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Editorial

Long-Chain PUFAs Reach the Plant Kingdom

The June 2004 issue of the *PUFA Newsletter* spans molecular methods to evolutionary human development. At the gene level, biotechnology is increasingly focused on consumer benefits, and that shift could yield a greater array of foods with long-chain polyunsaturated fatty acids (LC-PUFAs). The March 2004 *PUFA Newsletter* described research announcing the first transgenic mammal able to synthesize 20-carbon omega-3 PUFAs from omega-6 PUFAs. Here, we report the first transgenic plant able to synthesize arachidonic acid, an omega-6 PUFA, and eicosapentaenoic acid, an omega-3 PUFA. This remarkable feat by Dr. Baoxiu Qi and colleagues, then at the University of Bristol, U.K., was accomplished using three separate gene transfers and an alternate fatty acid synthetic pathway. Read about this accomplishment in the "Frontiers" section.

Also in Frontiers is a summary of Michael Crawford's insights from his decades of research on fatty acids and evolutionary development. He observes that in human development, placental and vascular tissue accumulates omega-6 fatty acids, while brain and eye, which first evolved in sea-dwelling organisms, preferentially accumulate docosahexaenoic acid, an omega-3 fatty acid. Crawford asserts that lipids are human beings' rapid response mechanism for adapting to environmental change. The ability of docosahexaenoic acid to alter the behavior of cell membranes, regulatory proteins, and genes has permitted the development of the brain and nervous systems as we know them today.

Crawford believes that today's wide departure from evolutionary dietary lipids is linked to the prevalence of chronic diseases of the vascular, endocrine, immune, and neural systems. Whereas our ancestors likely consumed a ratio of omega-6 to omega-3 fatty acids ranging from 1:1 to 1:3, western diets typically contain a ratio of 10:1 or more. Like his late distinguished compatriot, Hugh Sinclair, who pointed to fatty acid imbalance in the development of heart disease, Crawford extends this connection to other chronic diseases and predicts the consequences of today's dietary habits.

In "Cardiovascular Health," we describe a brief report showing the direct effect of omega-3 LC-PUFAs infused into the hearts of patients with ventricular tachycardia (episodes of excessively rapid heartbeats). In five of seven patients in whom tachycardia could be induced, infusion of omega-3 LC-PUFAs blocked the tachycardia. These pilot results confirm the antiarrhythmic properties of omega-3 LC-PUFAs in humans.

This issue also describes two facets of omega-3 LC-PUFAs and cancer that previously received little attention. One is a report of the protective association between fish consumption and risk of three types of lymph and blood system cancers. The beneficial effect was especially strong in men. The second summary reports two review papers, with one statistical meta analysis, of the literature on the putative association between alpha-linolenic acid consumption and risk of prostate cancer. Alpha-linolenic acid is the primary plant-based source of omega-3 fatty acids and is the precursor for the synthesis of omega-3 LC-PUFAs. Both reviews concur that the jury is still out on this controversial relationship.

The wealth of PUFA data emerging from laboratories around the world attests to the dynamism of PUFA research. It took some 30 years, but was well worth the wait.

We welcome your letters.

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Cardiovascular Health

Pilot Study Shows Dangerous Heartbeats Reduced with Infused n-3 LC-PUFAs

A Strong evidence indicates that long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) have anti-arrhythmic effects in humans. Largely because of this property, they are associated with significantly reduced mortality from sudden death. Direct effects of individual purified n-3 LC-PUFAs on abnormally rapid heartbeats, known as ventricular tachycardia, have been demonstrated in dogs in which the condition was induced. To see whether infused marine n-3 LC-PUFAs would be protective of ventricular tachycardia in humans, Schrepf and colleagues conducted a pilot study in 10 patients with implanted defibrillators who had repeated episodes of sustained ventricular tachycardia. Such patients are at high risk of sudden cardiac death.

At baseline, ventricular tachycardia could be induced by electrophysical stimulation in seven of the 10 patients. The three resistant patients consumed cold-water fish about two to three times/wk and had higher plasma phospholipid n-3 LC-PUFA concentrations than the remaining seven patients. These patients were not infused. The seven inducible patients were infused with 100 mL of marine triglyceride emulsion containing 3.8 g of n-3 LC-PUFAs*. After infusion, sustained ventricular tachycardia could be induced in two patients. Of the five resistant patients, it was possible to induce non-sustained ventricular tachycardia in three, but the two others remained resistant. All patients exhibited an extended refractory period of at least 20 minutes after n-3 PUFA infusion, indicating an increased period of protection against abnormal heartbeats.

Following infusion of n-3 LC-PUFAs, the concentration of free n-3 LC-PUFAs in plasma increased significantly (Table 1) from 0.3% ± 0.1% SD to 1.1% ± 0.8% SD of total free plasma fatty acids (P=0.01). This observation suggests that n-3 LC-PUFAs supplied in triglyceride form are rapidly hydrolyzed in the circulation to provide free fatty acids.

The investigators noted that the infused n-3 LC-PUFAs did not induce arrhythmia in any patient. Induction of ventricular tachycardia was reduced in five of seven patients, which suggests that these fatty acids are safe to use in patients who have implanted defibrillators and are at high risk of sudden cardiac death from uncontrolled heartbeats. This report is the first direct demonstration showing that n-3 LC-PUFAs can reduce the risk of potentially fatal uncontrolled heartbeats in subjects with implanted defibrillators. However, the authors emphasized the preliminary nature of this report and noted the absence of a placebo group, randomization, and open trial design.

Table 1. Changes in plasma free n-3 PUFA (%) after infusion of 3.8 g marine n-3 PUFA in high-risk patients with defibrillators who were induced for ventricular tachycardia.

Patient	Phospholipid n-3 PUFA (%)	Plasma free n-3 PUFA (%)	
	Baseline	*Baseline*	*After n-3 PUFA*
No inducible VT at baseline*†			
1	6.2	N/A	N/A
2	5.0	N/A	N/A
3	7.3	N/A	N/A
No inducible sustained VT after n-3 PUFA			
4	4.3	0.4	0.9
5	5.0	0.5	2.1
6	6.0	0.2	1.1
7	5.7	0.3	1.8
8	3.9	0.2	0.5
Inducible sustained VT after n-3 PUFA			
9	3.5	0.2	0.2
10	5.2	0.2	1.4

*VT, ventricular tachycardia

† Patients who could not be induced were not infused with n-3 PUFAs

In the accompanying editorial, Christine Albert points out that the implications of these data are limited given that suppression of ventricular tachycardia during

electrophysiological testing does not directly translate into a survival benefit when the same drugs are given chronically. Results from three randomized trials on the effect of fish oil supplementation on recurrent episodes of ventricular tachycardia in patients with implanted defibrillators are in progress or completed. It is hoped pending studies will provide clear and positive results.

*Omegaven, described by the manufacturer as containing 1.25-2.82 g EPA and 1.44-3.09 g DHA/100 mL emulsion

Schrepf R, Limmert T, Claus Weber P, Theisen K, Sellmayer A. Immediate effects of n-3 fatty acid infusion on the induction of sustained ventricular tachycardia. *Lancet* 2004;363:1441-1442.

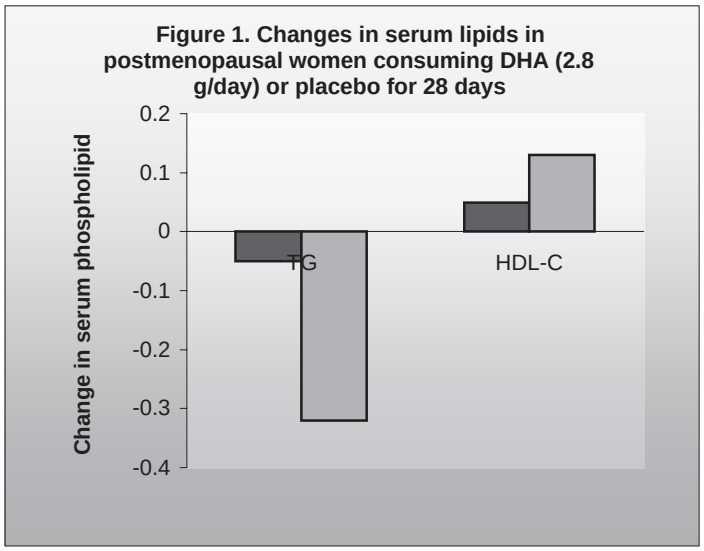
Albert C. Fish oil¾an appetizing alternative to anti-arrhythmic drugs? *Lancet* 2004;363:1412-1413.

DHA Supplementation Improves Some Heart Health Risk Factors in Postmenopausal Women

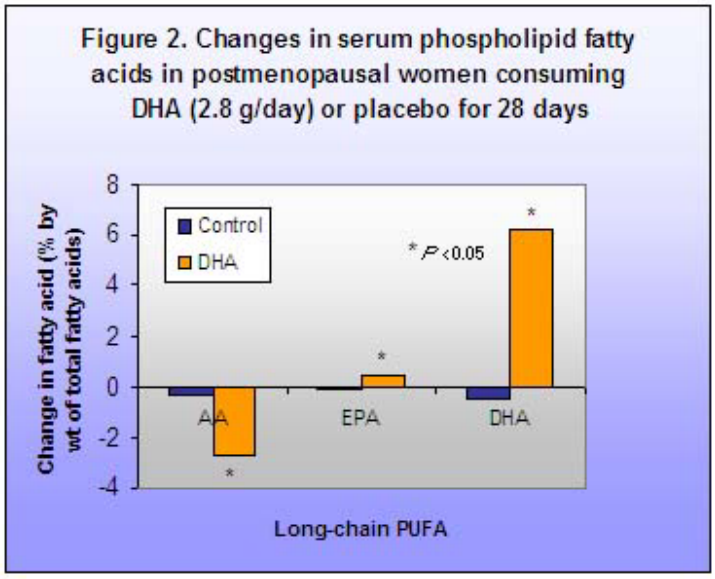
After menopause, the risk of cardiovascular disease and mortality in women increases. Hormone replacement therapy to reduce menopausal symptoms, once thought to reduce cardiovascular risk, is now believed to be without cardiovascular benefit and to increase risk of coronary disease and stroke. Instead, hormone treatment increases plasma triglycerides and C-reactive protein, both risk factors for heart disease. Consumption of docosahexaenoic acid (DHA) or fish oil, rich in long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), significantly reduces serum triglycerides in postmenopausal women, independent of hormone therapy status. Whether the effect of DHA on serum lipids and its retroconversion to eicosapentaenoic acid (EPA) is influenced by hormone therapy had not been investigated until Stark and Holub at the University of Guelph, Ontario, Canada, looked into it.

They conducted a placebo-controlled, double blind, crossover trial in 38 post-menopausal women, aged 45-70 years, 18 of whom received combined hormone replacement therapy of various regimens. Fourteen women not receiving hormones also completed the study. After a two-week pretrial period with no fish consumption, subjects were randomized to consume 12 capsules/day of DHA from algal oil or corn plus soy oil placebo for 28 days. Total DHA intake was 2.8 g/day. After a six-week washout period, subjects were switched to consume the other oil for another 28 days. Blood samples were obtained on days 0 and 28 of each dietary intervention.

At baseline, women receiving hormones had significantly higher concentrations of triglycerides (2.0 ± 0.3 vs. 1.0 ± 0.1 mmol/L, with and without hormones, respectively) and C-reactive protein (5.2 ± 1.2 vs. 2.0 ± 0.4 mg/L, with and without hormones, respectively) than those not receiving hormone therapy. All other clinical characteristics were similar in the two groups.



After 28 days of supplementation, women consuming DHA had a 20% reduction in triglycerides ($P<0.001$) and an 8% increase in high-density lipoproteins ($P<0.02$) compared with women consuming the control oil, independent of hormone status (Figure 1). The ratio of triglycerides to high-density lipoprotein cholesterol was also significantly reduced. DHA consumption was associated with a significant decrease in heart rate (71 ± 1.4 vs. 67 ± 1.1 beats/min) compared with the control supplement, with no differences between hormone treatment groups. C-reactive protein was unaffected by DHA supplementation. Other variables such as blood pressure, total cholesterol, glucose and insulin were not significantly affected by DHA consumption or hormone status.



Serum phospholipid fatty acid concentrations were affected by DHA consumption and hormone treatment. As has been reported often, consumption of DHA was accompanied by a significant decrease in arachidonic acid concentration (10.6 ± 0.4 at baseline vs.7.9 ± 0.2 at 28 days). As expected, DHA concentration also increased significantly, by 179% (Figure 2). The concentration of serum phospholipid EPA was significantly increased by DHA supplementation, suggesting retroconversion of DHA to EPA. Increases in EPA were significantly greater in women not receiving hormones than in those with them (0.7 ± 0.1% vs. 0.4 ± 0.1%, *P*=0.02). Consumption of DHA was also associated with a significant decrease in serum phospholipid n-3 docosapentaenoic acid, especially in women not receiving hormone therapy.

In summary, supplementation with 2.8 g/day DHA in healthy postmenopausal women, regardless of hormone therapy status, was associated with significant improvements in serum triglycerides (20% decrease) and high-density lipoproteins (8% increase), and significantly increased EPA and DHA concentrations in serum phospholipids. The authors interpreted the increased EPA concentration as evidence of retroconversion of DHA to EPA. Retroconversion was significantly greater in women not taking hormones than those receiving hormone therapy. Heart rate was significantly reduced (7%) with consumption of DHA, a change associated with reduced all cause mortality and sudden cardiac death in other studies. DHA consumption did not affect C-reactive protein levels, which were significantly greater in women receiving hormone therapy. It should be noted that the amount of DHA used in this study was substantially greater than could have been obtained through food sources alone.

Stark KD, Holub BJ. Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. *Am J Clin Nutr* 2004;79:765-773.

Short Takes

Study Refutes that Omega-3 Fatty Acids Reduce Blood Pressure in Mild Hypertension

Consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in amounts of 3 g/day or more have been associated with modest decreases in blood pressure in people with hypertension. Some studies suggest that of the two predominant n-3 LC-PUFAs in fish, docosahexaenoic acid, but not eicosapentaenoic acid (EPA), is effective in enhancing vasodilatory responses that may be linked to reduced blood pressure. Specific combinations of fatty acids may be more effective than others in lowering blood pressure, though trials in human subjects are few.

Dokholyan and colleagues tested a novel combination of LC-PUFAs for their effect on blood pressure in mildly hypertensive subjects. The study was a placebo controlled, randomized, double blind trial in103 mildly hypertensive subjects at two U.S. sites – Baltimore, Md. and Davis, Calif. Investigators aimed to find out if a daily fatty acid supplement containing 0.48 g EPA and 0.12 g gamma-linolenic acid* (18:3n-6) consumed for 12 weeks would affect blood pressure.

Prior to the study, subjects aged 30-54 years whose diastolic blood pressure was 85-94 mm Hg and cholesterol level less than 6.72 mmol/L (260 mg/L) were asked to consume 8 capsules/day of the olive oil control and discontinue all drugs affecting blood pressure for 28 days. Those who consumed less than two-thirds of the capsules or did not wish to participate

were excluded. Patients were randomized to consume either the fatty acid supplement or olive oil control capsules. Blood pressure was measured at the beginning of the study and at six and 12 weeks thereafter, with three measurements taken at 30-second intervals after patients were sitting for five minutes.

After six weeks, diastolic blood pressure was significantly reduced in the control group, but not in the fatty acid supplement group. At 12 weeks, diastolic blood pressure was significantly reduced in both groups, but more so in the olive oil control than in the supplement group. Systolic pressure was not significantly affected in either group.

Table 1. Change in systolic and diastolic blood pressure (mm Hg) after consumption of control oil (olive oil) or EPA plus gamma-linolenic acid for 12 weeks in 103 patients with mild hypertension.

Blood pressure	Control oil	P*	EPA+GLA	P*
Systolic				
Unadjusted	-1.56 ± 0.90	0.087	-1.21 ± 0.89	0.174
Adjusted	-1.54 ± 0.92	0.099	-1.23 ± 0.91	0.180
Diastolic				
Unadjusted	-3.35 ± 0.65	0.0001	-2.68 ± 0.64	0.0001
Adjusted	-3.45 ± 0.63	0.0001	-2.58 ± 0.62	0.0001

*** Change from baseline**

Differences between groups were not statistically significant regardless of whether the analysis included adjustment for confounding variables. At six weeks, systolic pressure was reduced more in the fatty acid group than the control group, but the effect was not sustained at 12 weeks and did not reach statistical significance. The authors concluded that the low dose EPA supplement with gamma-linolenic acid could not be recommended as adjuvant therapy to reduce blood pressure in patients with mild hypertension.

*The term "gamma-linoleic" acid in the original paper should have been "gamma-linolenic" acid, as confirmed by the first author.

Dokholyan RS, Albert CM, Appel LJ, Cook NR, Whelton PK, Hennekens CH. A trial of omega-3 fatty acids for prevention of hypertension. *Am J Cardiology* 2004;93:1041-1043.

Maternal and Infant Health

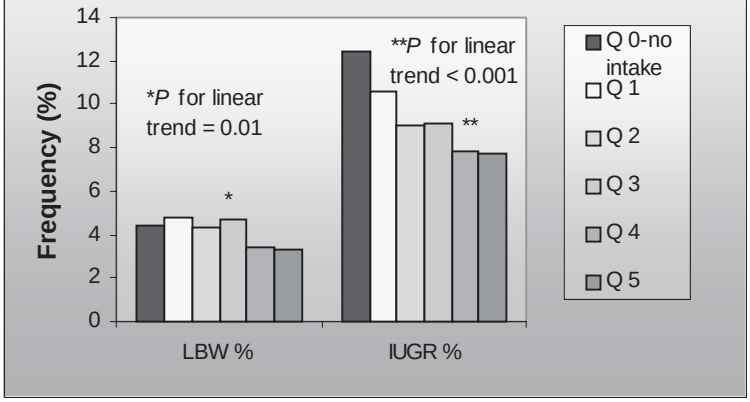
Fish Consumption in Pregnancy Reduces Intrauterine Growth Retardation and Low Birthweight

Pregnant and nursing women are encouraged to consume fish, especially fatty fish, during pregnancy and lactation to ensure adequate amounts of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) for the developing fetus and growing infant. Fish are the richest source of these fatty acids, which are essential for brain and eye development. Most Western diets provide very low amounts of n-3 LC-PUFAs, and alpha-linolenic acid, the vegetable-based precursor of n-3 LC-PUFAs, is poorly converted to the long-chain forms.

Studies comparing the length of gestation and infant birthweights of women who did or did not consume fish or fish oil during pregnancy have yielded inconsistent findings. Three observational studies reported increased birthweight or reduced risk of low birthweight in women who consumed fish, while another found no effect. Fish oil consumption has been associated with increased gestation time and birthweight in clinical trials, but not in high-risk pregnancies. Dr. Imogen Rogers and colleagues at the University of Bristol, U.K., examined the relationship between fish consumption in late pregnancy and birthweight and intrauterine growth in a cohort of 11,585 women living in southwest England.

Participants were part of a geographically based longitudinal study on pregnancy and childhood who were expected to give birth between April 1991 and December 1992. Questionnaires to assess frequency and type of fish consumed (i.e., white, other, and shellfish) were sent at 32 weeks' gestation. Fatty fish consumption was validated by docosahexaenoic acid (DHA) content of red blood cells in blood samples taken during pregnancy.

Figure 1. Frequency of low birth weight (%) and intrauterine growth retardation (%) according to quintile of maternal n-3 LC-PUFA intake in late pregnancy



DHA concentration in red blood cells increased with the frequency of fatty fish consumption ($P<0.001$). Estimates of n-3 fatty acid intake were calculated from fish consumption data and the cohort was divided into six groups based on n-3 fatty acid intake.

Response rate to the questionnaire was 87.9% of eligible women and 86.2% completed the information about eating fish. After excluding stillbirths, multiple births, fish oil consumption, and incomplete information on confounding variables the final sample was 10,040 births (71.0% of the original sample). Average fish consumption was 32.8 g/day with an estimated mean n-3 LC-PUFA intake of 0.15 g/day. White fish was consumed at least once in two weeks by 81.6% of women, while 57.4% and 19.3% consumed fatty fish and shellfish, respectively. Those consuming the most n-3 fatty acids were less likely to smoke, have low education, be single, in their teens, and weigh less than 50 kg (110 lb.), but were more likely to consume alcohol than women who consumed zero n-3 LC-PUFAs.

Frequency of low birthweight, preterm delivery, intrauterine growth retardation, birth weight and gestation were analyzed according to n-3 LC-PUFA and fish consumption. Intrauterine growth retardation was defined as birthweight for gestational age below the 10th centile, using the entire sample as a reference. As n-3 LC-PUFA intake increased, frequency of low birthweight ($P=0.01$) and intrauterine growth retardation ($P<0.001$) were significantly reduced (Figure 1). Birthweight was also slightly but significantly higher with increased n-3 LCPUFA consumption ($P<0.001$, total difference across quintiles 70 to 80 g). Frequency of preterm birth and mean gestation time were not associated with n-3 LC-PUFA or fish consumption. However, when the analyses controlled for confounding variables, the statistical significance of most associations disappeared, except for the lower frequency of intrauterine growth retardation associated with increased fish consumption ($P=0.017$). Overall, results were similar for fish and n-3 LC-PUFA intake.

The authors noted that their study found a positive association of n-3 LC-PUFA or fish intake with fetal growth rate but not with gestation time or frequency of preterm birth. Clinical trials using supplements have tended to find contrasting effects: increased gestation time and no effect on fetal growth. Unlike observational studies, supplementation trials can more easily control the dose and frequency of consumption, although it is possible that the outcomes in observational studies are related to other variables. In addition, a threshold effect may not be reached in free-living populations that do not consume fish often.

These findings from a large, predominantly Caucasian population provide some support for a possible positive effect of fish and n-3 LC-PUFA consumption on fetal growth rate; the data suggest a small increase in birth weight and reduced frequency of low birthweight infants. However, fish consumption was not associated with longer gestation or lower rates of preterm births. There were no adverse outcomes among women who consumed fish and fatty fish most often. It was previously reported that children of these mothers who consumed fatty fish during pregnancy were more likely to have achieved adult stereoacuity at three years of age.

Rogers I, Emmett P, Ness A, Golding J, ALSPAC Study Team. Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. *J Epidemiol Community Health* 2004;58:486-492.

Short Takes

Does the Form of LC-PUFAs Added to Infant Formula Matter?

Long-chain polyunsaturated fatty acids (LC-PUFAs) are present in breast milk but were not added to standard infant formulae until recently. The World Health Organization (1994) and European Society of Pediatric Gastroenterology and Nutrition (1991) recommend additions of arachidonic acid and docosahexaenoic acid (DHA) to infant formulae in Europe. These additions are permissible in the United States and Canada.

LC-PUFAs are available from various sources, including egg yolk lipids, fish oil low in eicosapentaenoic acid, and oils synthesized from fungal and algal organisms, which produce arachidonic acid and DHA, respectively. In egg yolk, LC-PUFAs are present mainly in phospholipids, whereas in fish oil and single cell oils, LC-PUFAs are in triglycerides. In breast milk, 85% of LC-PUFAs are in triglycerides. The different types of lipids are hydrolyzed, absorbed and incorporated into lipoproteins at different rates.

This study compared the fatty acid composition of plasma phospholipids, triglycerides, and cholesteryl esters at birth and three months in healthy term infants fed breast milk, or formula supplemented with similar amounts of LC-PUFAs from egg phospholipids or single cell oils. Arachidonic acid and DHA were provided at a concentration of 0.4 g and 0.1 g/100 g total fatty acids, respectively. Compared with breast milk, formula contained a similar concentration of arachidonic acid, but less than half the DHA (0.28 vs.0.11 and 0.12 g/100 g total fatty acids in human milk, egg phospholipid, and single cell formula, respectively).

At three months of age, plasma arachidonic acid and eicosapentaenoic acid concentrations were similar in all lipid fractions in all groups. DHA concentration, however, was significantly lower in both formula groups compared with breast-fed infants in all lipid fractions. In formula-fed infants, DHA concentration was a quarter that of breast-fed infants in plasma triglycerides, and less than two-thirds the concentration in phospholipids and cholesteryl esters. Fatty acid concentrations were similar in both formula groups, indicating that theses forms of LC-PUFAs had similar bioavailability.

The authors suggested that increasing the amount of DHA added to infant formula would provide a concentration that more closely resembles breast milk. However, DHA levels in breast milk depend on maternal DHA intake and, therefore, are notoriously variable, so this concentration is difficult to estimate. The authors noted that DHA levels in plasma phospholipids may be correlated with retinal and cerebral phospholipids, which underscores the importance of ensuring adequate dietary amounts for formula-fed infants.

Sala-Vila A, Castellote AI, Campoy C, Rivero M, Rodriguez-Palmero M, Lopez-Sabater MC. The source of long-chain PUFA in formula supplements does not affect the fatty acid composition of plasma lipids in full-term infants. *J Nutr* 2004;134:868-873.

Immune & Inflammatory Function

Consumption of Long-Chain Fatty Acid Intermediates Affects Lipid and Cell Composition

Increased consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) leads to their increased concentration in the membranes of red blood cells, platelets, and cells of the immune and nervous systems. By incorporating into cells, n-3 LC-PUFAs affect cell, tissue, and organ functions, frequently in ways that benefit human health. These fatty acids occur naturally mainly in fish, particularly fatty fish, but are also available in capsules and other foods, such as egg yolks from hens fed fish, flax, or algal oils. Only the 18-carbon precursors of LC-PUFAs are found in terrestrial plants.

The main precursor of n-3 LC-PUFAs, alpha-linolenic acid, is poorly converted to the long-chain forms partly because of the low activity of the first enzyme in the conversion pathway and partly because most is oxidized. The product of this first step, stearidonic acid (18:4n-3), is rapidly converted to eicosapentaenoic

acid (EPA) and docosapentaenoic acids. Little appears in docosahexaenoic acid (DHA), however. Thus, it is thought that plants with increased levels of stearidonic acid could provide a readily available plant-based source of EPA.

Figure 1. *Borago officinalis*. Photo copyright: Centre for Agricultural Strategy, The University of Reading. Reproduced with permission.

The corresponding pathway in the metabolism of omega-6 (n-6) PUFAs yields arachidonic acid. Concentrations of arachidonic acid are usually reduced by increased intake of EPA. This effect is beneficial in moderating inflammatory responses, but may be disadvantageous in other circumstances. Providing the precursor of arachidonic acid, gamma-linolenic acid, can increase arachidonic acid concentration in some lipid fractions, but may reduce the concentration of EPA. Thus, interactions between the types and amounts of n-3 and n-6 LC-PUFAs are complex.

Both stearidonic and gamma-linolenic acids occur naturally in certain specialty oils. Oils rich in gamma-linolenic acid include borage oil with about 25% gamma-linolenic acid, evening primrose oil (10%), and black currant oil (12%). Echium oil contains both gamma-linolenic acid (12%) and stearidonic acid (17%) as well as alpha-linolenic acid (33%). Borage oil comes from the plant *Borago officinalis* (Figure 1) and echium oil comes from the plant *Echium plantagineum* (Figure 2).

Figure 2. *Echium plantagineum*. Photo courtesy of photographer Rob Richardson and www.weedinfo.com.au.

Few studies have examined the effects of consuming long-chain fatty acid intermediates on lipid composition and cell function. In this report, Elizabeth Miles and colleagues at the University of Southampton, U.K., explored the effects of different LC-PUFA precursors on the fatty acid composition of plasma lipids and cell membranes of various types of cells in healthy subjects. Using seven treatment groups, the investigators provided capsules of dietary oils rich in the long-chain precursors stearidonic acid (n-3 pathway) from echium oil, gamma-linolenic acid (n-6 pathway) from borage and echium oils, EPA from an EPA-rich oil, plus three blends of these oils, and a placebo oil. The placebo oil consisted of an 80:20 mixture of palm and high linoleic sunflower oils. Blended oils numbered one to three contained increasing levels of stearidonic (1.0% to 8.1% per 100 g total fatty acids) and alpha-linolenic acids (0.8% to 24.2%) and decreasing levels of gamma-linolenic acid (12.1% to 7.8%), arachidonic acid (1.0% to 0.5%), EPA (13.9% to 7.1%) and DHA (6.1% to 3.0%). The amounts provided, about 2 g/day of each fatty acid, represented about 8 to 30 times the usual dietary intake of EPA, and at least 30 times the amount of precursor fatty acids, gamma-linolenic and stearidonic acids.

Subjects were healthy males aged 21 to 44 years who did not consume evening primrose oil, fish oil, or vitamin supplements, were not vegetarian, and did not eat more than two portions of oily fish/week. Each treatment group consisted of 8 to 12 subjects who consumed the oils in a parallel study design for 12 weeks. Blood samples were taken at baseline and every four weeks thereafter.

Consumption of stearidonic acid did not result in the appearance of stearidonic acid or its long-chain derivative, eicosatetraenoic acid, in plasma lipids. In contrast, consumption of gamma-linolenic acid resulted in significantly increased concentration of gamma-linolenic acid in all plasma lipid fractions after 12 weeks: triglycerides (185%), phospholipids (210%),

Table 1. Concentrations of gamma-linolenic and dihomo-gamma-linolenic acids in plasma lipids of healthy men consuming 2 g/day gamma-linolenic acid from borage oil for 12 weeks.

Fatty acid	Time	Triglycerides	Cholesteryl esters	Phospholipids
	(wk)	(g/100 g total fatty acids)		
Gamma-linolenic acid (18:3n-6)	0	0.42 ± 0.06	0.9 ± 0.1	0.08 ± 0.01
	12	1.21 ± 0.19*†	2.4 ± 0.2*†	0.25 ± 0.03*
Dihomo-gamma-linolenic acid (20:3n-6)	0	0.36 ± 0.05	0.74 ± 0.06	3.0 ± 0.1
	12	0.48 ± 0.08	1.20 ± 0.11*†	4.8 ± 0.3*†

*Significantly different from baseline, $P<0.05$.

†Significantly different from placebo group, $P<0.05$. Data not shown.

and cholesteryl esters (165%). The long-chain derivative of gamma-linolenic acid, dihomo-gamma-linolenic acid, was significantly increased in plasma cholesteryl esters and phospholipids of subjects consuming borage oil, the richest source of gamma-linolenic acid fed, but not in any other group (Table 1).

Consumption of EPA resulted in a significant increase of EPA in plasma cholesteryl esters (420%) and phospholipids (355%) and a statistically insignificant increase in triglycerides (55%), with maximal increases occurring after four weeks. Increases were dose related. DHA concentrations in cholesteryl esters and phospholipids also increased significantly after four weeks in this group, while arachidonic acid concentration decreased slightly, but not significantly (15% to 20%). Consumption of the EPA oil was accompanied by a significant decrease in dihomo-gamma-linolenic acid (about 30% on average) in cholesteryl esters and phospholipids. This decrease was not observed in the groups consuming the blended oils that provided less EPA.

The fatty acid composition of peripheral blood mononuclear cells, which are involved in immune function, did not increase in stearidonic acid, gamma-linolenic acid, or alpha-linolenic acid when oils rich in these fatty acids were consumed. However, consumption of the EPA and echium oils resulted in significant increases in EPA concentration in peripheral blood mononuclear cells compared with baseline values (Table 2). DHA content was unaffected by EPA consumption, and arachidonic acid content was not altered.

Subjects consuming the blended oils with different but lower levels of EPA than the EPA group also had significantly increased EPA concentration in their

Table 2. Fatty acid composition of total lipids extracted from peripheral blood mononuclear cells in healthy male subjects consuming 2 g/day of different dietary fatty acids.†

Fatty acid	Time	EPA	Echium oil	Blend 1	Blend 2	Blend 3
	(wk)	(g/100 g total fatty acids)				
EPA	0	0.7 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	1.0± 0.4	0.9 ± 0.3
	12	1.6 ± 0.3*	0.9 ± 0.2*	1.3 ± 0.2*	1.6 ± 0.4	1.5 ± 0.2
DHA	0	2.3 ± 0.3	1.8 ± 0.1	2.3 ± 0.3	1.7 ± 0.3	2.3 ± 0.2
	12	2.2 ± 0.2	2.9 ± 0.5*	2.9 ± 0.2	3.1 ±0.6*	2.4 ± 0.3

*Significantly different from baseline, $P<0.05$.

†Selected PUFA concentrations (g/100 g total fatty acids) in Echium, Blend 1, Blend 2, Blend 3 treatment oils, respectively: gamma-linolenic acid ,10.7%,12.1%, 9.1%, 7.85; alpha-linolenic acid, 33.4%, 0.8%, 15.7%, 24.2%; stearidonic acid, 11.7%, 1.0%,5.4%, 8.1%; EPA, 0.0%, 13.9%, 9.1%, 7.1%; DHA, 0.0%, 6.1%, 4.0%, 3.0%. EPA oil contained 25.7% and 10.9% EPA and DHA respectively.

peripheral blood mononuclear cells (Table 2). After 12 weeks, EPA concentration did not differ significantly among the blended oil and EPA oil groups. Of note, too, was the significant increase from baseline in EPA (125%) and DHA (61%) concentrations in those consuming echium oil, containing stearidonic acid but not EPA or DHA (Table 2).

This complex study confirmed an earlier report that stearidonic acid and its immediate 20-carbon derivative, intermediates in the synthesis of n-3 LC-PUFAs, do not accumulate in plasma lipids and cell membranes, but are efficiently converted to EPA. Compared with alpha-linolenic acid, the major dietary precursor of n-3 LC-PUFAs that is relatively poorly converted to long-chain forms, stearidonic acid provides an efficient plant-based source for the formation of EPA that is readily incorporated into plasma and cell lipids. These findings support the suggestion that stearidonic acid is efficiently utilized because it bypasses the need for the enzyme delta-6 desaturase, the first and limiting enzyme in the conversion of alpha-linolenic acid to n-3 LC-PUFAs.

Consumption of borage oil, rich in gamma-linolenic acid, increased the concentration of gamma-linolenic acid in plasma triglycerides, cholesteryl esters, and phospholipids (Table 1). Concentration of its 20-carbon derivative also increased in the latter two plasma lipids, indicating that gamma-linolenic acid was converted to long-chain forms. Arachidonic acid concentrations tended to increase as well, but not to levels of statistical significance.

When stearidonic acid or EPA was consumed with gamma-linolenic acid, as in the consumption of echium oil and the blended oils, conversion to the long-chain derivative in plasma lipids was inhibited. Others have also reported that n-3 LC-PUFAs inhibit the conversion of gamma-linolenic acid to long-chain n-6 derivatives.

In peripheral blood mononuclear cells, consumption of echium oil resulted in a trend toward higher concentrations of n-3 long-chain derivatives and DGLA and no significant change in arachidonic acid. Because increased arachidonic acid concentration may be undesirable, the investigators suggested that the functional effects of n-6 and n-3 LC-PUFAs might be obtained by consuming gamma-linolenic acid with stearidonic acid or EPA, which does not raise arachidonic acid levels. The authors noted that in a study in which both gamma-linolenic acid and stearidonic acid were consumed, concentration of the long-chain derivative of gamma-linolenic acid, dihomo-gamma-linolenic acid, increased, but the EPA and DHA concentrations were unaffected. In that report, gamma-linolenic acid was fed at a level five times greater than stearidonic acid. This suggests that the relative amount of these precursor fatty acids is an important consideration for influencing lipid and cell membrane composition.

This study offers further encouragement for the development of plant-sources of stearidonic acid. When consumed in judicious combination with the respective n-6 LC-PUFA intermediate, gamma-linolenic acid, such dietary fatty acids may achieve subtle, yet potentially functional changes in cell fatty acid composition. Studies are likely underway to determine if such dietary manipulations are associated with functional benefits.

Miles EA, Banerjee T, Calder PC. The influence of different combinations of y-linolenic, stearidonic and eicosapentaenoic acids on the fatty acid composition of blood lipids and mononuclear cells in human volunteers. *Prostaglandins Leukot Essent Fatty Acids* 2004;70:529-538.

EPA and DHA Intake Alter Fatty Acid Composition of Neutrophils and Plasma Phospholipids

The immune system exerts its protective effects through a variety of cell types and chemical substances. Mediators of immune and inflammatory responses include a family of substances called cytokines, such as various interleukins, tumor necrosis factor-alpha, and interferon-gamma. Eicosanoids, substances derived from long-chain polyunsaturated fatty acids (LC-PUFAs), are involved in stimulating or restraining inflammatory responses. The most common eicosanoids include prostaglandins and leukotrienes derived mainly from arachidonic acid. For this reason, arachidonic acid is usually considered pro-inflammatory. We need arachidonic acid for adequate eicosanoid production, but excess production is associated with exaggerated inflammatory responses. Excessive inflammatory responses underlie many immune-related

diseases, such as rheumatoid arthritis and asthma, and are associated with cardiovascular disease.

Consumption of omega-3 (n-3) LC-PUFAs reduces the production of pro-inflammatory eicosanoids and stimulates the synthesis of eicosanoids with weak inflammatory activity. Put simply, this concept underlies the moderating effect of n-3 LC-PUFAs in various immune and inflammatory conditions. To date, it is not clear whether the moderating effects of fish oils, rich in n-3 LC-PUFAs, in human subjects are due to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or both. Animal studies have suggested that both n-3 LC-PUFAs affect immune responses. However, different cell types and substances may respond to n-3 LC-PUFAs differently. For example, fish oil, but not DHA alone, suppressed lymphocyte proliferation and natural killer cell activity in healthy subjects. However, it was not clear whether the results depended on the presence of EPA or both EPA and DHA.

To answer this question, Kew and colleagues at the University of Reading, U.K., compared the effect of feeding purified EPA, DHA, and an olive oil placebo on the fatty acid composition of plasma phospholipids and neutrophils (a type of white cell), and immune cell responses under laboratory conditions. Forty-two healthy, non-vegetarian subjects aged 23 to 65 years, who were free of immune or inflammatory conditions, cardiovascular disease and other diseases and did not consume fish oil, evening primrose oil, or vitamin supplements were enrolled in the study. Subjects were stratified by age, body mass index, and fasting triglyceride levels, and randomized to consume placebo, EPA, or DHA for four weeks.

Each group contained 14 to 15 subjects. Those assigned to EPA consumed 4.75 g EPA/day plus 0.73 g DHA/day. Those assigned to DHA consumed 0.85 g EPA/day plus 4.91 g DHA/day. Fasting blood samples were obtained immediately prior to supplementation and after four weeks. Data were available for 10-15 subjects/group. Fatty acid measurements were obtained for plasma phospholipids and neutrophil total lipids. Immune cell function was assessed by the expression of monocyte adhesion molecules, phagocytic activity (particle engulfing) by neutrophils and monocytes, T lymphocyte activation in whole blood, and cytokine production by peripheral blood mononuclear cells.

Consumption of EPA or DHA significantly affected the plasma phospholipid concentrations of EPA, DHA, and dihomo-gamma-linolenic acid, an intermediate in the n-6 LC-PUFA pathway for arachidonic acid synthesis (Table 1). In contrast to many literature reports, EPA and DHA supplementation did not affect arachidonic acid concentrations. The proportion of EPA in plasma phospholipids in those consuming EPA or DHA was significantly higher than baseline and

Table 1. Fatty acid composition of plasma phospholipids in healthy subjects at baseline and after 4 wk supplementation with placebo, EPA*, or DHA.

Treatment	Time	AA	DGLA	EPA	DPA	DHA
		% by wt of total fatty acids				
Placebo	Baseline	13.8 ± 2.9	3.8 ± 0.7[a]	1.7 ± 0.8[a]	0.9 ± 0.2[a]	8.2 ± 1.9[a]
	4 wk	12.0 ± 2.6	3.8 ± 1.0[a]	1.3 ± 0.7[a]	0.8 ± 0.2[a]	7.3 ±1.6[a]
EPA	Baseline	12.5 ± 1.6	3.4 ± 0.9[a]	1.4 ± 0.5[a]	0.9 ± 0.1[a]	7.0 ± 1.8[a]
	4 wk	10.2 ± 1.0	2.3 ± 0.6[b]	7.2 ± 1.9[b]	1.8 ± 0.3[b]	7.6 ± 1.1[a]
DHA	Baseline	13.1 ± 1.7	3.4 ± 0.6[a]	1.5 ± 0.7[a]	0.9 ± 0.1[a]	6.0 ± 1.4[a]
	4 wk	10.2 ± 2.2	2.1 ± 0.9[b]	4.5 ± 1.3[c]	0.8 ± 0.2[a]	13.5 ± 3.1[b]

*Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; DGLA, dihomo-gamma-linolenic acid; DPA, docosapentaenoic acid.

[a]Values in the same column with different superscript letters are significantly different, $P<0.05$.

Table 2. Fatty acid composition of total lipids in neutrophils in healthy subjects at baseline and after 4 wk supplementation with placebo, EPA*, or DHA.

Treatment	Time	AA	DGLA	EPA	DPA	DHA
		% by wt of total fatty acids				
Placebo	Baseline	14.9 ± 2.7	2.4 ± 0.4	0.7 ± 0.3[a]	1.4 ± 0.7[a]	2.8 ± 1.2[a]
	4 wk	14.1 ± 1.8	2.4 ± 1.1	0.6 ± 0.3[a]	1.5 ± 0.5[a]	2.5 ± 0.9[a]
EPA	Baseline	13.5 ±5.2	2.0 ± 0.6	0.6 ± 0.1[a]	1.4 ± 0.4[a]	2.5 ± 0.6[a]
	4 wk	12.7 ± 2.1	1.7 ± 0.7	3.0 ± 1.1[b]	3.0 ± 1.0[b]	2.7 ± 0.9[a]
DHA	Baseline	15.9 ± 1.0	2.4 ± 0.8	0.7 ± 0.1[a]	1.3 ± 0.4[a]	2.8 ± 0.6[a]
	4 wk	12.2 ± 1.5	1.6 ± 0.2	1.8 ± 0.5[c]	2.0 ± 0.5[a]	5.3 ± 1.1[b]

* Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DGLA, dihomo-gamma-linolenic acid;
DPA, docosapentaenoic acid.

[a] Values in the same column with different superscript letters are significantly different, $P<0.05$.

placebo values. The proportion was higher in the EPA group compared with the DHA group (414% vs. 200% increase compared with baseline).

Consumption of DHA resulted in a significant increase in DHA concentration (125%). Consumption of either fatty acid resulted in significantly lower concentrations of dihomo-gamma-linolenic acid (32% and 38% decrease for EPA and DHA consumption, respectively). Consumption of EPA, but not DHA, was associated with a significant increase in the metabolite of EPA, docosapentaenoic acid.

Changes in the fatty acid composition of neutrophil total lipids paralleled those in plasma phospholipids (Table 2). Neutrophils are cells produced in bone marrow that protect against infectious agents. The main difference was that EPA and DHA had no significant effect on dihomo-gamma-linolenic acid levels, although DHA consumption decreased dihomo-gamma-linolenic acid concentration by a third. Consumption of EPA resulted in a significant increase in neutrophil docosapentaenoic acid concentration.

The effects of EPA and DHA consumption on T lymphocyte activation were assessed by examining two markers produced by the cells in response to stimulation. T lymphocytes are a type of white blood cell that mature in the thymus gland (whence the name "T") and attack foreign proteins or antigens. Consumption of EPA or DHA had no effect on T lymphocyte activation in terms of percentage of marker-responsive cells; however, there was a significant treatment effect for marker expression ($P<0.05$). Consumption of DHA, but not EPA, was associated with reduced marker expression.

Other measures of neutrophil and lymphocyte cell function showed no effect of dietary treatment. The ability of cells to engulf bacteria, generate cellular adhesion molecules, or produce an array of cytokines were unaffected by the consumption of EPA or DHA. Substantial variation in subject responses may have contributed to the lack of significant effects.

This study, like the preceding report by Miles and colleagues, showed that plasma phospholipids and neutrophil lipids are dramatically affected by the consumption of n-3 LC-PUFAs. Increased consumption of EPA or DHA resulted in increased concentration of these fatty acids in plasma phospholipids and neutrophil lipids. EPA appeared to be converted to its 22-carbon derivative, docosapentaenoic acid, but not further converted to DHA. Subjects consuming DHA appeared to retroconvert DHA to EPA, as indicated by the increased concentration of EPA in this group, but these subjects also consumed a small amount of EPA.

If so, the retroconversion process was not associated with accumulation of the intermediate, docosapentaenoic acid. Consumption of EPA or DHA decreased the concentration of dihomo-gamma-linolenic acid in plasma phospholipids, but not in neutrophils. This finding accords with the Miles study, but its biological significance remains unknown.

Assessment of immune cell function found no effect of EPA or DHA consumption on expression of monocyte adhesion molecules, engulfment of particles by monocytes or neutrophils, or production of cytokines by monocytes and lymphocytes. Whether these findings relate to the dose used, study duration, subject health status and variability, or measurements taken under laboratory rather than physiological conditions is unclear. DHA consumption was associated with reduced expression of an early marker of T lymphocyte activation, suggesting possible functional effects of DHA that are independent of lymphocyte proliferation. The inconsistency of the literature in this area suggests that both measurement and biological variables complicate the assessment of n-3 LC-PUFAs on immune function.

Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM, Yaqoob P. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. *Am J Clin Nutr* 2004;79:674-681.

Clinical Conditions: Cancer

Is Risk of Prostate Cancer Weakest Link in Alpha-Linolenic Acid Chain?

Alpha-linolenic acid, the 18-carbon precursor of the long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is found mainly in seeds and plants such as flaxseed, chia seeds, canola and soybean oils, and walnuts. Consumption of alpha-linolenic acid is associated with reduced risk of cardiovascular disease and mortality, although the evidence is not as strong as it is for fish oils and n-3 LC-PUFAs. Although the need for n-3 LC-PUFAs can theoretically be met from consuming alpha-linolenic acid, conversion to the long-chain forms is poor and may be insufficient to meet the needs of human infants during fetal growth and the first 18 months of life.

In addition, consumption of alpha-linolenic acid has been associated with increased risk of prostate cancer in six epidemiological studies. Another five epidemiological studies, four of which were case-control studies, found no association between dietary or serum alpha-linolenic acid levels. A twelfth study that examined fatty acids in prostate tissue of cases and controls reported a negative association between alpha-linolenic acid concentration in tissue and prostate cancer. The association reached borderline statistical significance, and has been interpreted to suggest a protective effect of alpha-linolenic acid. The real question is whether there is a health benefit from increasing the consumption of alpha-linolenic acid?

Two recent reports have tried to interpret these findings. Attar-Bashi and colleagues at RMIT University, Melbourne, Australia, analyzed the strengths and weaknesses of the published literature and included in vitro studies and links with other cancers as well. In view of the heterogeneity of prostate cancer, the protective potential of alpha-linolenic acid in cardiovascular disease, lack of intervention data, and studies in animal models, the authors conclude it would be premature to make recommendations about alpha-linolenic acid, an essential nutrient, and prostate cancer.

Brouwer and colleagues at Wageningen University, The Netherlands, approached the question by analyzing combined epidemiological studies using meta-analysis. In the first meta-analysis, only prospective studies were included to examine the association between dietary alpha-linolenic acid and risk of fatal heart disease. Comparing high and low intake of alpha-linolenic acid in five studies, the investigators calculated a relative risk of fatal heart disease, adjusting for confounding variables, of 0.79 (95% CI= 0.60-1.04) or a statistically insignificant 21% reduction in risk. The average high and low intakes of alpha-linolenic acid were 2.0 and 0.8 g/day, a mere difference of 1.2 g a day.

For prostate cancer, the team analyzed nine observational studies that included two prospective and seven case-

control reports. Analysis of the combined data yielded an estimated relative risk of prostate cancer with increased consumption of alpha-linolenic acid of 1.70 (95% CI=1.12-2.58), indicating an increased risk of 70%. The authors commented on the heterogeneity of the data and their uncertainty of whether a potential effect on prostate cancer is real. They noted that coronary heart disease is diagnosed six times more often than prostate cancer and occurs at a younger age. Someone with prostate cancer is unlikely to find this observation reassuring.

Brouwer's group noted that other epidemiological data have suggested that n-3 LC-PUFAs from fish were not associated with increased risk of prostate cancer and may be protective. Thus, they conclude that, "fish should be the first recommended source of (n-3) fatty acids."

While it is uncertain that alpha-linolenic acid is protective of cardiovascular disease, but seems risky for prostate cancer, the data cannot be dismissed. Incidence of, and in some countries mortality from, prostate cancer has increased since the 1960s and 1970s, although some of the increase can be attributed to improved screening for prostate specific antigen. The incidence of prostate cancer is highest in the United States and Canada, but it has also increased dramatically in England, Wales, and other countries. Hence, the case for additional research is not difficult to make.

Finally, a recent review of alpha-linolenic acid metabolism in men and women by Graham Burdge considers the conversion of alpha-linolenic acid to long-chain forms and the relative roles of these fatty acids in cell structure and function. He notes that "alpha-linolenic acid appears to be a limited source of longer-chain n-3 fatty acids in men, so adequate intakes of preformed n-3 polyunsaturated fatty acids, in particular docosahexaenoic acid, may be important for maintaining optimal tissue function."

Attar-Bashi NM, Frauman AG, Sinclair AJ. α-Linolenic acid and the risk of prostate cancer. What is the evidence? *J Urology* 2004;171:1402-1407.

Brouwer IA, Katan MB, Zock PL. Dietary α-Linolenic acid is associated with reduced risk of fatal coronary heart disease, but increased prostate cancer risk: A meta-analysis. *J Nutr* 2004;134:919-922.

Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care* 2004;7:137-144.

Proportion and Amount of Fish Consumed Associated with Lower Risk of Some Blood and Lymph Cancers

Consumption of fish has been associated with reduced risk of some cancers, particularly breast, prostate, and colon, but the data are inconsistent. Cancers of the lymph and hematopoietic system, which include leukemia, non-Hodgkin's lymphoma, and myeloma, have been more commonly associated with exposure to industrial chemicals than with dietary constituents. However, it has been reported that lymph and blood cancers are low among Circumpolar Inuit people whose traditional diet is rich in fat and marine lipids. Cancers of the lymph and blood system account for about 8% of all incident cancers in the United States.

Four studies reported that fish consumption was associated with reduced risk of lymph and hematopoietic (formation and development of blood cells) cancers, but others found no association or increased risk. It was observed that fish workers have reduced risk of leukemia and lymphoma independent of fish consumption.

A group of Australian and Canadian cancer researchers investigated the incidence of leukemia, multiple myeloma, and non-Hodgkin's lymphoma in a population database of 6,826 Canadians to determine whether fish consumption was associated with these cancers. Data for this case-control study were collected between 1994 and 1998 in a study of cancer risk among those in animal-related occupations. The sample included 1,418 cases of non-Hodgkin's lymphoma, 919 cases of leukemia, 287 myeloma cases, and 4,202 controls. Fish consumption was estimated from food frequency questionnaire data expressed as number of servings per week. Type of fish consumed was divided into two categories: fresh, frozen, or canned, and smoked, salted, or dried fish. Fish consumption was divided into quartiles ranging from zero servings per week to four or more.

After adjusting for age, sex, smoking, body mass index, and proxy respondent status, those who ate fresh, frozen, or canned fish more often were significantly

Table 1. Adjusted* odds ratios and 95% confidence intervals for risk of cancers of the lymph and blood system in Canadian men and women by fish intake.

Fish† Servings per Wk	All LH† Cancers	Leukemia	Myeloma	Non-Hodgkin Lymphoma
<0.5	**1.0** (0.86-1.17)	**0.97** (0.78-1.21)	**0.87** (0.60-1.23)	**1.05** (0.87-1.27)
0.5 to <4	**0.90** (0.77-1.05)	**0.80** (0.64-1.00)	**0.68** (0.47-1.00)	**1.01** (0.84-1.22)
4 or more	**0.81** (0.68-0.97)§	**0.72** (0.55-0.93)§	**0.76** (0.50-1.16)	**0.88** (0.71-1.30)

*Adjusted for age, sex, smoking, body mass index, and proxy respondent status.
†Abbreviations: Fish: fresh, frozen, or canned; LH: lymph and hematopoietic.
§ *P* value for trend <0.01.

less likely to develop any lymph or blood system cancers (*P* for trend=0.004). As shown in Table 1, the odds ratio for those consuming fresh fish four or more times weekly was 19% less compared with those who ate fewer than a half a serving per week (0.81 vs. 1.0).

For specific cancers, increased fish consumption was significantly associated with reduced risk of leukemia (odds ratio=0.72) for those eating fish four or more times a week. Fish consumption was not significantly associated with non-Hodgkin's lymphoma or myeloma, although odds ratios diminished as fish intake increased.

However, when the odds ratios were calculated on the basis of proportion of total fat or energy from fish, increased fish consumption was associated with significantly lower risk of all three cancers (*P* for trend <0.01 for all analyses). When the association with proportion of energy or fat from fish was analyzed separately for males and females, the relationship was much stronger in males and did not reach statistical

Table 2. Adjusted* odds ratios and 95% confidence intervals for risk of cancers of the lymph and blood system in Canadian men by percent dietary fat or energy from fish.

Percent Dietary Fat or Energy from Fish† by Quartile	All Lymph & Blood System Cancers	Leukemia	Myeloma	Non-Hodgkin's Lymphoma
Fat				
Q2	**0.79** (0.66-0.95)	**0.77** (0.60-0.99)	**0.82** (0.52-1.31)	**0.79** (0.63-0.99)
Q3	**0.76** (0.63-0.92)	**0.62** (0.47-0.82)	**0.69** (0.43-1.13)	**0.87** (0.69-1.10)
Q4	**0.63** (0.52-0.77)§	**0.56** (0.42-0.74)§	**0.51** (0.30-0.86)§	**0.71** (0.56-0.91)
Energy				
Q2	**0.79** (0.66-0.95)	**0.79** (0.61-1.02)	**0.79** (0.50-1.27)	**0.79** (0.63-1.00)
Q3	**0.73** (0.61-0.89)	**0.62** (0.78-0.82)	**0.64** (0.40-1.04)	**0.83** (0.66-1.04)
Q4	**0.66** (0.54-0.80)§	**0.54** (0.40-0.71)§	**0.57** (0.35-0.95)	**0.77** (0.60-0.97)

*Adjusted for age, smoking, body mass index, and proxy respondent status.
†Fish: fresh, frozen, or canned.
§ *P* value for trend <0.01.

significance in females (Table 2). Odds of all lymph and blood system cancers were reduced by 37% and 34% for proportion of dietary fat and energy from fish, respectively. The risk reduction was even greater for leukemia (45%).

It should be noted that cases and controls were similar in most respects except that control subjects were more likely to be current smokers and had a lower body mass index. Both groups tended to eat fish with similar frequency, but controls consumed less fat and energy overall, thus had a higher proportion of total fat and energy from fish. We note, too, that fat intakes of respondents seemed unusually low, averaging 51 to 55 g/day in the four groups. Comparatively few subjects consumed smoked, dried or salted fish. In the ones that did, a significant protective association was observed only for total lymph and blood system cancers.

This study contributes important information to the literature on fish consumption and reduced risk of cancer, particularly for th main cancers of the lymph and blood system. Fritschi and colleagues reported a significant inverse association between increased fish consumption and reduced likelihood of all three lymph and blood system cancers examined, particularly leukemia in men.

Fritschi L, Ambrosini GL, Kliewer EV, Johnson KC; Canadian Cancer Registries Epidemiologic Research Group. Dietary fish intake and risk of leukaemia, multiple myeloma, and non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev* 2004;13:532-537.

Short Takes

Review: Long-chain Omega-3s in Cancer Prevention

Ecologic studies have generally reported an inverse association between fish consumption and risk of breast, prostate, and some other cancers. Associations between the consumption of fish or long-chain omega-3 fatty acids (n-3 LC-PUFAs) and various cancers from case-control and cohort studies, however, have been inconsistent. Animal and laboratory studies have generally shown suppression of cancer by these fatty acids. This review describes various mechanisms by which n-3 LC-PUFAs may inhibit or retard cancer development and suggests reasons for the inconsistency of epidemiologic findings. Failure to consider the competing effects of dietary omega-6 (n-6) fatty acids, differences in n-3 LC-PUFA consumption versus total n-3 PUFA intake, and generally low intake of n-3 LC-PUFAs in Western countries are among many considerations that may work against detecting a protective association between fish or n-3 LC-PUFA intake and cancer.

Most of the potential mechanisms by which n-3 LC-PUFAs may inhibit cancer development occur in the early stages of the disease during promotion and progression. These mechanisms include inhibition of arachidonic acid-derived eicosanoid synthesis; regulation of transcription factor activity, such as the peroxisome proliferator-activated receptor; regulation of gene expression for this receptor; altered estrogen metabolism; and changes in the production of reactive oxygen species and free radicals, substances that harm cells.

The authors present a hypothetical scheme to illustrate potential regulatory influences of n-3 and n-6 LC-PUFAs on the promotion or suppression of cancer. This review, and a previous one by the same group, collect and discuss the evidence for the involvement of LC-PUFAs in different types of cancer. The evidence presented suggests that the balance between n-6 and n-3 fatty acids may be even more important than commonly thought. The possible effects of intermediates in the metabolic pathways of LC-PUFAs remain to be added to this emerging canvas.

Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. *Am J Clin Nutr* 2004;79:935-945.

Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. *Am J Clin Nutr* 2003;77:532-543.

Gamma-Linolenic Acid Exerts Anti-Estrogenic Effects in Hormone Sensitive Breast Cancer Cells

Regular consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) has been associated with reduced incidence of some cancers, especially of the breast and prostate. The omega-6 LC-PUFA, gamma-linolenic acid, an intermediate in the conversion of linoleic acid to arachidonic acid, has also been linked to suppression of estrogen-dependent tumor growth in breast cancer cells, and improved liver function in patients with liver cancer. Gamma-linolenic acid has been further shown to augment the effect of tamoxifen, an anti-estrogenic drug used to inhibit the recurrence of breast cancer, in reducing the expression of estrogen receptors in breast cancer patients.

In this lengthy letter to the editor, Menendez and colleagues described their studies and literature findings on the myriad of anti-estrogenic effects of gamma-linolenic acid in various models of hormone sensitive breast cancer in humans. They presented evidence that gamma-linolenic acid blocked the estradiol-dependent activation of estrogen receptors. Further, it inhibited the expression of estrogen dependent genes, such as the cancer-promoting gene *c-fos*. Both gamma-linolenic acid and tamoxifen reduced the synthesis of a key estrogen receptor protein by interfering with the translation of the gene for this protein. Tamoxifen combined with gamma-linolenic acid reduced gene expression more than either agent by itself, and could completely inhibit gene expression in the cultured cells.

The authors presented additional evidence for the effects of gamma-linolenic acid in suppressing estrogen receptor production and activity and for enhancing the effectiveness of anti-estrogenic substances such as tamoxifen. They previously showed that gamma-linolenic acid enhanced the effectiveness of docetaxel and other chemotherapeutic agents used to treat breast cancer. The evidence summarized in this letter suggests several ways in which gamma-linolenic acid may reduce the onset, growth, and recurrence of breast cancer. Regarding the latter, it may enhance the effectiveness of current pharmacological treatments without incurring nasty side effects.

Menendez JA, Colomer R, Lupu R. ω-6 Polyunsaturated fatty acid y-linolenic acid (18:3n-6) is a selective estrogen-response modulator in human breast cancer cells: y-linolenic acid antagonizes estrogen receptor-dependent transcriptional activity, transcriptionally represses estrogen receptor expression and synergistically enhances tamoxifen and ICI 182,780 (Faslodex) efficacy in human breast cancer cells. Letter. *Int J Cancer* 2004;109:949-954.

Clinical Conditions: Asthma

Two Omega-6, but No Omega-3, PUFAs Associated with Asthma Risk in Adults

Consumption of fatty fish or fish oils containing long-chain omega-3 fatty acids (n-3 LC-PUFAs) has been associated with reduced symptoms in several immune and inflammatory conditions including asthma, but results have been inconsistent and modest. Consumption of fish, especially fatty fish, has been associated with reduced risk of asthma and lower prevalence of wheeze in children. However, a review of controlled clinical trials of dietary n-3 PUFAs and asthma outcomes did not find significant differences between diets low and high in n-3 PUFAs. In this report, researchers at Monash University and the Alfred Hospital in Melbourne, Australia, investigated whether plasma concentrations of n-3 LC-PUFAs were associated with the prevalence of asthma or atopy (allergic reactions) in adults aged 20-44 years (mean age 34.6 ± 7.1 years).

Subjects were selected randomly from the Melbourne area electoral roll and screened by questionnaire for presence of asthma and allergies. A total of 3,194 respondents completed the screening questionnaire and 1,601 subjects completed the respiratory questionnaire. Blood samples for fatty acid measurements in plasma phospholipids were obtained from 1,090 subjects. Forty-one subjects reported taking fatty acid or fish oil supplements within the previous six months and were excluded from the analyses. The final sample consisted of 1,049 subjects, of whom 986 (94.0%) currently had asthma or clinically confirmed bronchial hyperreactivity and 63.3% had allergies (atopy), as determined by a wheal 3 mm or greater in response to skin prick testing of any of 12 airborne or food allergens.

Table 1. Adjusted* odds ratios and (95% confidence intervals) for associations between plasma fatty acids (per 1% increase) and asthma or atopy in young adults.

Plasma Fatty Acid	Current asthma (n=986)	Any asthma in past yr (n=1049)	Dr. diagnosed asthma (n=1049)	Atopy (n=1049)
n-6 PUFAs				
Eicosadienoic acid, 20:2	**2.5** (0.24-26.06)	**10.23** (1.42-73.46)†	**11.10** (1.64-75.61)†	**1.23** (0.20-7.42)
Dihomo-gamma-linolenic acid, 20:3	**1.30** (1.06-1.60)†	**1.34** (1.13-1.60)†	**1.25** (1.06-1.48)†	**1.06** (0.91-1.24)
Arachidonic acid, 20:4	**0.97** (0.88-1.06)	**1.05** (0.98-1.14)	**0.94** (0.87-1.02)	**0.97** (0.91-1.04)
n-3 PUFAs				
Alpha-linolenic acid, 18:3	**0.72** (0.09-5.84)	**0.14** (0.02-0.86)†	**1.05** (0.19-5.83)	**1.23** (0.24-6.16)
Eicosapentaenoic acid, 20:5	**1.01** (0.71-1.45)	**1.00** (0.75-1.34)	**0.94** (0.71-1.26)	**0.98** (0.76-1.26)
Docosahexaenoic acid, 22:6	**0.96** (0.86-1.07)	**1.05** (0.96-1.15)	**0.97** (0.89-1.06)	**0.96** (0.88-1.04)

* Adjusted for age, sex, smoking, body mass index, region of birth, family history of asthma, total energy intake.

† $P<0.05$

Subjects were separated into four categories according to whether they met definitions for atopy as defined above, current asthma (self-reported wheeze in previous 12 months, plus bronchial hyperreactivity in response to methacholine (a drug), asthma (any asthma attack or use of asthma medicine in past 12 months), and doctor-diagnosed asthma (ever had asthma and had asthma confirmed by a doctor). Subjects who did not meet any of these definitions served as controls. Plasma fatty acids were compared between the four categories of cases and controls, and odds ratios calculated for associations between inflammatory condition and plasma fatty acids.

Cases did not differ significantly from controls in any plasma fatty acid measurements. No associations between n-3 LC-PUFAs or the ratio of n-6 to n-3 PUFAs and any category of asthma or atopy were observed. However, the odds ratios for all categories of asthma, but not for atopy, were significantly associated with plasma levels of dihomo-gamma-linolenic acid, an omega-6 fatty acid intermediate in the conversion of linoleic acid to arachidonic acid (Table 1). These odds ratios remained significant when the analysis controlled for potential confounding factors.

Odds ratios associated with another omega-6 fatty acid, eicosadienoic acid (20:2n-6) were greatly increased in all categories of asthma subjects, except those with current asthma. Eicosadienoic acid is the direct elongation product of linoleic acid and is present only in very small amounts in tissues and foods. However, the apparent effect of alpha-linolenic acid in subjects reporting asthma in the past year was not observed in any other category of asthmatics and included wide variation among subjects. This may be a statistical anomaly. No fatty acids were associated with allergic responses in adjusted analysis.

These findings add to the literature casting doubt on the association between n-3 LC-PUFAs and asthma, but suggest instead that dihomo-gamma-linolenic acid increases risk of asthma. Even though the odds ratio

was greatly increased for eicosadienoic acid, it is difficult to attribute biological significance to this observation, given the rarity of the fatty acid in biological systems. The dramatic increase in odds of developing asthma associated with this fatty acid, however, calls for examining this observation more closely.

Woods RK, Raven JM, Walters EH, Abramson MJ, Thien FC. Fatty acid levels and risk of asthma in young adults. *Thorax* 2004;59:105-110.

Frontiers

Urgently Needed: Brain, Not Brawn

For several decades, Dr. Michael Crawford, Institute of Brain Chemistry, London Metropolitan University, U.K., has focused his research activities and penetrating insights on the involvement of fatty acids in brain development and evolution. In this thought-provoking paper, Crawford highlights the critical importance of long-chain polyunsaturated fatty acids (LC-PUFAs) in brain development and reproduction and offers a sinister warning: by the year 2020, the top three health burdens will be cardiovascular disease, perinatal conditions, and mental illnesses. All are linked to our distorted pattern of fatty acid consumption.

DHA is concentrated in the brain and photoreceptor cells of fish, amphibians, reptiles, birds and mammals, where it has been involved in neural signaling for the past 600 million years. DHA originated in plants (marine algae) and became involved in photoreception and neural systems during evolution. As Crawford notes, "The trick used by the first primitive air-breathing systems that originated in our own line, was to convert sunlight not into carbohydrate and proteins but into electricity and ultimately the brain." It was the availability of oxygen, not natural selection, that made possible the evolution of all 32 phyla [categories of animals] in a short period of geologic time, he asserts.

The brain and vision first evolved in the sea, according to Crawford. Oxygen made possible the genetic variation that developed to exploit the high energy oxygen-using systems. Fish required n-3 fatty acids for their reproduction, while flowering plants developed seeds rich in omega-6 (n-6) fatty acids. About the same time, mammals evolved, requiring n-6 fatty acids for their reproduction. The dominant essential fatty acids for vascular and placental development are the n-6 PUFAs.

As land-based animals grew larger, their brain capacity diminished relative to their body size. In humans, however, brain size remained 1.9% to 2.0% of body weight, similar to that of small mammals like the squirrel. Crawford believes the relative scarcity of DHA in the land-based food chain resulted in the collapse of relative brain size. He points out that animals deprived of essential fatty acids, including DHA, have reduced brain cell number and severe behavioral pathology. Further, n-3 LC-PUFAs in brain are involved in the regulation of over 107 genes, which would make them critical to brain development and function.

In just one century, average height, weight, and disease patterns in the United Kingdom and other western countries have changed more dramatically than can be explained by changes in the human genome. The protein composition of tissue membranes, which is genetically determined, has remained fixed, but lipid composition has changed with our nutritional environment. Consumption of lipids from the marine food chain has fallen, while intake of saturated fatty acids and n-6 food oils has escalated. An evolutionary diet probably had a 1:1 to 3:1 ratio of n-6 to n-3 fatty acids. Western diets today, at least in the United States, have an average ratio of 10:1, and in some individuals, may be as high as 25:1.

Crawford points out that fish-eating populations and those consuming predominantly wild foods have substantially lower rates of cardiovascular mortality and mental illness than non-fish eating populations. Both diseases increase with the adoption of Western dietary patterns. It is the type of fat, not the amount, that is key, as studies of the Greenland Inuit showed vividly. To restore a more healthful balance among fatty acid families, Crawford calls for the necessary investment to stop the plundering and pollution of marine resources and a refocus of our food supply on the patterns reflected in wild foods. If, like cardiovascular disease, mental illness rates soar in this century, the bell will toll further.

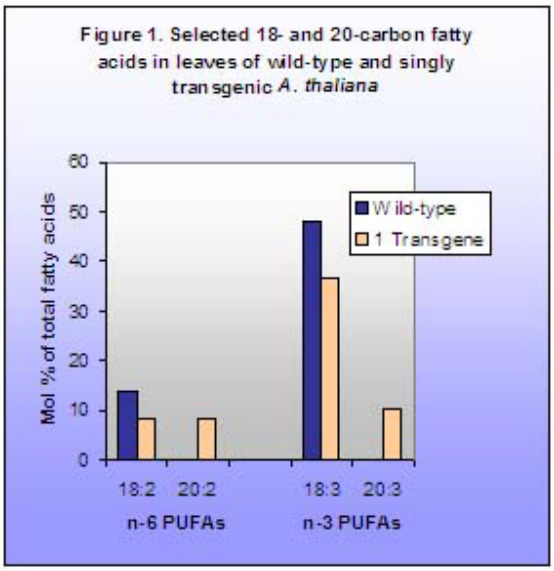
Crawford MA. Docosahexaenoic acid and the evolution of the brain--a message for the future. *Lipid Technology* 2004;16:53-56.

Long-Chain PUFAs Produced in Transgenic Cress, *Arabidopsis thaliana*

Figure 1. *Arabidopsis thaliana*. Photo © Thomas Schoepke, www.plant-pictures.com

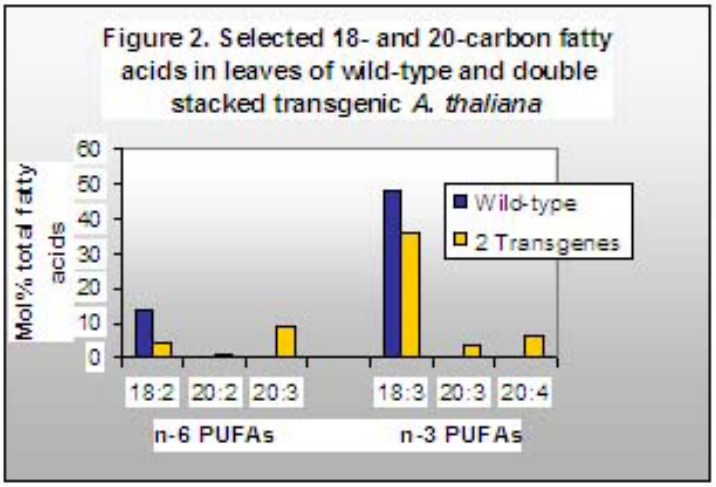
Applications of biotechnology to increase the production of long-chain polyunsaturated fatty acids (LC-PUFAs) in animals and plants have been reported in mice, canola, and now in mouseear cress (*Arabidopsis thaliana*). *A. thaliana*, the first plant to have its genome sequenced, is widely used in plant biotechnology research. Plants make the 18-carbon precursors of LC-PUFAs of both the omega-3 (n-3) and omega-6 (n-6) families, but are unable to synthesize the LC forms essential to the growth of several fish species and humans. Algae, however, can synthesize n-3 LC-PUFAs. Mammals convert the 18-carbon precursors, linoleic and alpha-linolenic acids, to 20- and 22-carbon LC-PUFAs, but conversion to n-3 LC-PUFAs occurs at a very low rate. Arachidonic acid appears to be sufficiently synthesized from dietary linoleic acid and is found in meats. Fish is the richest food source of n-3 LC-PUFAs.

In this report, the research team of Baoxiu Qi, then at the University of Bristol, U.K., transferred into *Arabidopsis* plants three genes encoding for different enzymes in the elongation and desaturation pathway that converts linoleic and alpha-linolenic acids to arachidonic and eicosapentaenoic acids, respectively. A unique aspect of their research is use of an alternative pathway for the synthesis of 20-carbon LC-PUFAs. The predominant pathway for the conversion of 18-carbon PUFAs to their 20- and 22-carbon PUFA derivatives begins with desaturation, the removal of two hydrogen atoms, followed by elongation of two carbons and a further desaturation step.

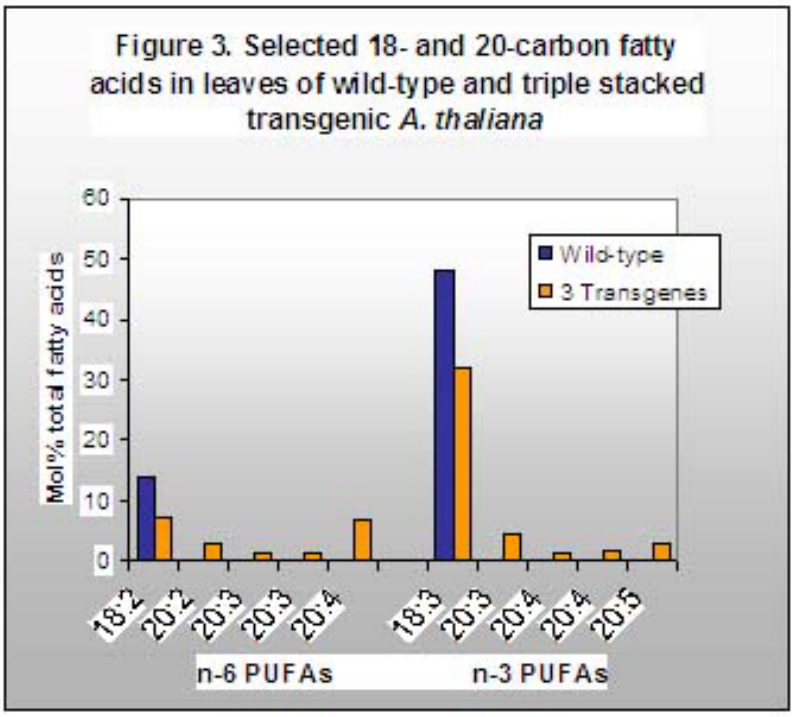


Qi and colleagues began by introducing into *A. thaliana* plants a gene from a marine microalga, *Isochrysis galbana*, that encodes for a delta-9-specific elongation enzyme. With this enzyme, the plants were able to convert linoleic and alpha-linolenic acids to their 20-carbon derivatives, eicosadienoic acid (20:2n-6) and eicosatrienoic acid (20:3n-3), respectively. These fatty acids were not present in the wild-type plants (Figure 1). The transgenic plants accumulated 8.4% and 10.4% mol percent of total fatty acids in 20:2 and 20:3, respectively, representing conversions of 51% and 22% of their 18-carbon parent compounds.

After growing a stable line of these transformed plants, the researchers next inserted a gene from the microscopic pond organism, *Euglena gracilis*, which encodes



for the enzyme delta-8 desaturase, thereby adding another double bond to the fatty acid. With this second genetic transformation, the *A. thaliana* plants produced the fatty acids dihomo-gamma-linolenic acid (20:3n-6) and eicosatetraenoic acid (20:4n-3), respectively (Figure 2). Synthesis of these fatty acids represented a conversion of 88% and 63% of their respective 20-carbon precursors.



In the third genetic transformation, a gene from the fungus, *Mortierella alpina*, which encodes for the enzyme delta-5 desaturase, enabled the plants to introduce a second double bond into the 20-carbon fatty acids, converting them to arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3), respectively (Figure 3). These triple stacked transgenic plants produced four fatty acids not detected in the double stacked plants. Two of these fatty acids were arachidonic and eicosapentaenoic acids. The other two were identified as sciadonic and juniperonic acids. The total content of 20-carbon fatty acids was 22 mol percent of all fatty acids. After three genetic transformations, the plants were visually indistinguishable from wild-type plants.

The relative amounts of these 20-carbon fatty acids compared with the wild-type *A. thaliana* plants after each genetic transformation are shown in Figures 1 to 3. Fatty acids with the same label have the same number of double bonds, but are located in different positions. Overall yields of arachidonic and eicosapentaenoic acids were 6.6 and 3.0 mol percent of total fatty acids, respectively, or 29% and 13% of all 20-carbon PUFAs. The investigators confirmed the identity of the fatty acid elution peaks by mass spectrometry.

The authors noted that the alternate elongation and desaturation pathway not normally present is viable in higher plants and appears to be appreciably more efficient than the usual desaturation and elongation pathway characteristic of mammals and yeast. They suggested that the rate-limiting step in the alternate pathway is the first elongation step. Their results also showed that incorporation of LC-PUFAs into chloroplast and non-chloroplast membrane lipids had no adverse effects on plant growth and development. Therefore, application of the alternate pathway for producing 20-carbon PUFAs in oilseed crops might be an efficient and appropriate approach for enriching seed oils with these LC-PUFAs. Such a development would greatly expand the availability of LC-PUFAs in food and feed for human and animal nutrition. This would be quite an accomplishment.

Qi B, Fraser T, Mugford S, Dobson G, Sayanova O, Butler J, Napier JA, Stobart AK, Lazarus CM. Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. *Nature Biotechnology* 2004:22:739-745.

Letters to the Editor

Transgenic Mice: New Opportunities for Diet-Tissue-Disease Relationships

Editor:

Successful formation of transgenic mice expressing the fat-1 gene [reported] by Kang et al.[1] stimulates more study of the impact on health of n-6 and n-3 fats commonly present in animal and human food. The added gene gave dramatically less dominance of the 20-carbon n-6 arachidonic acid (AA, 20:4n-6) over the 20- and 22-carbon n-3 acids, eicosapentaenoic,

docosapentaenoic and docosahexaenoic (EPA, 20:5n-3; DPA, 22:5n-3; DHA, 22:6n-3). Ratios for these two types of highly unsaturated fatty acids (HUFA) in liver, kidney and heart were near 12 for wild type mice compared with about 1 for transgenic mice (presumably eating standard lab chow). Since laboratory rats fed different diets had n-6 HUFA/n-3 HUFA ratios in phospholipids from liver, plasma, and red cells below 1.0 (e.g., 0.40, 0.48, 0.69, 0.83)[2], an important next step is to see if such diets will create ratios below 0.1 in the transgenic mice.

Kang's results raise the question of what cultural considerations led the experimenters to create such a great predominance of arachidonic acid in the tissues of their experimental animals. A provocative, but unsupported, claim for an "ideal n-6/n-3 ratio of about 1"[1] was noted in the context of possible tissue-disease relationships for humans. However, HUFA analyses of healthy Japanese yielded ratios ranging from 0.50 to 0.69[3,4] well below those reported for the transgenic mice. In addition, the strong tissue-disease association reported for n-6 HUFA proportions with coronary heart disease[5] shows no ideal value. Rather, lower CHD mortality per 100,000 population parallels lower ratios of n-6/n-3 HUFA from USA (160 and 4) to Spain (80 and 1.5) to Japan (38 and 0.6) to Greenland (20 and 0.5)[5].

Production of transgenic domestic animals will likely provide future opportunities to balance n-3 and n-6 HUFAs in human tissues. Nevertheless, the need to better understand tissue-disease relationships in humans will continue to depend on rationally managing the diet-tissue relationship[6,7].

Rigorous handling of existing evidence shows that "ideal" ratios for human health are likely much lower than currently recognized by official committees[8]. For humans, as with mice, the results raise the important question of what cultural considerations are leading so many people to ingest diets that create a great predominance of arachidonic acid in tissue HUFAs. New opportunities for future changes from the current diet-tissue-disease relationships for humans should be welcomed.

William E. Lands, MD, PhD
College Park, MD

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Dr. Kang replies:

The objective of our study was to demonstrate the feasibility of converting n-6 to n-3 fatty acids in mammals by gene transfer, rather than define the optimal value of n-6/n-3 ratio. In the study, both transgenic and wild type mice were maintained on a diet containing 5% fat—safflower oil high in n-6 fatty acids (76% linoleic acid) but low in n-3 fatty acids (~0.1% of total fat supplied). Feeding such a high n-6/n-3 diet allowed us to readily identify the phenotype. Under this dietary regime, wild-type mice had little or no n-3 fatty acid and thereby a very high n-6/n-3 ratio in their tissues. With this as reference, even a small change in the ratio (conversion of n-6 to n-3) could be easily observed in the transgenic mice. According to our recent experiments, the conversion of n-6 to n-3 fatty acids in transgenic mice varies, dependent to some extent on the dietary ratio of n-6 to n-3 fatty acids—a higher n-6/n-3 ratio, a greater conversion.

The high n-6HUFA/n-3HUFA ratios in the tissues of our wild type mice were mainly due to the deficiency of n-3 fatty acids, rather than extremely high levels of n-6HUFA. (In fact, the tissue levels of arachidonic acid were comparable to or even lower than other laboratory animals). The n-6HUFA/n-3HUFA ratios (around 12) are not uncommon in humans (1), and much higher ratios have been found in some populations such as urban Indians (2) and even some Europeans and Americans (3). A high ratio is also found in tissues of farm animals fed with grains without supplementation with fishmeal or other n-3 feeds (4). The low ratios (<1) observed in the laboratory rat tissues, as mentioned by Dr. Lands, are because these animals had been fed a diet supplemented with n-3 fatty acids. In fact,

many rodent diets contain high levels of fish oil n-3 fatty acids. Of course, supplementation can make a difference. In contrast, our transgenic mice can balance their tissue n-6/n-3 ratios with no need of supplementation.

I agree that the diet-tissue-disease relationships are important and require intensive investigations. Although the optimal n-6/n-3 ratio remains to be defined, many lines of evidence support an ideal n-6/n-3 ratio of about 1 (1). Whether running to the other extreme of n-6/n-3 ratio (much lower than 1 as suggested by Lands) is necessary and practical is a question that requires future research. I hope that our fat-1 transgenic mice can contribute to the elucidation of the ideal n-6/n-3 ratio for good health.

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