# PUFA NEWSLETTER

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# Editorial

## PUFAs and Gene Expression

In February 2004, a fundamental constraint on omega-3 polyunsaturated fatty acid (PUFA) synthesis was breached. Using genetically engineered mice, Jing Kang and colleagues at the Massachusetts General Hospital, Boston, Mass., USA, showed that transgenic mice with the *fat-1* gene could synthesize the omega-3 (n-3) family of PUFAs from linoleic acid, an omega-6 (n-6) fatty acid. Normally, mammals cannot perform this fatty acid conversion. Even though the transgenic mice were fed a diet deficient in n-3 fatty acids, their tissues had abundant long-chain n-3 PUFAs. Compared with wild-type mice, the content of n-3 fatty acids was increased at the expense of n-6 fatty acids. These transgenic mice should prove useful for studying the functional effects of n-3 PUFA metabolism, and possibly increasing the long-chain n-3 fatty acid content of food animals. Kang's research is described in a section we've called Frontiers.

Another article linked genetic mutations to inflammatory responses in atherosclerosis. James Dwyer and colleagues at the University of Southern California, USA, studied subjects with genetic mutations affecting the enzyme 5-lipoxygenase. This enzyme converts arachidonic acid to leukotrienes, substances that mediate inflammation. Dwyer et al. wondered whether patients with altered enzyme expression had enhanced inflammatory responses in their carotid arteries. They reported that subjects carrying mutations had significantly greater carotid artery intima-media thickness than those with the common genotype. Further, those with mutations responded more severely to high levels of arachidonic and linoleic acids. Consumption of long-chain n-3 PUFAs abolished the effect on artery thickness.

This issue of the newsletter includes two reports on the effects of dietary alpha-linolenic acid. In a study of 4400 middle-aged subjects, increased consumption of alpha-linolenic acid was associated with significantly lower triglyceride levels. At the highest level of alpha-linolenic acid intake, a mean of 1.2 g/day, triglycerides were reduced by 17% compared with the lowest intake of 0.4 g/day. The literature is inconsistent on whether alpha-linolenic acid lowers triglycerides.

In a study from the Netherlands, the consumption of nearly 3 g/day alpha-linolenic acid did not increase plasma docosahexaenoic acid levels in pregnant women or their newborns and had no effect on maternal cognition during pregnancy. This report adds to the findings that alpha-linolenic acid is an ineffective means of increasing maternal and infant docosahexaenoic acid levels during fetal and infant development.

This issue also includes three reports on the effects of fish or n-3 LC-PUFA consumption on behavior and mental health. Readers will appreciate the challenge involved in establishing satisfactory experimental designs and controls in this research field.

We welcome your comments.

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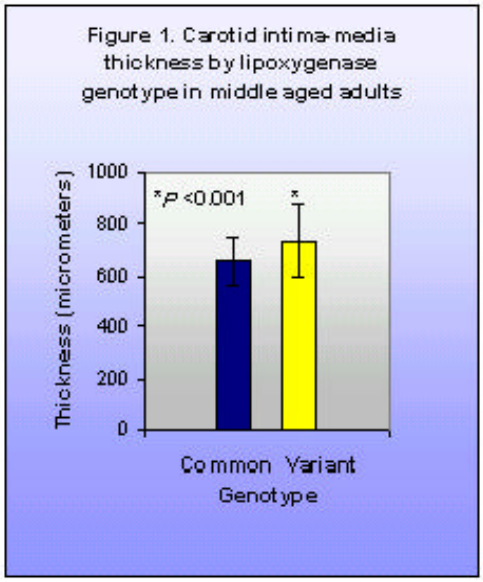
# Cardiovascular Health

## Links Between Dietary LC-PUFAs, Genetic Variability, Inflammation, and Atherosclerosis

A relative newcomer to the list of conditions that increase the risk of atherosclerosis and cardiovascular disease is inflammation. Markers of inflammation, especially highly sensitive C-reactive protein, are strongly associated with increased risk of cardiovascular disease and mortality. Anti-inflammatory substances such as long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) may reduce risk of atherosclerosis and coronary thrombosis by stabilizing atherosclerotic plaques in blood vessels and reducing the thickening of arterial walls. Studies often examine the thickness of the carotid artery in the neck using ultrasound because this artery is more accessible than coronary arteries.

Substances that elicit inflammatory responses are produced from the conversion of LC-PUFAs into a family of eicosanoids called leukotrienes. These products stimulate cells in the blood and blood vessel walls to make compounds that generate inflammatory responses. Leukotrienes produced from the omega-6 (n-6) LC-PUFA, arachidonic acid, are pro-inflammatory, whereas those produced from omega-3 (n-3) LC-PUFAs, mainly eicosapentaenoic acid (EPA), are only weakly inflammatory. Both families of fatty acids compete for the enzyme that produces leukotrienes. The activity of this key enzyme is critically involved in the inflammatory processes associated with atherosclerosis. Thus, LC-PUFAs may be a key link between diet, leukotriene production, and inflammatory responses.

The insightful question that Dwyer and colleagues at the University of Southern California, USA, asked was would people with increased production of leukotrienes that was genetically linked also have thicker artery walls and higher levels of C-reactive protein? To find out, Dwyer's group determined the genotype of the enzyme responsible for leukotriene production in a random sample of 470 healthy, middle-aged, men and women of diverse ethnicity enrolled in the Los Angeles Atherosclerosis Study. In addition, they measured carotid artery intima-media thickness, inflammatory markers such as plasma C-reactive protein, and dietary intake of n-6 and n-3 PUFAs.




In the sample, 6.0% or 28 subjects were carriers of two variant alleles for the leukotriene-producing enzyme. Variant genotypes were significantly more prevalent among blacks, Asians or Pacific Islander and other racial groups than in Hispanic or non-Hispanic whites ($P<0.001$). Those carrying the variant genotype had significantly thicker carotid artery intima-media than those with the common genotype (736 ± 141 m vs. 661 ± 95 m, respectively, mean ± sd, $P<0.001$) (Figure 1). Differences remained highly statistically significant in multivariate analysis, including up to 15 relevant covariables.

Highly sensitive C-reactive protein level was double in those with the variant genotypes (2.6 vs. 1.3 mg/L, $P=0.007$). Although plasma low-density lipoprotein (LDL) levels were similar in both groups, subjects with the highest LDL levels and the variant genotypes had greater carotid thickening than those who had the common genotype. For this reason, the authors concluded that LDL status was more atherogenic in subjects with the variant genotype.

Increased intima-media thickness was significantly and positively associated with the intake of arachidonic acid and linoleic acid, its metabolic parent, but only in subjects carrying the variant genes. Consumption of marine n-3 LC-PUFAs was significantly and negatively associated with reduced intima-media thickness only in those having the variant genes. Saturated and monounsaturated fatty acid intake was not related

to thickness, but at the highest third of saturated fat intake, carotid intima-media thickness was significantly higher in subjects with the variant genotype.

This investigation provides strong evidence for diet-gene interactions affecting leukotriene synthesis, inflammatory responses, and the development of atherosclerosis, as manifest by carotid arterial intima-media thickening in subjects with genetic variants affecting leukotriene production. These observations suggest that disorders of leukotriene production could stimulate or accelerate the development of atherosclerosis and that such disorders may be ameliorated by dietary n-3 LC-PUFA intervention and reduction in linoleic and arachidonic acids. The authors considered the atherogenic effect of the variant genes equivalent to type 2 diabetes, a disease that increases risk of cardiovascular disease by three times or more. Additional evidence from intervention studies linking disordered leukotriene production to increased development of atherosclerosis would leave little room to doubt the consequences of exaggerated inflammatory responses in cardiovascular disease.

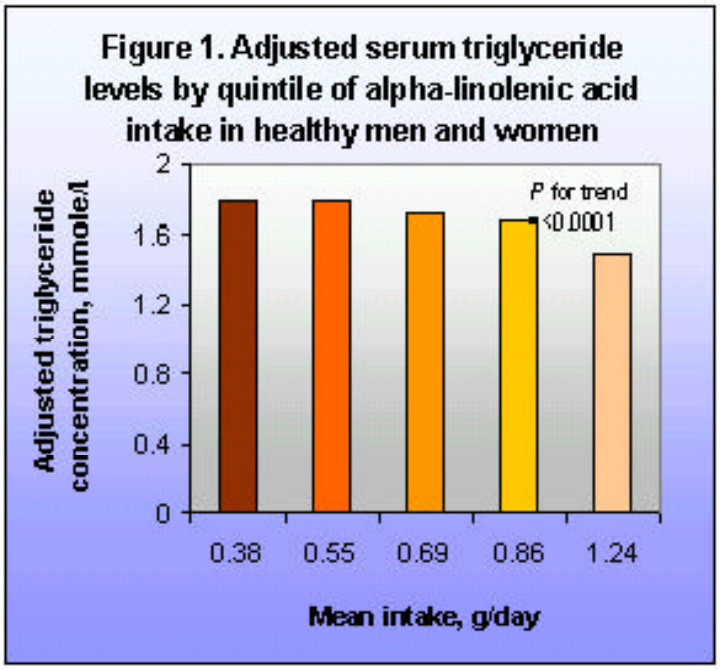
Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, Lusis AJ, Mehrabian M. Arachidonate –5 lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *New Eng J Med*. 2004;350:29-37.

De Caterina R, Zampolli A. From asthma to atherosclerosis–5-lipoxygenase, leukotrienes, and inflammation. (Editorial). *New Eng J Med*. 2004;350:4-7.

## Dietary Alpha-Linolenic Acid Associated with Lower Serum Triglycerides in Adults

Consumption of alpha-linolenic acid has been associated with lower risk of coronary artery disease and mortality in some epidemiological studies, but it is not known whether alpha-linolenic acid itself, or its long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) derivatives are responsible for the reduced risk. The question is important because of the public health implications for increasing the dietary availability of different n-3 PUFAs.

Many studies have reported that consumption of alpha-linolenic acid is without effect on circulating triglyceride levels, whereas n-3 LC-PUFAs are widely known for their triglyceride-lowering effect. A recent trial using alpha-linolenic acid-enriched margarine resulted in increased triglyceride concentrations after two years. This question was examined by Djousse and colleagues at Boston University, USA, using data from a large multi-center, population-based study designed to evaluate genetic and nongenetic determinants of cardiovascular disease and its risk factors. Subjects were participants in the National Heart Lung and Blood Institute's Family Heart Study and included 4440 white men and women whose average ages were 52 ± 14 years. Subjects included those chosen at random and those at high-risk for coronary artery disease. Dietary consumption of alpha-linolenic acid was determined by a semiquantitative, validated, food-frequency questionnaire and was estimated over the previous year. Fasting blood samples were obtained on the first clinic visit.




Mean daily consumption of alpha-linolenic acid ranged from 0.13 to 3.48 g/day, with an average of 0.81 ± 0.35 and 0.69 ± 0.29 g/day for men and women, respectively. Consumption of alpha-linolenic acid was divided into quintiles and related to serum triglyceride levels after adjusting for confounding variables (Figure 1). Total alpha-linolenic acid intake was inversely and significantly related to serum triglyceride levels. Triglycerides were not related to total cholesterol, low- or high-density lipoprotein cholesterol levels. The relationship was independent of fish consumption.

This report adds useful data to the collected findings on the effects of dietary alpha-linolenic acid, but does not explain the inconsistent findings on triglyceride levels of studies in which different amounts of alpha-linolenic acid have been consumed. Thus, the effect of alpha-linolenic acid on triglyceride levels remains an open question.

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Djousse L, Hunt SC, Arnett DK, Province MA, Eckfeldt JH, Ellison RC. Dietary linolenic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Clin Nutr*. 2003;78:1098-1102.

## Maternal and Infant Health

### Alpha-Linolenic Acid Supplementation in Pregnancy Has No Effect on Maternal Cognition or DHA in Maternal and Infant Plasma

The long-chain polyunsaturated fatty acids (LC-PUFAs), arachidonic acid and docosahexaenoic acid (DHA) of the omega-6 (n-6) and omega-3 (n-3) families, respectively, are important constituents of cell membranes, especially in the nervous system. Maternal stores, dietary intake, and potential conversion of 18-carbon fatty acid precursors provide these fatty acids to the developing fetus at a cost of reduced LC-PUFA status in the mother. Supplementing maternal diets with LC-PUFAs aims to ensure adequate supplies for fetal development and protect maternal stores. To date, most studies in pregnant women have emphasized DHA status and supplementation.

In two studies from Maastricht University, The Netherlands, de Groot and colleagues examined the effect of maternal consumption of alpha-linolenic acid, the 18-carbon precursor of n-3 LC-PUFAs, on maternal cognitive function and plasma fatty acid levels in mothers and infants. From the 14th week of pregnancy through eight months post-partum, women consumed margarine enriched with alpha-linolenic acid and linoleic acid. By using only alpha-linolenic acid as an addditional source of n-3 LC-PUFAs, the study indirectly addressed the question of the conversion of alpha-linolenic acid to its long-chain derivatives, particularly DHA.

The rationale for examining maternal cognitive function came from previous studies by the authors, which showed decreased cognitive function in women during pregnancy. Higher n-3 LC-PUFA status has also been linked with reduced likelihood of Alzheimer's disease in older subjects, in which memory and cognition are impaired.

Subjects included 56 healthy pregnant women of an average age of 29 to 30 years, whose gestational age was less than 14 weeks at enrolment. Women with high blood pressure, multiple pregnancy, high fish consumption, or who used LC-PUFA supplements were excluded. Alpha-linolenic acid was provided to 30 subjects by an enriched margarine containing 2.82 g and 9.02 g alpha-linolenic acid and linoleic acid, respectively, in a 25 g daily portion. Control subjects (26) consumed margarine that provided 0.03 and 10.94 g alpha-linolenic acid and linoleic acid, respectively, per 25 g daily portion. Five different cognitive tests were administered in five parallel versions randomly allocated to subjects at weeks 14, 17, 29, 36 and 32 weeks post-partum. Blood samples were collected at weeks 14, 26, 36, delivery and 32 weeks post-partum. Neonatal fatty acids were measured in umbilical plasma and vessel walls.

Provision of alpha-linolenic acid during pregnancy from the 14th week of gestation through eight months post-partum had no effect on any measure of cognitive function. Supplementation significantly increased plasma alpha-linolenic acid levels in the experimental group from week 26 through delivery until eight months post-partum (Table 1). DHA and arachidonic acid levels were similar in the two groups throughout the study and both declined until delivery. DHA continued to decrease at eight months post-partum, but the change did not reach statistical significance. Osbond acid (docosapentaenoic acid, 22:5n-6), a specific biochemical marker of functional DHA status, increased in the control group during pregnancy and was significantly higher than in the experimental group at delivery. The authors interpreted this difference as indicative of a better functional DHA status in those consuming alpha-linolenic acid.

Table 1. Plasma PUFA levels (mean ± SD) during pregnancy and post partum in 56 pregnant women who consumed margarine during pregnancy enriched with 14.2% alpha-linolenic acid or <0.2% alpha-linolenic acid (control).

| Fatty acid %wt/wt | Control/ Experimental | Week 14 29/29 | Week 36 28/29 | Delivery 26/27 | 32 Wk Post-partum 28/28 |
|---|---|---|---|---|---|
| Alpha-linolenic acid[a] | C | 0.22 ± 0.03 | 0.21 ± 0.06 | 0.20 ± 0.07 | 0.19 ± 0.06 |
| | E | 0.22 ± 0.03 | 0.39 ± 0.13 | 0.33 ± 0.14 | 0.20 ± 0.07 |
| Arachidonic acid | C | 9.71 ± 1.73 | 8.25 ± 1.22 | 8.73 ± 1.26 | 10.10 ± 1.71 |
| | E | 9.56 ± 1.70 | 7.80 ± 1.51 | 8.19 ± 1.36 | 9.54 ± 1.93 |
| DHA | C | 3.97 ± 0.85 | 3.64 ± 0.92 | 3.45 ± 0.84 | 3.10 ± 1.01 |
| | E | 4.48 ± 1.01 | 3.95 ± 0.87 | 3.94 ± 0.96 | 3.10 ± 0.80 |
| Osbond acid[b] (22:5n-6) | C | 0.36 ± 0.16 | 0.44 ± 0.14 | 0.47 ± 0.19 | 0.21 ± 0.09 |
| | E | 0.30 ± 0.08 | 0.30 ± 0.11 | 0.33 ± 0.11 | 0.21 ± 0.08 |

[a]Significant difference between groups at weeks 26 (not shown), 36, delivery, and 32 weeks post partum ($P$=0.05)
[b]Significant difference between groups at weeks 26 (not shown), 36, and delivery ($P$=0.05)

In the infants, there was no difference in DHA or alpha-linolenic acid concentrations in plasma or vessel wall phospholipids between experimental and control groups (Table 2). However, plasma eicosapentaenoic acid and vessel wall docosapentaenoic acid levels, both n-3 LC-PUFAs, were significantly higher in the infants of mothers consuming alpha-linolenic acid. Osbond acid was lower in both plasma and vessel walls of infants whose mothers consumed alpha-linolenic acid, but this difference was statistically significant only for the arterial wall phospholipids.

This study has not eliminated the possibility that n-3 LC-PUFA may affect maternal cognitive function during pregnancy, but it has shown that alpha-linolenic acid is ineffective in improving maternal DHA plasma level and in preventing the loss of DHA that occurs during pregnancy. Further, it showed that pregnant women apparently do not convert dietary alpha-linolenic acid to DHA efficiently or effectively. Interestingly, supplemental alpha-linolenic acid prevented the increase in maternal and infant plasma Osbond acid that results when consumption of n-3 PUFAs is low.

Table 2. Neonatal phospholipid-associated fatty acid concentrations (mean ± SD) in infants of mothers consuming control or alpha-linolenic acid-enriched margarine.

| Fatty acid | Control/ Experimental[a] | Plasma *mg/L* | *Arterial wall mg/kg tissue* |
|---|---|---|---|
| Alpha-linolenic acid | C | ND | 0.11 ± 0.03 |
| | E | ND | 0.12 ± 0.03 |
| Arachidonic acid | C | 17.34 ± 1.35 | 13.23 ± 2.20 |
| | E | 16.05 ± 1.43[b] | 12.89 ± 1.60 |
| Eicosapentaenoic acid | C | 0.13 ± 0.10 | ND |
| | E | 0.26 ± 0.12[c] | ND |
| Docosapentaenoic acid | C | 0.41 ± 0.29 | 0.19 ± 0.08 |
| | E | 0.51 ± 0.21 | 0.29 ± 0.09[c] |
| Docosahexaenoic acid | C | 5.37 ± 1.39 | 4.84 ± 0.87 |
| | E | 5.65 ± 1.47 | 5.21 ± 0.94 |
| Osbond acid (22:5n-6) | C | 0.71 ± 0.32 | 3.10 ± 0.53 |
| | E | 0.58 ± 0.23 | 2.70 ± 0.46[d] |

[a]Control subjects for plasma and arterial wall = 26 and 28, respectively, experimental subjects = 28 in both
[b]Significantly different from control, $P$<0.01
[c]Significantly different from control, $P$<0.001
[d]Significantly different from control, $P$<0.05

de Groot RH, Adam J, Jolles J, Hornstra G. Alpha-linolenic acid supplementation during human pregnancy does not effect cognitive functioning. *Prostaglandins Leukot Essent Fatty Acids.* 2004;70:41-47.

de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of á-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. *Am J Clin Nutr*. 2004;79:251-260.

## Fish Oil Consumed in Pregnancy Reduces Immune and Clinical Responses to Allergens in Infants Predisposed to Allergies

The anti-inflammatory properties of fish oils and other long-chain polyunsaturated fatty acids (LC-PUFAs) have been used to treat people with various immune and inflammatory disorders such as rheumatoid arthritis, and in infants at high risk for developing allergies. Fish oil consumption has been associated with reduced levels of some substances that promote inflammatory responses, but clinical improvements have tended to be modest. Intervention with anti-inflammatory agents such as omega-3 (n-3) LC-PUFAs in high risk subjects, prior to the development of allergic responses, might be a more effective approach. Could allergic conditions be thwarted before they develop?

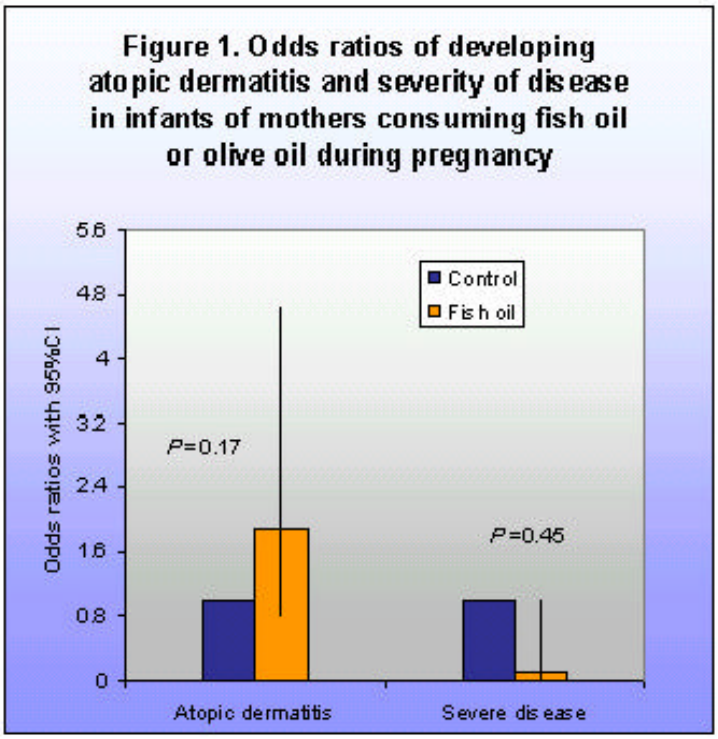
To find out, Dunstan and colleagues at the University of Western Australia examined the effect of n-3 LC-PUFA supplementation during pregnancy in women who had clinically diagnosed allergic conditions (asthma or allergic rhinitis) and were thus more likely to have infants at high risk of developing such conditions. They wanted to see if maternal n-3 LC-PUFA consumption would modify neonatal immune responses and the development of allergic symptoms at one year of age.

Ninety-eight pregnant women who consumed less than two fish meals/week were randomized to consume four fish oil capsules providing 3.7 g n-3 LC-PUFAs/day or 4.0 g/day olive oil capsules from 20 weeks gestation until delivery. Of the 98 women in the study, 83 mothers and infants completed the study, 40 in the fish oil group, and 43 in the control group.

Fish oil consumption was associated with a significantly higher proportion of n-3 LC-PUFAs in neonatal red blood cell membranes compared with infants of control mothers (17.8% ± 1.8% vs. 13.7% ± 1.2%, $P<0.001$). Total neonatal n-6 PUFAs were significantly lower in the infants of mothers consuming fish oil.

Investigators evaluated neonatal immune responses by measuring production of various cytokines – protein mediators of inflammation – in response to several common allergens such as house dust mite, egg albumin, and cat hair extract. Responses were examined in mononuclear cells (a fraction of white cells) from cord blood. In general, immune responses were weaker in fish oil neonates compared with controls, but differences were not statistically significant, with two exceptions. The cytokine interleukin-10 response to cat allergen was significantly lower in infants of fish oil mothers, and plasma levels of the cytokine interleukin-13 were significantly lower compared with control infants. When the data for all infants were combined, the relative amount of arachidonic acid in neonatal red blood cell membranes was positively associated with stronger interferon-gamma responses to three of the four allergens tested ($P<0.05$). In contrast, the n-3 LC-PUFA eicosapentaenoic acid was negatively associated with interferon-gamma responses to the PHA allergen ($P<0.01$; $P<0.05$ after adjustment for potential confounders). This suggests that arachidonic acid, an n-6 LC-PUFA, may facilitate some allergic reactions.

At one year of age, however, differences in allergic conditions between the two groups were more pronounced. Infants in the fish oil group were three times less



likely to be sensitized to egg allergen as determined by skin prick test. The odds ratio for sensitivity to egg allergen in the fish oil infants compared with the control infants was 0.34 (95% CI= 0.11-1.02, *P*=0.055). Among control infants, 37.8% were sensitized to egg albumin, while 17.1% in the fish oil group were sensitized. This difference represents a 55% risk reduction in egg sensitivity. Children in the fish oil group were consistently less likely to develop a range of allergy features such as wheezing, asthma, food allergy, or anaphylaxis compared with the control group, although differences were not statistically significant.

One exception to these findings was the chance of developing atopic dermatitis (Figure 1). Paradoxically, infants of mothers consuming fish oil were nearly twice as likely to develop atopic dermatitis as those of control mothers (odds ratio for the fish oil compared with control group infants was 1.88, 95% CI=0.77-4.65, *P*=0.17). However, children who did develop atopic dermatitis were 10 times less likely to have severe disease compared with the control children. Overall, susceptibility to various allergies was lower in the infants of fish oil mothers and responses were milder, although differences seldom achieved statistical significance. The study suggests that it may be possible to reduce the severity of certain allergic responses in infants predisposed to allergies before the conditions become fully established. Studies with more infants would be needed to verify and extend this possibility.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: A randomized, controlled trial. *J Allergy Clin Immunol*. 2003;112:1178-1184.

Dunstan JA, Mori TA, Barden A, Beilin LJ, Taylor AL, Holt PG, Prescott SL. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clin Exp Allergy*. 2003;33:442-448.

## Mental Health

### Is Fish Consumption Related to Hostile Behavior?

There is a growing literature describing the effects of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in various psychiatric and behavioral disorders including depression, Alzheimer's disease, bipolar disease, schizophrenia, and others. In children, some but not all reports have linked n-3 LC-PUFAs with improvements in attention deficit hyperactivity disorder and cognition. Some evidence has linked LC-PUFA status to personality characteristics such as aggressive behavior and hostility. The concentration of docosahexaenoic acid (DHA), one of two major n-3 LC-PUFAs, in neural cells and certain regions of the brain suggests the plausibility of a connection between LC-PUFA status and behavior.

Dr. Carlos Iribarren at Kaiser Permanente, Calif., USA, and colleagues at four other US medical institutions drew upon data collected in the CARDIA study, a longitudinal investigation of heart disease risk in black and white men and women 18 to 30 years of age at baseline in 1985-86. Information on personality characteristics using the Cook-Medley Scale and diet histories were collected at years five and seven, respectively. Fish consumption was estimated on the basis of a food frequency questionnaire and expressed as eating occasions per week for all fish consumed or those rich in n-3 LC-PUFAs.

Study participants were significantly heterogeneous across sex and race categories in all study variables. Mean hostility scores from highest to lowest were: black men, black women, white men, and white women, ranging from 20.9 to13.4 (*P*=0.0001). Total fish consumption did not differ across sex and race groups and averaged 1.4 occasions/week. However, black subjects consumed n-3-rich fish more frequently than white subjects and had significantly higher consumption of all n-3 fatty acids.

In bivariate analysis, total energy intake was consistently and positively correlated with hostility across sex and race groups, while alcohol consumption was significantly

and positively related to hostility in black subjects. Significant inverse associations were observed between hostility and the consumption of total n-3 PUFAs (black males only), linoleic acid (black males and overall), total n-6 PUFAs (black males and overall), alpha-linolenic acid (black males only), eicosapentaenoic acid (EPA, black population and overall), DHA (black males only), and eating occasions per week of n-3-rich fish (black males and overall). Except for total energy (r = <- 0.14 overall), most significant correlation coefficients were 0.10 or below, indicating that only 1% of the variation in hostility is explained by the respective variables. Part of the inverse association of hostility with n-3-rich fish was accounted for by the DHA content. Multivariate odds ratios for selected variables and the highest quartile of hostility score are shown in Table 1.

Table 1. Multivariate-adjusted odds ratios with 95% confidence intervals for the highest quartile of hostility score associated with one standard deviation increase in selected dietary variables in the CARDIA study, 1990-1991.

| Dietary variable | Odds ratio (95% CI) | *P* |
|---|---|---|
| Total energy (kcal) | 1.25 (1.15-1.35) | 0.0001 |
| DHA (kcal/1000 kcal) | 0.90 (0.82-0.98) | 0.02 |
| EPA (kcal/1000 kcal) | 0.93 (0.85-1.01) | 0.09 |
| Alpha-linolenic acid (kcal/1000 kcal) | 0.97 (0.89-1.05) | 0.43 |
| All n-6 fatty acids (kcal/1000 kcal) | 0.94 (0.86-1.02) | 0.11 |
| Alcohol (kcal/1000 kcal) | 1.09 (1.01-1.18) | 0.02 |
| All fish consumption, >0 but <2/wk | 0.94 (0.64-1.40 | NS |
| n-3-rich fish, >0 but <1/wk | 0.81 (0.68-0.97) | - |

This study is among the few to have examined whether n-3 LC-PUFAs may be associated with aggressive or hostile behavior, particularly in young adults. Any consumption of n-3-rich fish was an independent and significant predictor of reduced likelihood of a high level of hostile personality characteristics. Alpha-linolenic acid, EPA, and n-6 PUFAs were not significantly associated with the highest level of hostile characteristics. These findings warrant caution because of the observational nature of the study, the two-year difference in measurement of diet and personality characteristics, and the underlying assumptions about the measurement of hostility characteristics. However, they suggest a potentially useful area for more detailed and controlled investigation of the interaction of diet and behavior.

Iribarren C, Markovitz JH, Jacobs DR, Schreiner PJ, Daviglus M, Hibbeln JR. Dietary intake of n-3, n-6 fatty acids and fish: Relationship with hostility in young adults-the CARDIA study. *Eur J Clin Nutr*. 2004;58:24-31.

## Seafood Consumption Associated with Lower Lifetime Prevalence of Bipolar Disorders in 12 Countries

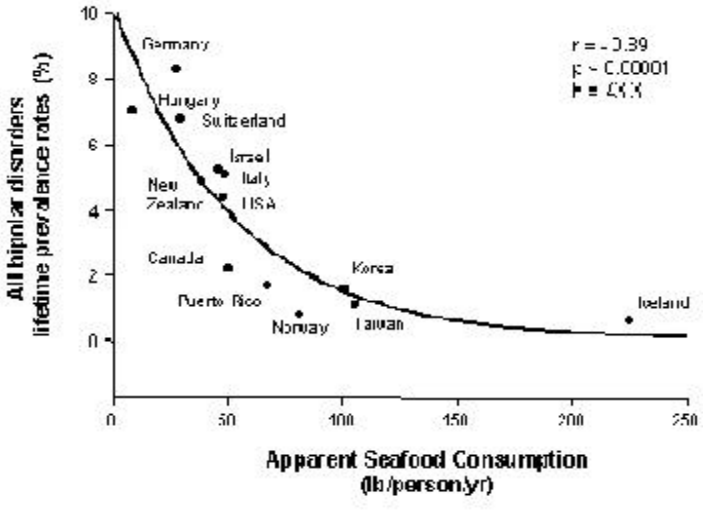
Expanding scientific literature describes a protective association between the risk of various mental disorders and the consumption of fish or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). Beneficial associations with n-3 LC-PUFA consumption have been reported for reduced risk of Alzheimer's disease, depression, and cognitive decline in older people. Treatment with these fatty acids has also been linked to improvements in patients with bipolar disorder. It is useful to ask, therefore, whether the occurrence of these disorders differs among countries having variable seafood intakes.

Noaghiul and Hibbeln from Columbia University and the National Institute on Alcohol Abuse and Alcoholism, USA, respectively, examined lifetime prevalence rates of bipolar disorders and schizophrenia from published epidemiological studies having large, clearly defined samples and comparable diagnostic and sampling procedures. Samples included people aged 18-64 years and were weighted for age and sex. Seafood consumption was estimated from national food balance data available from the United Nations' Food and Agriculture Organization. These data measure disappearance

of seafood from the market and serve as a surrogate for individual consumption. They do not account for distribution of fish intake within the population and other ways product can be lost from the marketplace. Thus, they are a crude approximation of intake. The investigators used the occurrence of schizophrenia as a control condition because prevalence rates of schizophrenia are not associated with seafood consumption.

Prevalence and seafood consumption (i.e., disappearance) data were available for up to 12 countries for different types of bipolar disorder. Prevalence rates of bipolar spectrum disorder ranged from 0.2 to 6.5 cases per 100,000 population, while apparent seafood consumption varied from about 6 lb/person/yr to about 225 lb/person/yr. Lifetime prevalence of all bipolar disorders and seafood consumption is shown in Figure 1. Lower prevalences of bipolar spectrum and bipolar II disorders were significantly correlated with higher seafood consumption (P= 0.02 and 0.04, respectively) using linear regression analysis. Using nonlinear analysis, all three types of bipolar disorder were even more strongly inversely correlated with seafood consumption. In contrast, prevalence of schizophrenia was unrelated to seafood consumption.

**Figure 1. All bipolar disorders, lifetime prevalence and seafood consumption by country**






The curvilinear relationship between seafood consumption and prevalence of bipolar disorders suggested a threshold level of consumption below which bipolar disorders increased sharply. This level was around 50 lb/person/year, which the authors calculated may represent as much as 300 g of seafood/person/day. More detailed dietary studies would be required to quantify possible threshold effects. Other cautions in interpreting these data include lack of adjustment for confounding variables, such as socioeconomic and marital status, alcoholism, smoking, family history, and other demographic factors. Data were unavailable to permit more refined statistical analysis.

Although cross-national studies cannot demonstrate causal relationships, this study included countries with diverse food habits and a wide range of seafood consumption. The study revealed a highly significant inverse correlation between lifetime prevalence of bipolar disorders and seafood intake. These observations are consistent with other studies reporting a significant relationship between low seafood consumption and increased rates of depressive or bipolar disorders.

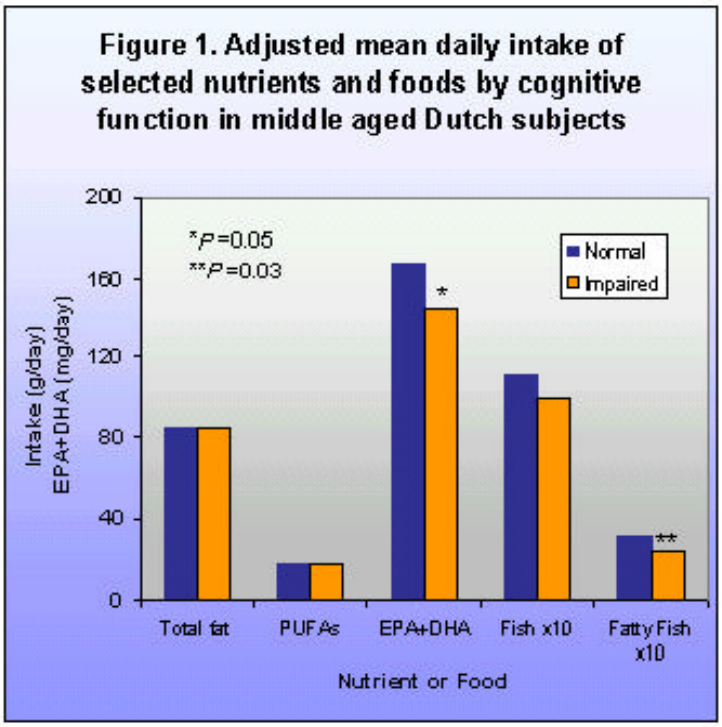
Noaghiul S, Hibbeln JR. Cross-national comparisons of seafood consumption and rates of bipolar disorders. *Am J Psychiatry* 2003;160:2222-2227.

## Fish Consumption Linked to Higher Cognitive Function in Middle Age

From prenatal life to old age, fish is proving to be "brain food." Not only are the long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in fish incorporated into the brain during development, their presence is associated with improved cognitive function and lower risk of mental impairment and diseases of aging. Fish consumption blurs the line between mental and physical health, as it benefits both.

In this report of a cross-sectional analysis or "snapshot" view of habitual fish consumption and cognitive function in middle aged subjects, investigators from Utrecht and Maastricht Universities and government research agencies in The Netherlands and the United States studied 1613 Dutch subjects, aged 45-70 years. They administered a battery of cognitive tests reflecting memory, psychomotor speed, cognitive flexibility, and overall cognition. Dietary fat intake and fish consumption were assessed by a food frequency questionnaire

from which the intake of n-3 LC-PUFAs was calculated. Diet was assessed upon enrolment and six years later at the time of cognitive testing. Subjects were divided into two groups on the basis of cognition scores.



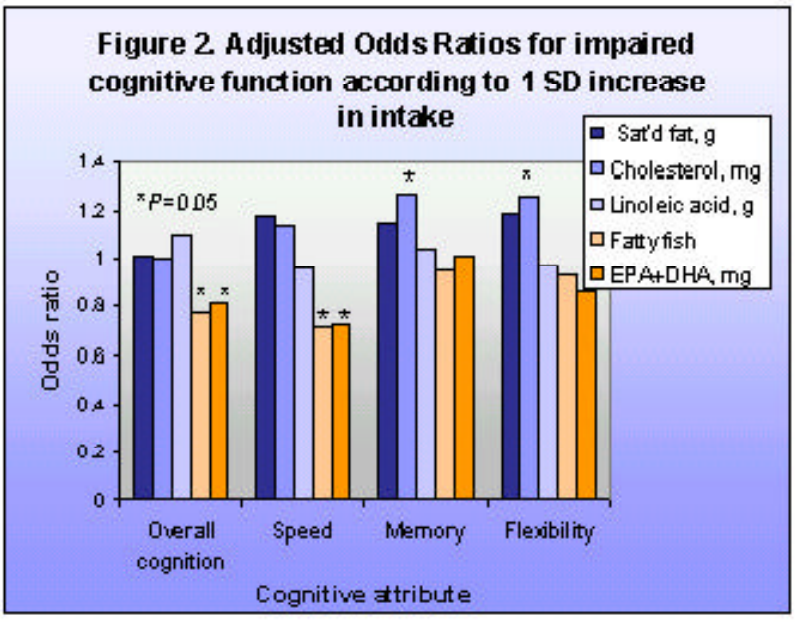

Those scoring the lowest 10% were defined as impaired. The sample included 1450 normal and 163 impaired subjects.

Compared with normal subjects, those in the lowest cognition category were predominantly men (58.6%, P=0.006) who had a history of heart disease (8.1% vs 4.3%, *P*=0.04), lower education (4.5% vs. 20.8%, *P*=<0.001), and were older (59.4 yr. vs. 56.2 yr, *P*=<0.001). Fatty fish consumption, (Figure 1), but not total fish intake was significantly lower among impaired subjects than normal ones (2.3 g/day vs. 3.06 g/day, *P*=0.03). Data were adjusted for age, sex, and education. Intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main n-3 LC-PUFAs, were also significantly less.

Over the six-year study, total fat consumption tended to increase in both groups while cholesterol intake tended to decline. The changes were not statistically significant.

Investigators calculated odds ratios for impaired cognitive performance according to an increase of one standard deviation in the intake of various lipids and fatty fish. The only variables significantly associated with lower odds of impaired overall cognition and speed of cognitive processes were fatty fish and EPA plus DHA consumption (Figure 2). Cholesterol and saturated fat intake were associated with increased odds of impaired cognitive function, but only cholesterol intake reached statistical significance for impaired memory and speed of cognitive processes. No other fat or fatty acid variables were linked to risk of cognitive impairment.

Cross-sectional studies – those that examine relationships at a single time point – say nothing about what happens long term. We know, however, that subjects with mild cognitive impairment are likely to advance to dementia or Alzheimer's disease, so factors that might prevent or delay such progression are of keen interest, especially as the proportion of older subjects is increasing. This study adds to those studies showing a link between moderate fish consumption and reduced risk of cognitive decline. Folk wisdom has long advised, "use it or lose it" to maintain mental function. One might add, "use it" to eat fish regularly.




***S. Kalmijn, van Boxtel MPJ, Ocké M, Verschuren WMM, Kromhout D, Launer LJ. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age.* Neurology *2004:62:275-280.***

### LC-PUFAs in Schizophrenia

Several lines of evidence suggest that long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) are involved in the development or treatment of schizophrenia. The quantity and quality of existing data leave considerable room for uncertainty (see also the Noaghiul study, reviewed above), and large well-controlled trials are lacking. Abnormalities in LC-PUFA content of cell membranes in schizophrenic patients have been reported and results from treatment studies using eicosapentaenoic acid (EPA) have been encouraging. Two of four randomized trials of treatment with EPA reported significant benefits and two did not. Methodological limitations plague much of this research. A Cochrane review in 2000 suggested a positive effect of EPA treatment, but warned of the limitations of the data.

Emsley and colleagues at the University of Stellenbosch, South Africa, provided an updated critical review of the literature and concluded that EPA remains to be tested properly as a "stand-alone" antipsychotic agent in schizophrenia. Perhaps this careful review will stimulate the support needed for a definitive trial of EPA or n-3 LC-PUFAs in this debilitating condition.

Emsley R, Oosthuizen P, van Rensburg SJ. Clinical potential of omega-3 fatty acids in the treatment of schizophrenia. *CNS Drugs* 2003;17:1081-1091.

## Clinical Conditions: Type 1 Diabetes

### Cod Liver Oil in First Year Reduces Risk of Type 1 Diabetes

Many readers remember taking cod liver oil as children, mainly to obtain vitamin D. The practice is still widespread in Norway, where much of the world's cod liver oil is produced. Today's emphasis is on the long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in cod liver oil, rather than the content of vitamins A and D. However, both n-3 LC-PUFAs and vitamin D, among other substances, have been implicated in the development of juvenile or type 1 diabetes, the most severe form of the disease.

In northern countries the consumption of supplemental vitamin D is recommended during winter months. In Norway, the need for vitamin D may be met with either cod liver oil or vitamin D supplements. From results of a pilot study that linked the consumption of cod liver oil during pregnancy or the child's first year of life with a lower risk of type 1 diabetes, Stene and colleagues at the Diabetes Research Centre and Ullevål University Hospital in Oslo conducted a country-wide case-control study of all children with type 1 diabetes born between 1985 and 1999. Three thousand randomly selected children born in the same period served as controls. Of all eligible subjects, 73% of cases and 56% of controls responded to the survey questionnaire, yielding a sample of 545 cases and 1668 controls. Odds ratios for development of type 1 diabetes were calculated in relation to several maternal and infant variables, including breastfeeding, introduction of solid foods, parent or sibling type 1 diabetes, and other descriptors. Not surprisingly, existence of type 1 diabetes in a parent or sibling increased the risk of the disease six- to seven-fold.

In contrast to the results of their earlier pilot study, consumption of cod liver oil or vitamin D supplements by mothers during pregnancy was not associated with the likelihood of having a child with type 1 diabetes. Infants who consumed cod liver oil during their first year of life were significantly less likely to develop type 1 diabetes than those who did not consume cod liver oil (Table 1). The odds ratios for type 1 diabetes were significantly lower regardless of whether potentially confounding factors, including family history of the disease, were included in the analyses. In addition, the age at which cod liver oil consumption was initiated appeared to have no effect on the incidence of diabetes. The consumption of vitamin D supplements was not significantly related.

Although other studies have reported an association between the consumption of vitamin D supplements and reduced risk of type1 diabetes, this study did not support such an association. Risk of type 1 diabetes has been associated with aberrations in prostaglandin metabolism and the authors speculate that n-3 LC-PUFAs may modify prostaglandin synthesis in ways that reduce the risk of type 1 diabetes. Would continued

Table 1. Use of cod liver oil or vitamin D supplements during the first year of life by infants with type 1 diabetes (cases) and infants without type 1 diabetes (controls).

| Supplement | Cod liver oil | | Vitamin D | | Odds Ratio for cod liver oil (95% CI) | |
|---|---|---|---|---|---|---|
| Frequency of use | Cases n=545 | Controls n=1668 | Cases n=545 | Controls n=1668 | Unadjusted | Adjusted |
| None | 318 | 834 | 171 | 628 | 1.00 | 1.00 |
| 1-4x/wk | 60 | 224 | 76 | 248 | 0.70 | 0.81 |
| ≥5x/wk | 137 | 553 | 235 | 693 | 0.65 | 0.74 |
| *P* for trend | | | | | <0.001 | 0.04 |

consumption of cod liver oil by these children sustain the reduced risk?

Stene LC, Joner G; Norwegian Childhood Diabetes Study Group. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr*. 2003;78:1128-1134.

## Age-Related Macular Degeneration

### Fish Consumption Linked to Lower Risk of Age-Related Macular Degeneration

Among thual loss can result. ance of Among the ravages of age, declining eyesight is a particular bane. A major cause of impaired vision and blindness in middle and late age is age-related macular degeneration. In the United States, an estimated 8 million people have intermediate stage macular degeneration, and if untreated, more than a million of these will develop advanced disease with severe loss of vision. Age-related macular degeneration is also a leading cause of blindness in Australia. Supplemental nutrition with antioxidant vitamins and zinc, along with the dietary carotenoids lutein and zeaxanthin, are associated with reduced risk. Increased fish consumption is also linked to lower risk.

As age-related macular degeneration progresses, treatment options become limited and irreversible visual loss can result. For this reason, prevention of the condition, as well as strategies to halt its progress are most urgent. Seddon and colleagues at the Massachusetts Eye and Ear Infirmary in Boston, USA, previously reported the benefits of particular dietary substances, including antioxidants and zinc, in reducing the risk of age-related macular degeneration. In this study, they evaluated the association between fat intake, specific types of fat, and certain foods including fish on the rates of progression of age-related macular degeneration in a population of white subjects 60 years or older with the disease. From 366 subjects enrolled, complete data were available from 261 whose mean age was 73 years.

Dietary intake was assessed using a modified food-frequency questionnaire adapted for use among elderly eye patients. Use of vitamins and supplements was recorded. Subjects were given annual dilated eye examinations. Demographic and lifestyle information was collected by a trained interviewer who was unaware of the subject's ocular status. The average followup time was 4.6 years during which 101 patients progressed to advanced disease status.

Subjects were divided into quartiles on the basis of fat intake and into tertiles according to frequency of fish consumption: less than once/week, once/week, and two or more times/week. Relative risks for disease progression were adjusted for age-sex group by decade, log energy, and quartile of protein intake. Additional adjustments for confounding variables were performed in several multivariate analyses.

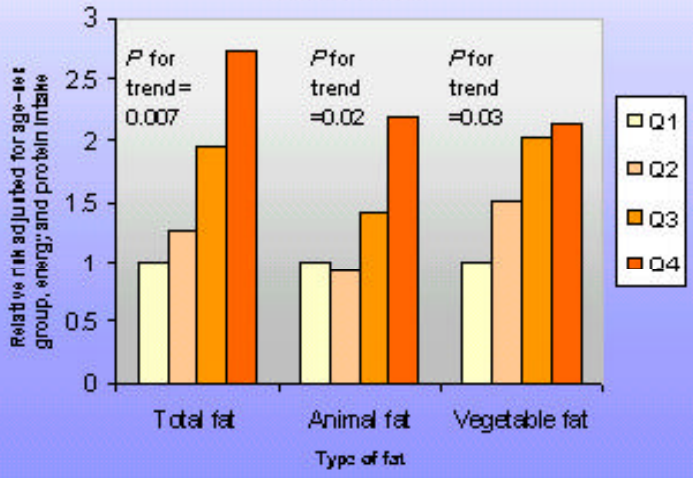
Median fat intake among the four quartiles ranged from 24.4 g/day to 70.1 g/day. Relative risk of disease progression was significantly associated with increased fat intake (*P* for trend = 0.007) with a relative risk in the highest fat group of 2.74 (Figure 1). When additional factors were included in the analysis, the increased risk for total fat intake remained significant at 2.90 (*P* for trend = 0.01, not shown). the most confounding variables. Trans fatty acids were strongly associated with an approximately 2.5-fold greater risk of disease progression in all statistical analyses (*P* for trend = <0.001 for adjusted relative risk).

What about fish consumption? When risk was analyzed by frequency of fish consumption there appeared to be no effect on risk of disease progression. However, when the effect of fish consumption was examined according to the intake of linoleic acid, the major n-6 PUFA in the diet, a protective effect of fish consumption emerged for those whose intake of linoleic acid was below the median (Figure 2). In patients whose intake of linoleic acid was greater than 4.9 g/day fish consumption had no effect. Current intake of linoleic acid in the United States has been estimated at 11-16 g/day. In Figure 2, it is assumed that 1 serving/week includes amounts greater than one but less than 2 servings/week.

These findings confirm earlier results from a pilot study and show that specific types of dietary fat and the ratio of n-6 to n-3 PUFAs can have a strong effect on the progression of age-related macular degeneration.

Monounsaturated and trans fatty acids, prevalent in processed and baked foods, were strongly linked with increased risk of disease progression.

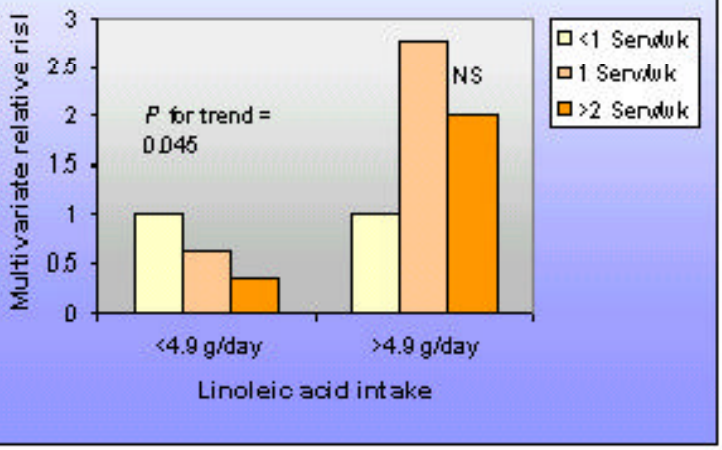
**Figure 1. Relative risk for progression of age-related macular degeneration in patients with moderate disease according to amount (quartiles) and type of fat consumed**




When type of fat was examined, risk of disease progression was significantly increased about two-fold at the highest level of both animal and vegetable fat consumption. Upon multivariate analysis the significance of animal fat disappeared, but for vegetable fat, risk of disease progression increased to nearly four-fold and was of stronger statistical significance (*P* for trend = 0.003).

With further detailed analysis according to families of fatty acids, a different picture emerged. First, saturated fatty acids were not significantly associated with risk. Monounsaturated fatty acids were significantly associated with a doubling of risk in all statistical analyses, but polyunsaturated fatty acids (PUFAs) were significantly associated with risk only in the model that controlled for

**Figure 2. Relative risk for progression of age-related macular degeneration in patients with moderate disease by fish consumption and linoleic acid intake**



Protective foods were fish and nuts, although fish was protective only when linoleic acid consumption was moderately low. This study has shown clearly that composition of dietary fat strongly influences the progression of age-related macular degeneration, but leaves open the question of its function in the onset of this disease. The study provides some evidence that current high intakes of linoleic acid may be linked to the development or progression of macular degeneration and may compromise the benefits associated with fish consumption. To see may be the key to believing.

Seddon JM, Cote J, Rosner B. Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake. *Arch Ophthalmol*. 2003;121:1728-1737.

## Attention, Hyperactivity, and Disruptive Behaviors

### PUFA Supplementation Improves Some Behaviors in Children with Attention and Activity Disorders

The most prevalent mental disorder in children is attention deficit/hyperactivity disorder, known commonly as AD/HD. Children with the condition have difficulty maintaining focus, finishing tasks, planning, and sitting still. Their fidgety behavior is disruptive in the classroom. This condition is estimated to affect as many as 2 million children in the United States.

Children with AD/HD may also have symptoms affecting skin and thirst. Forty percent of boys with AD/HD were reported to have significantly lower plasma phospholipid levels of arachidonic acid, a long-chain omega-6 polyunsaturated fatty acid (n-6 LC-PUFA) and docosahexaenoic acid (DHA), an omega-3 (n-3) LC-PUFA. These observations, and the participation of LC-PUFAs in central nervous system function and other mental disorders, suggest the involvement of LC-PUFAs in AD/HD. However, three trials using different LC-PUFA supplements in children with hyperactivity disorders reported mixed results. This pilot study using supplementation with mixed LC-PUFAs selected AD/HD subjects with skin or thirst symptoms, which suggest potential association with LC-PUFAs. Fifty healthy children with AD/HD, aged 6-13 years, were randomly assigned to consume 8 capsules/day containing LC-PUFAs and 3 mg vitamin E, or an olive oil placebo. Groups were balanced by gender and medication status. Children receiving the intervention were compared at baseline with a reference sample of 24 children without AD/HD. Each capsule contained 60 mg DHA, 10 mg eicosapentaenoic acid, 5 mg arachidonic acid, and 12 mg dihomo-gamma-linolenic acid. Blood samples were obtained at baseline, two months, and four months after intervention. Primary outcome measures of behavior assessed by parents and teachers were obtained at baseline and the end of intervention using the Conners' Abbreviated Symptom Questionnaires and the Disruptive Behavior Disorders Rating Scale. The former test is widely used and has high validity; the latter is phrased differently from the former and assesses hyperactivity, attention, conduct, and oppositional defiant behavior. Parents completed three-day diet records and evaluated thirst and skin symptoms by questionnaire for their children at baseline and after four months. Thirty-three subjects completed the study, 18 in the LC-PUFA group and 15 in the placebo group.

Placebo and LC-PUFA treatment groups were similar at baseline except for significantly lower performance measures in two of 17 behavioral assessments in the placebo group and one in the LC-PUFA group. Plasma and red blood cell fatty acid composition was significantly affected by LC-PUFA supplementation compared with the placebo group with significant increases in the concentration of total n-3 PUFAs and DHA at the end of four months in both plasma and red blood cells. In red cells, eicosapentaenoic acid level was also significantly increased at four months. Significant decreases in plasma and red cells in the LC-PUFA group were observed for arachidonic acid and the ratio of total n-6 PUFAs/n-3 PUFAs. In red cells, total n-6 PUFAs were also significantly diminished at four months compared with the placebo group. Duration of the study was a significant factor in all the fatty acid changes. In spite of the provision of arachidonic acid in the supplement, increased consumption of n-3 PUFAs was associated with significantly reduced arachidonic acid concentration in plasma and red cells. These changes are consistent with many other

reports on the effects of dietary n-3 PUFAs on blood and red cell lipids.

Findings from the behavioral and cognitive tests showed no effect of treatment, but there were significant changes over the 4-month period within each group. Parents' evaluations in both groups showed significant improvement in nearly all assessments, whereas teachers' assessments showed significant improvement only for attention within the LC-PUFA group. In the parents' evaluations, the greatest difference between placebo and LC-PUFA treated groups was observed in conduct (9.9% vs 42.7% in placebo and LC-PUFA groups) and this difference reached statistical significance ($P$=0.05) in intention-to-treat analysis.

It is always troubling when there are substantial changes in a control group because it reduces the effect of treatment and suggests that study participation itself has significant effects. Other factors may also cloud the results. However, as the authors point out, the size of the behavioral improvement scores, 10% to 43% in parents' evaluations, in both groups across diverse behavioral tests, suggests that LC-PUFAs may be linked to these changes. It was noted that some LC-PUFAs increased in the plasma of placebo and LC-PUFA subjects. The authors also noted that the behaviors that did improve significantly with LC-PUFAs, conduct and attention, are highly valued. Further, the authors suggested several potential confounding variables that could be controlled using more rigorous criteria.

Stevens L, Zhang W, Peck L, Kuczek T, Grevstad N, Mahon A, Zentall SS, Arnold LE, Burgess JR. EFA supplementation in children with inattention, hyperactivity, and other disruptive behaviors. *Lipids*. 2003;38:1007-1021.

## FRONTIERS

### Genetically Modified Mice Synthesize Long-Chain Omega-3 PUFAs from Omega-6 PUFAs

Researchers Jing Kang and colleagues at the MasResearchers Jing Kang and colleagues at the Massachusetts General Hospital, Boston, USA, used molecular biological techniques to give mice the ability to make long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) from omega-6 fatty acids. This is a critical biochemical step that mammals cannot naturally do. For that reason, n-3 PUFAs, which are essential for normal human development, must be obtained from the diet.

In this landmark application of transgenic biotechnology, Kang and colleagues transferred the *fat-1* gene that encodes an n-3 fatty acid desaturase enzyme from the roundworm *C. elegans* into mice. Mammals lack this enzyme. They fed the transgenic mice and comparable unmodified (wild-type) mice a diet high in n-6 PUFAs, but deficient in n-3 PUFAs, for eight weeks. At the end of the feeding period several body tissues were analyzed for n-6 and n-3 fatty acids. The authors calculated the ratio of n-6 to n-3 PUFAs, including the 18-carbon PUFAs, linoleic acid (n-6) and alpha-linolenic acid (n-3). Their paper showed a sample tracing of the fatty acid analyses to illustrate the increase in n-3 fatty acids at the expense of n-6 fatty acids in the transgenic mice compared with the wild-type mice.

When the total n-6 to n-3 fatty acid ratios in the wild-type and transgenic mice were compared, the ratios in all tissues of the transgenic mice were significantly reduced compared with the wild mice (Table 1). When just the LC-PUFA ratios were compared, the same pattern was observed. Wild-type mice contained about 98% of their PUFAs as linoleic acid, an n-6 PUFA. However, milk from transgenic mothers contained a higher ratio of arachidonic acid to n-3 LC-PUFAs than other tissues, suggesting that availability of this important fatty acid may not have been compromised by the transgene. Without the actual fatty acid values, one cannot determine whether this is so.

Change in the n-6 to n-3 ratios in the transgenic mice resulted from a decrease in linoleic and arachidonic acids and an increase in all n-3 PUFAs. The authors noted that in all tissues except skeletal muscle, DHA was the predominant n-3 LC-PUFA. In muscle, eicosapentaenoic acid was the main n-3 LC-PUFA. The investigators also reported that n-3 LC-PUFAs were consistently raised in four generations of transgenic mice bred from one or both parents with the transgene

Table 1. Ratios of n-6:n-3 PUFAs in wild-type and transgenic mice carrying the *fat-1* gene for n-3 fatty acid desaturase.

| TISSUE | Ratio n-6:n-3 PUFAs* | | Ratio AA:n-3 LC-PUFAs† | |
|---|---|---|---|---|
| | *Wild* | *Transgenic* | *Wild* | *Transgenic* |
| Skeletal muscle | 49.0 | 0.7 | 11.3 | 0.4 |
| Red blood cell | 46.6 | 2.9 | 27.0 | 1.6 |
| Heart | 22.8 | 1.8 | 14.3 | 0.9 |
| Brain | 3.9 | 0.8 | 3.6 | 0.7 |
| Liver | 26.0 | 2.5 | 12.5 | 0.9 |
| Kidney | 16.5 | 1.7 | 11.9 | 1.2 |
| Lung | 32.3 | 2.2 | 19.8 | 1.2 |
| Spleen | 23.8 | 2.4 | 17.3 | 1.5 |
| Milk§ | 32.7 | 5.7 | 15.7 | 2.5 |

*n-6 fatty acids include n-6 18:2, 20:4, 22:4, 22:5; n-3 fatty acids include 18:3, 20:5, 22:5, 22:6

†Ratio based on AA (20:4n-6) to 20:5 (EPA)+22:5 (DPA)+22:6 (DHA)

§Milk obtained from stomach contents of 5-day old mice born to wild or transgenic mothers

and that the mice were healthy. How the fatty acid patterns would be affected by a diet that included n-3 PUFAs remains to be determined.

Transfer of the *fat-1* gene overcomes a major metabolic block to the availability of n-3 LC-PUFAs in mammals by enabling them to convert n-6 PUFAs to n-3 PUFAs. The implication of this transformation is that food animals might be developed with the ability to convert dietary n-6 PUFAs to n-3 LC-PUFAs. As it is now, food animals can only be enriched in both eicosapentaenoic acid and DHA through the inclusion of fish meal or oil in their diet. Animals fed linseed or flax oil are enriched in alpha-linolenic acid and eicosapentaenoic acid, but not DHA. This genetic strategy, if accepted, would significantly reduce the demand on marine resources for animal feed and improve the nutritional quality of such animal foods for human consumption. These transgenic mice should also prove valuable for the investigation of several animal models of human disease.

Kang JX, Wang J, WuL, Kang ZB. *Fat-1* mice convert n-6 to n-3 fatty acids. *Nature* 2004:427:504.

## DHA Function in Membranes

It is widely understood that docosahexaenoic acid (DHA) one of two major long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) found in fish oil, is incorporated into cell membranes. It is abundant in the cell membranes of brain grey matter, the retina, testis, heart, liver, kidney, skeletal muscle and red blood cells. But does the presence of this highly unsaturated, long-chain molecule affect the cell's metabolic activity, and if so, how?

DHA is longer (22 carbon atoms) and more unsaturated (six double bonds) than most fatty acids in the body. The numerous double bonds cause the molecule to bend and occupy more space. When incorporated into a cell membrane, this property makes the membrane more flexible, more permeable to water and ions, and more able to fuse with other membranes. It also affects the activity of proteins residing in the membrane. Some membrane proteins are enzymes that affect chemical activity; others are recognition sites for immune cells and hormones outside the cell. Other membrane proteins bind charged atoms (ions) such as sodium, potassium, and calcium and control their flow or channeling across the membrane. These ions are essential for the transmission of nerve impulses, skeletal muscle contraction, and bone remodeling.

Several hypotheses have been put forward to explain how DHA may affect processes such as nerve transmission,

oxidative stress, communications between cells, interactions of cells with constituents in their environment, and electrical and enzyme activity. Nigel Turner and colleagues at the University of Wollongong, Australia, approached the question of how membrane DHA may affect cellular function by comparing the DHA content in heart, kidney, and brain of diverse mammals and birds of widely differing size. These tissues contain different concentrations of DHA in their membranes and are highly metabolically active. Mammalian species included mice, rats, sheep, pigs and cattle, while birds ranged from small zebra finches and sparrows, to starlings and pigeons, and to geese and emus.

As a reflection of metabolic activity, the investigators examined the activity of the membrane-bound enzyme, sodium-potassium ATPase, which occurs in all tissues selected. This enzyme releases energy inside the cell to fuel the transport of sodium out and potassium into the cell. It is a critical enzyme for providing cellular energy.

Of the tissues examined, the highest concentration of membrane DHA was found in the brains of both birds and mammals. Concentration ranged as high as 40%. DHA content varied as much as 80-fold across tissues and species. Heart and kidney contained less DHA, but concentrations varied widely with the tissue and species. Likewise, enzyme activity was highest in brain samples, but values ranged across samples from about 1,500 activity units in bird and mammal heart samples to nearly 30,000 in mammalian brain. Enzyme activity varied substantially between and within tissues.

When enzyme activities from all species and tissue samples were compared together, DHA content was significantly correlated with sodium-potassium ATPase activity in the heart ($P$=0.02) and kidney ($P$=0.0004), but not in the brain. The authors interpret these broad findings as consistent with the idea that DHA content in membranes may act as a "pacemaker" of a species' metabolic rate. The broad correlation observed for diverse mammals and birds is consistent with membrane regulation of enzyme activity, particularly those enzymes involved in energy production and electrical conductivity.

Although membrane DHA content was not correlated with enzyme activity in brain, the authors speculated that membrane DHA may facilitate optimal interactions between membrane lipids and proteins in maintaining cognitive function and the activity of membrane-bound receptors. In time a more detailed understanding may emerge to explain how changes in membrane composition affect various membrane functions and their links to clinical conditions such as depression and diabetes.

Turner N, Elses PL, Hulbert AJ. Docosahexaenoic acid (DHA) content of membranes determines molecular activity of the sodium pump: implications for disease states and metabolism. *Naturwissenschaften* 2003;90:521-523.

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