 0.08
22:4	0.09 ± 0.02[c]	0.08 ± 0.02
22:5	0.05 ± 0.02	0.04 ± 0.01

[a]Significantly different from DHA group P<0.0001.
[b]Significantly different from DHA group P<0.001.
[c]Significantly different from DHA group P<0.004.

Although infant plasma phospholipid DHA concentration was higher in the infants of DHA-supplemented mothers, visual acuity scores did not differ between the two groups. However, the amplitude of the VEP was significantly lower in the infants of DHA-supplemented mothers at 4 months of age, but not at 8 months. The biological significance of this finding seemed unclear.

The main finding of this study, the significantly higher Bayley Psychomotor Development score in infants of mothers supplemented with DHA, also appears difficult to interpret. This is because the score was well above the expected mean in both groups and was unrelated to the infants' phospholipid DHA concentration or visual function. Whether this observation will be confirmed in future studies or turns out to be a harbinger of other health effects later in life is unknown.

Jensen CL, Voigt RG, Prager TC, Zou YL, Fraley JK, Rozelle JC, Turcich MR, Llorente AM, Anderson RE, Heird WC. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr *2005;82:125-132.*

Is DHA Required for Brain Development? A Critical Look at the Evidence

With the U.S. Food and Drug Administration's approval in 2001 of the addition of docosahexaenoic acid (DHA) and arachidonic acid to infant formula in the U.S., the question of the desirability of these long-chain polyunsaturated fatty acids (LC-PUFAs) in early human development appeared to be answered affirmatively in American minds. Many studies have reported that the addition of these two LC-PUFAs supports visual and cognitive development that is often indistinguishable from breast-fed infants. The question of requirement, however, is tricky, partly because critical needs vary with time. Stephen Cunnane's discussion of essential fatty acids in the June 2005 issue of the *PUFA Newsletter* bears reading.

McCann and Ames' overview of the evidence that DHA is required in human development examines three different study designs used in animal and human research, summarizes the findings from each or identifies recent reviews of them, and discusses the interpretation and limitations of each type of study. Organizing the wealth of published data in an orderly way enables the reader to approach this topic relatively unencumbered.

The review concentrates on three types of study: 1) observational studies comparing breast or formula feeding in humans; 2) randomized controlled trials in infants fed either unsupplemented or LC-PUFA-supplemented formulas; 3) rodent studies in which PUFAs are restricted at different times for varying periods with or without refeeding. The authors note that the addition of arachidonic acid in most supplementation protocols means that DHA is not the only variable in question. Also, when it comes to assessments, global performance tests, such as Bayley Scales of Infant Development, differ substantially from specific function tests, such as language and communications skills or problem solving. This means that findings from different types of studies may be difficult or inappropriate to compare. These differences in outcome measurements apply to human and animal studies.

Observational studies comparing breast-fed with formula-fed infants are subject to a number of confounding variables because women who breastfeed differ in many ways from women who do not. The reviewers urge more comprehensive analysis of cofounders and cite the example of maternal intelligence quotient (IQ) as a frequently overlooked known confounding variable. Since 1999, some 30 breastfeeding studies have been reported and these are tabulated in the review.

Results from randomized controlled trials have been mixed. Two Cochrane reviews published in 2004 concluded that available evidence did not support neural developmental benefits from LC-PUFA supplementation. Others have noted that tests of visual attention have been consistently positive in both preterm and term infants.

Of the 20 animal studies published since 1999 and listed in the review, almost all reported significant deficits in performance of omega-3 (n-3)-PUFA-restricted animals compared with controls. However, differences were not always large, were inconsistent across different tests, and varied across multiple test sessions. Other problems in interpreting animals studies exist, including confounding variables, overlap between performance and behavioral characteristics, and other effects of alpha-linolenic acid restricted diets, such as extremely high omega-6 PUFA content, that could have inflammatory consequences. Statistical issues abound. For example, sample size is inflated by using number of pups instead of litters, and correlations between group means does not necessarily translate to individual animals. Other issues exist in animal studies such as the relevance of severe depletion of brain DHA to more modest deprivation.

Given the weaknesses inherent in observational studies, the authors asked why the results from randomized controlled trials, the most rigorous experimental approach, have been mixed. They suggested several possible avenues for further examination and the need to determine whether false negatives or false positives have occurred. It is also noted that healthy term infants are born with about 1 gram of DHA in their fat stores and, if breastfed, continue to accumulate DHA.

The review concludes with a brief discussion of how well the evidence fits the considerations for whether the evidence is sufficient to deduce causality, articulated by Sir Austin Bradford Hill in 1965 and recently elaborated. The reviewers conclude that within the context of specific experimental designs, changes in brain concentrations of DHA are positively associated with changes in cognition and behavior. This is a scientist's careful way of saying DHA nourishes the noggin.

McCann JC and Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr *2005;82:281-295.*

Immune Function

Healthy Fish Eaters Have Lower Levels of Inflammatory Markers

Polyunsaturated fatty acids (PUFAs) are linked to immune system function through the production of pro- and anti-inflammatory substances. For example, arachidonic acid, an n-6 long chain (LC)-PUFA, is the precursor of inflammatory prostaglandins, thromboxanes, and leukotrienes, but LC omega-3 PUFAs (n-3 LC-PUFAs) are converted to immuno-restraining versions of these substances. Novel anti-inflammatory mediators, such as resolvins and neuroprotectins, are also made from n-3 LC-PUFAs, providing additional armaments to combat excessive inflammation. Philip Calder described these developments in the September 2004 issue of the *PUFA Newsletter*.

Whether the regular consumption of n-3 LC-PUFAs modulates the production of inflammatory mediators in healthy people, possibly reducing the risk of inflammatory conditions, is unclear. There are data supporting and refuting such effects. From a public health perspective, it is relevant to ask whether regular fish consumption has any effect on markers of inflammation among healthy people. Residents of Attica, Greece have provided an answer.

Antonis Zampelas and colleagues at Harokopio University in Athens, Greece examined a variety of inflammatory markers in a population-based sample of 3,042 healthy middle-aged men and women (age 18-89, average age 46 years) living in the region of Attica. Participants were free of cardiovascular and atherosclerotic diseases and chronic viral infections upon enrollment. Diet was assessed by food frequency questionnaire and fasting blood sampled upon entry to the study. The investigators divided fish consumption into four categories: none (<4 servings/mo.), rare (< 4 servings or about 150 g/wk), moderate (4 to 12 servings or 150 to 300 g/wk), and frequent (>12 servings or >300 g/wk).

There was a significant inverse association between the amount of fish consumed and blood levels of C-reactive protein, interleukin-6, and tumor necrosis factor-alpha, all indicators of inflammatory responses (Table 1). Amyloid A protein levels and white blood cell counts were also significantly lower in people consuming fish most frequently. Amyloid deposits in brain and cerebral blood vessels are associated with Alzheimer's disease and dementia. In the Greek study, there were no differences between men and women in these associations. Significant inverse associations between fish consumption and hypertension, systolic blood pressure, and triglyceride concentration were also reported.

The investigators repeated the analysis, adjusting for several potential confounding factors including age, gender, pack-

Table 1. Inflammatory markers in healthy adults in Greece according to weekly fish consumption

	Fish consumption (g/week)				P*
	Almost none	<150	150 to 300	>300	
No. participants	319	1,719	745	259	-
C-reactive protein, mg/L	2.7 ± 1.2	2.0 ± 1.1	2.0 ± 2.1	1.8 ± 1.1	0.004
Interleukin-6, ng/ml	1.5 ± 0.5	1.3 ± 0.6	1.2 ± 1.1	1.0 ± 0.3	0.03
Tumor necrosis factor-alpha, mg/dL	5.3 ± 3	5.1 ± 2	4.7 ± 3	4.2 ± 2	<0.001
Amyloid A, mg/dL	6.4 ± 4	5.9 ± 4	5.3 ± 4	4.6 ± 3	0.004
White blood cells, x1,000 counts	6.8 ± 3	6.7 ± 4	6.6 ± 4	6.5 ± 3	0.04

*Probability values derived from analysis of variance. Values are means ± SD.

years of smoking, physical activity status, body mass index, blood pressure, total cholesterol, low and high density lipoprotein cholesterol, triglycerides, use of medication, and average weekly frequency of food group consumption. After these adjustments, all markers remained significantly inversely related to fish consumption.

When participants were stratified by clinical characteristics, fish consumption was inversely associated with lower inflammatory markers in people with diabetes (10% to 25% lower) and hypertension (5% to 15% lower), but not in those with hypercholesterolemia ($P<0.05$). Further, cut-off analysis indicated that consumption of 600 mg/day of n-3 LC-PUFAs would be the optimal intake for having inflammatory markers in the lowest or middle category. This amount is similar to the 500 mg/day of n-3 LC-PUFAs recommended by the International Society for the Study of Fats and Lipids for reduction of cardiovascular risk in healthy people.

While this study reports a positive association between fish consumption and reduced markers of cardiovascular risk, it is worth remembering that observational studies such as this are merely a snapshot, not proof of cause, as the authors themselves commented. That said, these findings are consistent with the expectation that regular fish consumption, as reported in this free-living population, reduces markers of the risk of cardiovascular disease and mortality.

Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysohoou C, Skoumas Y, Stefanadis C. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease. The Attica Study. J Am Coll Cardiol *2005;46:120-124.*

Clinical Conditions

Alzheimer's Disease

DHA Protects Against Key Protein Loss and Amyloid Deposits in Transgenic Mice with Alzheimer's Disease

Consumption of fish and the omega-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) docosahexaenoic acid (DHA) have been linked to reduced risk of developing Alzheimer's disease. Because this disease afflicts some four million mostly older people in the U.S. alone, and is the leading cause of dementia, ways to reduce its onset or the severity of its symptoms are urgently sought. To date, strategies to prevent the condition remain undiscovered.

Alzheimer's disease is a progressive brain disorder that begins with memory loss and then erodes a person's ability to reason, communicate, and function. The condition has a genetic component, but environmental factors, especially DHA, are critically involved. Three papers cited here describe key advances in understanding the disease and the function of DHA in reducing its consequences. A transgenic mouse model of Alzheimer's disease (T_g2576) was used in these studies. This genetic modification triggers the overproduction of one of the human mutant amyloid precursor proteins, HuAPPsw, whose accumulation impairs memory in Alzheimer's disease and defines the disease.

In the first paper cited below, researchers focused their attention on postsynaptic activity of the dendrites in nerve cells. Dendrites are projections from the nerve cell (neuron) that bring information to it. They receive this information from chemical transmitters that are released into the gap or synapse between the dendrite of one neuron and the axon or transmitting portion of another. In Alzheimer's disease, synapses and certain proteins associated with them become dysfunctional and are lost. For example, 70% to 95% of the developmentally regulated brain protein, drebrin, is lost in Alzheimer's disease. This protein is involved in regulating dendritic spine actin, a protein that becomes abnormal in Alzheimer's and other types of mental retardation. Drebrin and other regulatory proteins are used as markers of synaptic activity and are related to cognitive deficits in the mouse model of Alzheimer's disease. Note that synapses are rich in DHA.

In aged transgenic mice, which exhibit symptoms of Alzheimer's disease such as memory loss and beta-amyloid deposits, concentrations of drebrin were significantly decreased in brain cortex by 48% ($P<0.001$) compared with non-transgenic mice. At the same time, a breakdown fragment of actin known as fractin was increased by 71% (expressed as the ratio of fractin to actin) in the transgenic animals. These observations are consistent with increased activity of the degradation enzyme caspase that breaks down actin and other cellular proteins released upon cell death. It is also known that lipid peroxidation is higher in brains of

transgenic mice with Alzheimer's disease and this further depletes DHA content.

The effect of dietary DHA on dendritic regulatory proteins and actin breakdown was investigated by feeding "old" (17 months) transgenic and non-transgenic control mice diets depleted of n-3 PUFAs (0.06% alpha-linolenic acid) or supplemented with 0.6% DHA for 103 days (about 15 weeks) and comparing brain LC-PUFA levels with mice fed control diets containing 0.18% alpha-linolenic acid. Results for the mean content of DHA, docosapentaenoic acid (n-6), and arachidonic acid in the frontal cortex of the different groups are shown in Table 1.

observations are consistent with DHA function in the regulation of caspase, an enzyme that cleaves dendritic actin. Similarly, drebrin and another synapse-linked protein, PSD-95, were reduced by 85% and 77%, respectively, in the n-3 PUFA-depleted transgenic mice compared with control mice. The authors also reported that n-3 PUFA depletion and transgene expression did not cause significant neuron loss in either the cortex or hippocampus of mouse brains. Further, the loss of postsynaptic proteins was DHA-dependent because DHA repletion suppressed the loss of drebrin and PSD-95.

To explore possible mechanisms underlying the loss of

Table 1. Content of selected LC-PUFAs* in frontal cortex of control and transgenic Alzheimer's mice fed control, low n-3 PUFA, and DHA-supplemented diets from 17 months of age

Diet	Control mice			Transgenic mice		
	DHA	DPAn-6	AA	DHA	DPAn-6	AA
Control	19.2 ± 0.3	0.52 ± 0.02	9.6 ± 0.1	19.5 ± 0.5	0.59 ± 0.03	9.5 ± 0.1
Low n-3 PUFA	18.0 ± 0.5	1.43 ± 0.12[a]	9.8 ± 0.1	16.4 ± 0.8[ab]	1.81 ± 0.20[a]	9.9 ± 0.2
Low n-3 PUFA + 0.6% DHA	20.5 ± 0.5[c]	0.29 ± 0.05[c]	8.4 ± 0.2[c]	21.3 ± 0.6[c]	0.22 ± 0.02[c]	7.9 ± 0.2[c]

Abbreviations: DHA, docosahexaenoic acid, 22:6n-3; DPA, docosapentaenoic acid, 22:5n-6; AA, arachidonic acid, 20:4n-6

*Expressed as mean percent of total fatty acid ± SEM

[a] $P<0.01$ compared with control diet within transgene

[b] $P<0.05$ compared with non-transgenic control within low n-3 PUFA diet

[c] $P<0.01$ compared with low n-3 PUFA group within transgene

Diets depleted in n-3 PUFAs significantly reduced frontal cortex DHA concentration in transgenic mice, but not in control mice. Compensatory n-6 docosapentaenoic acid (DPA) was significantly increased in both control and transgenic mice consuming the n-3 depleted diet. Supplementation with DHA prevented the loss in DHA and increase in docosapentaenoic acid, although arachidonic acid was reduced in both groups of mice compared with the low n-3 PUFA diet.

In addition, transgenic mice depleted of n-3 PUFAs exhibited a 3.2-fold increase in the ratio of fractin to actin compared with mice fed control diets (2.1-fold increase), but provision of DHA prevented the increase in this ratio. These

postsynaptic proteins, the investigators turned to the PI3-kinase (phosphoinositide) pathway that is a critical cell signaling pathway in nerve cells. The enzyme is an important regulator of cell proliferation, survival and growth. The question asked was whether DHA regulated the activity of PI3-kinase in neurons. Researchers found that n-3 PUFA depletion was accompanied by a 95% reduction in the protein subunit of PI3-kinase in transgenic mice compared with control mice. A 49% decrease in this subunit was also observed in the brains of Alzheimer's patients. Additional experiments provided evidence to support the view that DHA regulates PI3-kinase signaling, which triggers subsequent events that limit caspase activation, thereby preserving dendritic synaptic function.

The investigators also explored the effects of n-3 PUFA depletion on behavioral performance using the Morris water maze. Mice capable of finding the visible platform used in the test were evaluated. Animals from all groups were capable of learning the visible platform, indicating the absence of major sensory-motor problems. Swim speed and anxiety in the DHA-depleted transgenic mice was comparable to that of control mice. However, stark differences were apparent during hidden platform testing. Transgenic mice on the low n-3 PUFA diets had significantly longer latencies and showed signs of increased circling and a tendency to stay close to the walls of the maze. These responses did not occur in non-transgenic mice on the low n-3 PUFA diet or in DHA supplemented transgenic mice, indicating an interaction between dietary DHA and the transgene. These responses are consistent with memory deficits associated with Alzheimer's disease.

Also note that in another test of memory retention, DHA supplementation was unable to overcome the deficit observed in the depleted transgenic mice.

The results of these experiments show that DHA affects the expression of at least one gene linked to Alzheimer's disease, and when present in sufficient amounts, DHA can prevent some deleterious effects of the disease. However, a large retention deficit remained in the transgenic mice that was not corrected by DHA. The evidence indicates that DHA affects the PI3-kinase pathway, caspase activity, and oxidative stress.

Another implication of these findings is that Alzheimer's disease related to genetic factors may be particularly susceptible to dietary deficits of n-3 PUFAs, particularly DHA. There is also the suggestion that deficits in DHA may contribute to the Alzheimer's disease associated with diabetes, as signaling pathways for DHA are the same as those for insulin. DHA deficits in transgenic Alzheimer's mice appear to be independent of synapse loss that occurs in the disease because compensatory n-6 docosapentaenoic acid is produced in DHA-deficient transgenic mice. DHA may also be contributing to oxidative stress that further damages postsynaptic proteins and leads to cleavage of actin proteins and decline in cognitive function. It is a complicated and detailed picture, but one that is relevant to positive interventions with DHA for deterring and retarding the progress of Alzheimer's disease in people.

Subsequent studies by this group (Lim *et al.* 2005) have shown that in the same transgenic mouse model of Alzheimer's disease, feeding DHA significantly reduced beta-amyloid by more than 70% compared with animals fed low DHA or chow diets. Overall plaque burden was reduced by 40% with the largest reductions occurring in the hippocampus and parietal cortex regions of the brain. These findings provide additional evidence of the protective effects of DHA in gene-linked Alzheimer's disease. In a 2005 study by Calon *et al.*, the group showed that n-3 PUFA deficient transgenic Alzheimer's mice compared with deficient nontransgenic mice also had decreased NMDA receptors, a type of glutamate receptor, and reduced CaMKII protein kinase, an enzyme involved in the function of synapses. Diminution of these receptors correlated with PI3 kinase levels and with increased amounts of protein fragments linked to caspase activity. Supplementation with DHA partly protected against the loss of NMDA receptors and accumulation of protein fragments and fully protected against the decrease in CaMKII protein kinase.

Together, these studies provide insight into the mechanisms underlying the cognitive deficits of Alzheimer's disease and show the importance of DHA in regulating many activities that keep neuronal communication functioning well. In both transgenic Alzheimer's mice and in gene-linked Alzheimer's disease DHA shows substantial potential to deter the onset of the disease and perhaps slow its insidious progression. If confirmed in humans, this would be a major breakthrough in preventing and treating this blemish on the golden years.

Calon F, Lim GP, Yang F, Morihara T, Teter B, Ubeda O, Rostaing P, Triller A, Salem N Jr, Ashe KH, Frautschy SA, Cole GM. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. Neuron *2004; 43:633-645.*

Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N Jr, Frautschy SA, Cole GM. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci *2005;25:3032-3040.*

Calon F, Lim GP, Morihara T, Yang F, Ubeda O, Salem N Jr, Frautschy SA, Cole GM. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. Eur J Neurosci *2005;22:617-626.*

Parkinson's Disease

PUFA Consumption Linked to Lower Risk of Parkinson's Disease

Among the neurodegenerative diseases that afflict the elderly, Parkinson's disease is second in occurrence only to Alzheimer's disease. Named for the British physician who first described it in 1817, Parkinson's disease is a progressive degenerative condition in the brain that announces itself with shaking limbs, slowed movement, rigidity, and poor balance. In the U.S., about 1.5 million people, mostly over the age of 65, have the condition. It strikes both men and women equally.

Parkinson's disease results from deterioration in a part of the brain called the substantia nigra, a region that produces the chemical messenger, dopamine. This chemical is involved in regulating coordinated movement. Parkinsonism is frequently treated but not cured by providing dopamine. Its primary cause is unknown.

Since the mid 1990s, intakes of animal fat, total fat, cholesterol, and a few other nutrients have been associated with increased risk, while PUFA consumption has been linked to lower risk, but data are inconsistent. There is a reasonable basis for examining the potential involvement of long-chain (LC) PUFAs, particularly omega-3 (n-3) LC-PUFAs in Parkinson's disease. n-3 LC-PUFAs are important in the structure of neural membranes and they have neuroprotective and anti-inflammatory properties. In addition, the n-6 PUFA, arachidonic acid is involved in the synthesis of cannabinoids, pain-deadening substances produced in the brain that also function in the control of movement. Of particular interest is the involvement of lipid metabolism in regulating the toxicity of alpha-synuclein, the abnormal protein involved in the neurodegeneration of Parkinsonism. However, other epidemiological studies have found no association between the consumption of PUFAs and risk of Parkinson's disease.

Because Parkinson's disease occurs mainly in older people, a long-term prospective study in the Netherlands designed to identify the determinants of diseases of the elderly afforded the opportunity to examine diet's contribution to the condition. In the Rotterdam study, de Lau and colleagues assessed the links between fat and fatty acids and the development of Parkinsonism.

Residents of Rotterdam 55 years and older enrolled in the study from 1990 to 1993. A total of 5,289 people free of dementia and Parkinsonism were eligible. Dietary intake was assessed by home interview and food frequency questionnaire, with emphasis on total energy and fat intake, various fatty acids, alcohol, dairy products, and coffee.

Table 1. Hazard ratios for intake of dietary fat components and risk of Parkinson's disease expressed per standard deviation of energy-adjusted intake (g/day)

Dietary component*	Standard deviation of energy-adjusted intake (g/day)	Hazard ratio adjusted for age and sex (95% confidence interval)	Hazard ratio with added adjustments for vitamin E & smoking (95% confidence interval)
Total fat	13.2	**0.67 (0.51-0.89)†**	0.69 (0.52-0.91)
Saturated fat	7.2	0.82 (0.61-1.10)	0.82 (0.61-1.10)
Cholesterol	0.06	0.81 (0.61-1.08)	0.81 (0.59-1.10)
MUFA	5.4	**0.67 (0.49-0.91)†**	0.68 (0.50-0.94)
PUFA	6.7	0.77 (0.58-1.03)	0.66 (0.46-0.96)
n-6 PUFA	6.6	0.79 (0.59-1.06)	**0.69 (0.47-1.00)†**
Linoleic acid	6.6	**0.79 (0.59-1.06)†**	0.68 (0.47-1.00)
Arachidonic acid	0.04	0.96 (0.72-1.28)	0.96 (0.72-1.27)
n-3 PUFA	0.6	**0.66 (0.46-0.94)†**	0.68 (0.48-0.97)
Alpha-linolenic acid	0.6	**0.65 (0.45-0.95)†**	0.64 (0.44-0.92)
DHA	0.1	1.02 (0.79-1.31)	1.03 (0.45-2.38)
EPA	0.09	1.02 (0.81-1.29)	1.03 (0.81-1.31)

*Abbreviations: MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.
†Statistically significant (bold) using Cox proportional hazards regression analysis.

Participants were followed for an average of 6 years during which 77 cases of Parkinsonism were identified. The investigators used Cox proportional hazards regression analysis to evaluate the association between energy-adjusted fat intake and risk of Parkinson's disease.

After adjusting for age and sex, consumption of total fat, monounsaturated fat, and omega-3 (n-3) PUFAs were significantly associated with a lower risk of developing Parkinson's disease (Table 1). Analysis after additional adjustments for smoking and vitamin E intake revealed a significant association between n-6 PUFA consumption and lower chance of Parkinsonism. Among individual fatty acids, only alpha-linolenic and linoleic acids were linked to lower risk.

As shown in Table 1 and highlighted in bold, consumption of total and 18-carbon PUFAs of both n-6 and n-3 classes was associated with a lower likelihood of developing Parkinson's disease. In contrast with some other reports, saturated fat and cholesterol intake were not associated with development of the disease. As the authors noted, dietary assessment took place at enrollment before disease occurrence, thereby avoiding the possibility of the condition affecting food habits.

These findings support those from the Honolulu-Asia study published in 2003, in which Japanese men with higher intakes of PUFAs were significantly less likely to develop Parkinsonism. Evidence is accumulating that PUFAs are involved in and disturbed by the development of Parkinsonism. Soluble fractions from brains of Parkinson patients contained elevated PUFA levels and were associated with increased formation of alpha-synuclein oligomers, the harbingers of Parkinsonism. Where PUFAs were elevated, the authors postulated that this was the result of alpha-synuclein pathology, not the cause of it. These observations suggest that PUFAs may be involved in regulating the conversion of alpha-synuclein from the soluble (benign) to the insoluble (harmful) form characteristic of the disease. n-3 PUFA-deficient diets have also been associated with decreased production of dopamine. The de Lau study suggests that PUFA consumption is beneficial in maintaining neuronal function later in life.

de Lau LML, Bornebroek M, Witteman JCM, Hofman A, Koudstaal PJ, Breteler MMB. Dietary fatty acids and the risk of Parkinson disease. The Rotterdam Study. Neurology *2005;64:2040-2045.*

Cancer

Lung Cancer Cells Less Invasive With Reduced Omega-6 to Omega-3 Ratio

Biotechnology was put to work again by Jing Kang's research team at the Massachusetts General Hospital in Boston, USA. Using a transgenic technique pioneered in their laboratory, Kang and colleagues developed mice with an enzyme that converts omega-6 polyunsaturated fatty acids (n-6 PUFAs) to omega-3 (n-3) PUFAs. Mammals cannot normally interconvert n-6 and n-3 PUFAs. However, mice carrying the *fat-1* gene from the round worm *Caenorhabditis elegans* express the enzyme n-3 desaturase that converts n-6 to n-3 PUFAs. A recent report of these transgenic mice was described in the March 2004 issue of the *PUFA Newsletter*.

Table 1. PUFA content of cultured human lung cancer cells expressing marker protein (control*) or marker protein and the *fat-1* gene

Fatty Acid (mol %)	Control	Transformed cells with *fat-1* gene*
n-6 PUFAs		
Linoleic acid (18:2)	3.5	1.4
Arachidonic acid (20:4)	15.1	3.5
Docosatetraenoic acid (22:4)	2.1	0.6
Docosapentaenoic acid (22:5)	1.6	0.4
n-3 PUFAs		
Alpha-linoleic acid (18:3)	0.3	0.6
EPA (20:5)	0.4	7.6
Docosapentaenoic acid (22:5)	1.2	3.3
DHA (22:6)	3.1	4.5
n-6/n-3 Ratio†	4.5	0.4

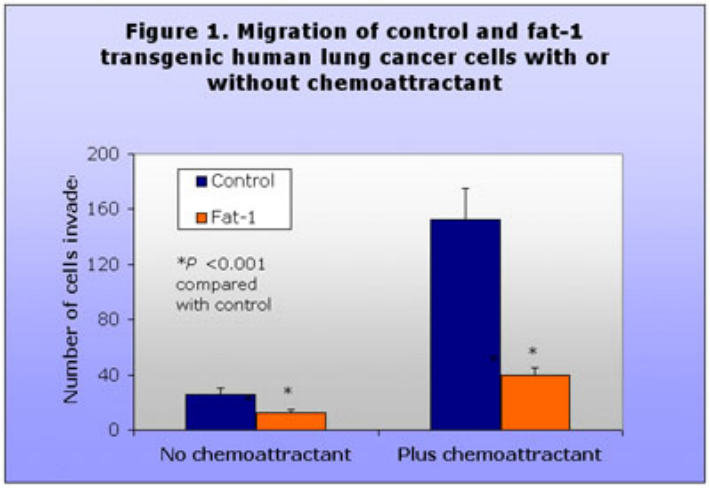
*Significant difference between control and transformed cells for each PUFA, $P<0.01$.
†Ratio: 20:4/EPA+DHA

Kang's team used this genetic modification to explore the effects of reduced n-6 to n-3 PUFA ratio on the potential tumor invasiveness and metastasis of human lung cancer cells. The thinking behind these studies reflects findings that n-6 fatty acids stimulate carcinogenesis, tumor growth, and metastasis, while n-3 suppress these processes in animal and cell culture studies. In human studies, data are suggestive but less clear.

The investigators used cultured human lung cancer cells (line A549) that were transformed to express the *fat-1* gene. About 90% of the treated cells expressed the transgene, as verified by the expression of the green fluorescence marker and confirmed by immunofluorescence staining. Control cells expressed only the green fluorescence marker protein. Total tissue fatty acids were analyzed 48 hours after the cells were transformed and eight PUFAs, four in each n-6 and n-3 class, were detected in control and transformed cells. Compared with control cells, those with the *fat-1* gene contained significantly less ($P<0.01$) n-6 PUFAs and significantly more of each of the n-3 PUFAs (Table 1). The ratio of arachidonic acid to EPA plus DHA decreased 10-fold in the cells with the *fat-1* gene.

Potential for invasiveness was evaluated by four assessments: 1) adhesion of cells after fresh transfer to a new culture plate, 2) invasion through a matrigel matrix and polycarbonate filter using a commercial invasion kit, 3) apoptosis or numbers of dead cells, and 4) expression of adhesion- and invasion-related genes. Comparisons between control and transformed cells in adhesion and growth at 6 and 24 hours, illustrated in the publication by micrographs, showed marked differences in attachment to the culture medium and growth at both times. Cells with the *fat-1* gene were markedly less able to attach and form colonies, reflecting impaired growth and adhesion.

Invasiveness of the cells containing the *fat-1* gene was also significantly impaired compared with control cells. As shown in Figure 1[†], the number of cells migrating through the filter barrier was significantly lower ($P<0.001$) in the transformed cells compared with controls in both the presence and absence of a chemoattractant (a chemical lure). This finding indicates that the transformed cells



Reproduced by permission of Oxford University Press from *Carcinogenesis* 2005;26:779-784.

were less able to detach and migrate—requirements for metastatic invasion of tumor cells and the spread of cancer—suggesting that they might be less likely to invade under a similar fatty acid environment *in vivo*.

Another means of inhibiting the growth of cancer cells and thereby, their potential to spread, is to hasten their death. Kang's team first looked at the growth rate of control, transformed, and untreated tumor cells. After 4 days, the growth of cells with the *fat-1* gene fell by about 50%, those containing the marker protein declined about half as much, and cells with no treatment continued to grow. When the

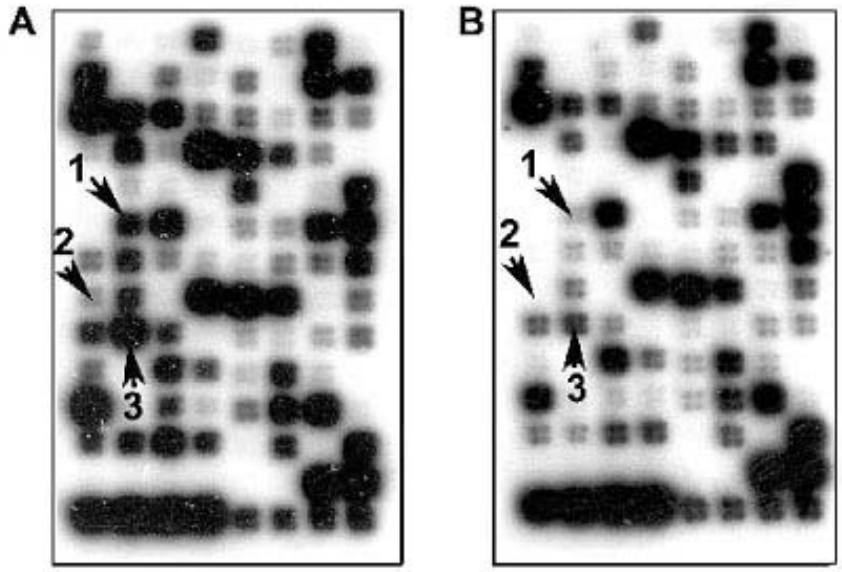


Figure 2. Microarray images of expression of 96 cancer-related genes in control (A) and transgenic *fat-1* human lung cancer cells (B). Downregulated genes are: 1) integrin-alpha2, 2) MMP-1, 3) NM23-H4. Reproduced by permission of Oxford University Press from *Carcinogenesis* 2005;26:779-784.

investigators counted the number of dead cells after 4 days, cell colonies with the *fat-1* gene contained about three and a half times as many dead cells as those bearing only the marker protein, a statistically significant increase ($P<0.05$). The investigators concluded that expression of the *fat-1* gene induced apoptosis in the tumor cells.

Finally, the team examined the effect of the *fat-1* gene on the expression of cancer-related genes. Screening the cancer cells with a low-density microarray containing 96 cancer genes, they observed downregulation of eight genes in the *fat-1* transgenic cells, among them three involved in the adhesion or metastasis (spread) of cancer cells. As indicated by the arrows in Figure 2, these genes were were suppressed by 2-to 4-fold in the *fat-1* transgenic tumor cells (B) compared with the control cells (A). The suppression of these genes was confirmed by polymerase chain reaction, a technique that enables researchers to measure quantities of precise genetic sequences.

It is tempting to make the leap to human lung cancer, a disease with high mortality from the invasion and spread of tumor cells. Could dramatic dietary change in PUFA consumption help combat the disease? What about other highly invasive cancers? Of course, these questions linger now, but this study presents a credible and encouraging hypothesis for designing studies in humans. We hope Dr. Kang's group continues this work using animal models and other types of cancers, so that treatment of human disease, if not prevention of it, might be more effective.

Xia S-H, Wang J, Kang JX. Decreased n-6/n-3 *fatty acid ratio reduces the invasive potential of human lung cancer cells by downregulation of cell adhesion/invasion-related genes.* Carcinogenesis *2005;26:779-784.*

Developmental Coordination Disorder

Children With Coordination Disorder Benefit from PUFA Supplements

One way of dealing with a clumsy child is to overlook his awkwardness, assuming he will outgrow the problem. Alas, 5% to 6% of children worldwide lack several basic movement skills, a difficulty they do not outgrow. In 1994, the American Psychiatric Association and the World Health Organization recognized the syndrome as a distinct disorder and named it developmental coordination disorder (DCD). It is also known as developmental dyspraxia.

Once DCD was recognized as separate condition, it became apparent that children so afflicted often had difficulties in academic performance, social skills, behavior, and self-esteem and these problems persisted into adulthood. Effective treatments using diverse interventions may be slowly emerging, but many challenges remain.

Little is known about the causes of this disorder, but its similarities to other motor and behavioral impairments, such as attention deficit hyperactivity disorder and dyslexia, raise the possibility that fatty acid imbalances in brain may contribute to the condition. There is mixed evidence that polyunsaturated fatty acids (PUFAs) are involved in many of these related conditions of behavior and learning. Because PUFAs are important to brain structure and function, there is reason to examine the effects of PUFAs in children with DCD.

Accordingly, Alex Richardson and Paul Montgomery at the University of Oxford, U.K., designed a randomized, controlled, partial crossover trial for three months to test the effect of daily supplementation with a combination of fish and evening primrose oils compared with an olive oil placebo. PUFA supplementation consisted of 732 mg/day of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA), omega-3 (n-3) PUFAs, and 60 mg gamma-linolenic acid, an omega-6 (n-6) PUFA and the first desaturation product in the conversion of linoleic acid to arachidonic acid. At the end of three months, children in the placebo group were switched to the PUFA treatment. However, children originally receiving the PUFA capsules were not switched to placebo treatment. For this reason, the study has a partial crossover design. The study continued for another three months with both groups consuming PUFAs. Comparison of the effects of the first three months of parallel interventions was the primary focus of the study.

Mainstream school children 5 to 12 years of age, who met the diagnostic criteria for DCD, had confirmed impairments in academic achievement and daily living, and were not receiving any treatment, were recruited from schools in County Durham, U.K. Of the 117 students (78 boys, 39 girls) eligible for the study, 110 completed the full 6-month study. Students' average age was 9 years. Performance assessments included motor function by the Movement

Assessment Battery for Children, reading and spelling by the Wechsler Objective Reading Dimensions, and teacher-rated attention symptoms using Conners' Teacher Rating Scales. Because data were not normally distributed, the nonparametric Mann-Whitney statistical analysis was used.

After 3 months, motor skills improved from below the 6th percentile at baseline to the 12th percentile, but changes between the PUFA treatment and placebo groups were not statistically significant.

At baseline, reading and spelling assessments, expressed as developmental chronological age, lagged the children's actual age by about 1 year. After 3 months' intervention, both reading and spelling age scores increased significantly in the PUFA than in the placebo group (Table 1). Reading age increased by an average of 9.5 months ($P < 0.004$) and spelling by 6.6 months ($P<0.001$) in the PUFA-treated children. These gains were 3 times the expected normal gain in reading and twice that for spelling, which brought the children close to normative values.

Reading and spelling continued to improve in the following 3 months in both groups, with the placebo group consuming PUFAs scoring the same as the original treatment group. At the end of 6 months, children switched from placebo to PUFA improved by an average of 13.5 months in reading age and 6.2 months in spelling age. Children continuing active treatment improved in reading an average of 10.9 months in age and in spelling by 5.3 months.

In the assessments of attention, children consuming PUFAs for 3 months showed significant improvements in all 7 global scores on the Conners' Rating Scales. In the subscales, the PUFA group had significantly improved scores in 4 of the 6 subscales: opposition, cognitive problems, hyperactivity, and anxiety/shyness. Subscales for perfectionism and social problems did not improve significantly. In the following 3 months, children continuing to consume PUFAs showed some additional improvement, while those switched from the placebo to PUFAs improved their scores to values similar to those in the original PUFA group.

These observations demonstrated that consumption of specific PUFAs from both n-3 and n-6 classes for 3 months was associated with significant, measurable, and sustained improvements in reading, spelling, and behavior in school children with DCD. No significant effects on motor skills were observed. These results are consistent with findings from at least two other clinical trials of PUFAs in children with dyslexia and inattention. The authors noted the parallels between the positive effects of PUFAs in children with DCD and those in children whose primary diagnosis was dyslexia. Because the gender balance in this study favored boys (67%) it was not possible to determine whether gender affected the results. In a study of Japanese children 9 to 12 years of age, DHA-enriched fish oil consumed for 3 months improved impulsivity and physical aggression in girls, but had no effect on boys.

The Oxford Durham study raises cautious hope that a simple, safe, and effective dietary treatment could provide meaningful and positive improvements in children with DCD and closely related disorders such as dyslexia and inattention. The long-term implications for such a therapy point to improved quality of life for these children and their families with potentially sustained benefits. However, this possibility remains to be more firmly established. While not a cure, PUFA treatment

Table 1. Reading and spelling ages (mean ± SD) at baseline and after 3 months consumption of PUFAs or placebo in children aged 5-12 years with developmental coordination disorder

	Baseline		3 months		0-3 month comparison
	PUFA*	Placebo	PUFA	Placebo	P†
Reading age (mo.)	93.6 ± 18.6	99.8 ± 25.5	103.2 ± 28.4	103.2 ± 27.1	<0.004
Spelling age (mo.)	92.2 ± 16.3	95.5 ± 17.6	98.8 ± 22.0	96.7 ± 17.9	<0.001

*PUFAs: 732 mg/day of EPA and DHA, and 60 mg/day gamma-linolenic acid.
†Statistical comparison of groups by Mann-Whitney test.

appears to match current pharmacological therapy for DCD with minimal risk of harm. The study adds to the evidence that fish really is brain food, but it remains to be discovered how it is so in children with these types of behavioral and cognitive disabilities.

Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics *2005;115:1360-1366.*

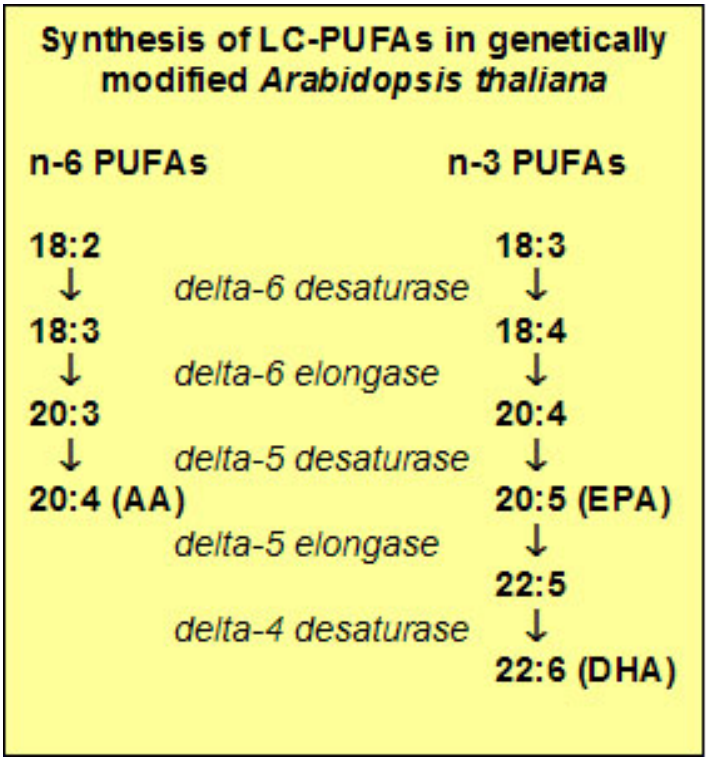
Frontiers

Another Step Closer to Long-Chain PUFAs in Plants

The desirability of diversifying the sources of long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in foods and supplements is driven by worldwide recommendations to increase consumption of these fatty acids, the limited number of foods that contain them, and the rapid decline of wild fisheries. As humans are very inefficient in making long-chain fatty acids from the 18-carbon precursor, alpha-linolenic acid, scientists have turned to plants to make these PUFAs for us. It is not an easy task.

Bio-engineering oil-producing plants such as canola and soybean to produce n-3 LC-PUFAs was first demonstrated in canola that was genetically modified to produce stearidonic acid, the first product in the conversion of alpha-linolenic acid to eicosapentaenoic acid. Stearidonic acid is rapidly converted to eicosapentaenoic acid (EPA) in humans. However, further chain elongation and desaturation to docosahexaenoic acid (DHA) did not ensue. Consequently, the quest for DHA synthesis in plants has continued.

In 2004, Dr. Qi and colleagues reported the synthesis of EPA and arachidonic acid in transgenic cress, *Arabidopsis thaliana*, a widely used model plant. This achievement required three separate transformations using genes for the synthesis of three different enzymes. To accomplish the synthesis of DHA from EPA, teams of scientists in Germany and France identified and cloned genes for specific conversion enzymes from algae and moss and used them to transform yeast cells. The genetically engineered yeast synthesized small amounts of DHA via a more direct pathway than is available in mammals. Not surprisingly, the investigators encountered bottlenecks along the way, some related to enzyme-substrate specificity and the need to shuttle intermediate products from one lipid pool to another so that the appropriate enzyme could function. This shuttling required additional enzyme activity and compromised efficiency. In spite of the hurdles, Ernst Heinz and his team succeeded in demonstrating DHA synthesis in linseed (flax).



An Australian team under the direction of Dr. Allan Green has also been working on the synthesis of DHA in seed oil. These investigators developed another pathway for DHA synthesis that avoided the need for both acyl-CoA and acyl-phosphatidylcholine substrates. They accomplished this by selecting enzymes with more flexible substrate preferences and the ability to use a single fatty acid pool. Using the model plant *Arabidopsis thaliana*, they achieved the synthesis of EPA, DHA, and arachidonic acid plus several of their intermediates.

To enable EPA synthesis, plants were genetically modified to express the enzymes delta 5-/delta 6-desaturase from zebrafish, *Danio rerio*, and delta-6 elongase from the nematode *Caenorhabditis elegans*. As shown in the illustration, the delta 5-/delta 6-desaturase first converted alpha-linolenic acid (18:3n-3) to stearidonic acid (18:4n-3).

Following elongation, the desaturase converted 20:4n-3 to EPA (20:5n-3). The same enzyme also converted linoleic acid to gamma-linolenic acid and subsequently, to arachidonic acid (illustration). Transformed plants synthesized EPA (0.4 to 2.3% total fatty acids) and arachidonic acid (0.2 to 1.4% total fatty acids) in their seed, with one line producing 3.2% EPA and 1.6% arachidonic acid.

The highest-yielding transformed plants were further genetically modified to express two genes from the microalga, *Pavlova salina*, for the enzymes delta 5-elongase and delta 4-desaturase. The former enzyme converts EPA (20:5n-3) to 22:5, which is then desaturated by the latter to 22:6n-3 or DHA. Two lines of the transgenic plants yielded 0.2 and 0.5% DHA, respectively. The line synthesizing the greatest amount of DHA was shown to be homozygous (two identical genes for the same trait) for the genes leading to EPA synthesis and thus, had more EPA available for conversion to DHA. The investigators further showed that 90% of the newly synthesized EPA and DHA occurred in the triglycerides of the seed oil.

Several intermediates in the pathway to EPA and arachidonic acid were present in amounts ranging from 0.4 to 1.6% of the total triglyceride fatty acids. For example, gamma-linolenic acid was found at 0.4% and stearidonic acid at 1.6%. Concentrations of total n-3 and n-6 PUFAs were similar in the phospholipid and triglyceride fractions.

The investigators also examined the relative efficiencies of the transgenic enzymes, observing a preference for alpha-linolenic acid (32%) compared with linoleic acid (14%) in the initial desaturation step. Conversion to EPA and 22:5n-3 (89%) was twice as efficient as that for arachidonic acid (45%). Further along the DHA synthesis pathway, only 17% of EPA was elongated to 22:5n-3, but virtually all of that was converted to DHA. The authors suggested that DHA production could be boosted by improving the efficiency of the conversion pathway using different enzymes, providing more substrate, or employing other promoters for gene expression. Interestingly, the precursor pool of linoleic and alpha-linolenic acids was not appreciably reduced by these conversions, but oleic acid declined significantly.

This elegant research increased the production of DHA in seeds from previous work and improved the efficiency of the conversion process. An important key to these achievements was the specificity of the enzymes involved—desaturation and chain elongation—and the ability to use a single form of substrate (acyl-CoA). Finding the long-chain PUFAs in the triglyceride pool means that these fatty acids accumulate in a highly bioavailable form in seed lipid droplets from which seed oils are extracted. These results bring us closer to the development of commercial oilseeds that could provide these LC-PUFAs for an array of foods.

Robert SS, Singh SP, Zhou X-R, Petrie JR, Blackburn SI, Mansour PM, Nichols PD, Liu Q, Green AG. Metabolic engineering of Arabidopsis to produce nutritionally important DHA in seed oil. Functional Plant Biology *2005;32:473-479.*

Singh SP, Zhou X-R, Liu Q, Stymne S, Green AG. Metabolic engineering of new fatty acids in plants. Curr Opinion Plant Biol *2005;8:197-203.*