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EDITORIAL

Fast Away the Old Year Passes

It is now the month of December, when the greatest part of the city is in a bustle. Loose reins are given to public dissipation; everywhere you may hear the sound of great preparations . . .

—Seneca, the Younger, circa 50 C.E.

So went the Roman Saturnalia, a time of festivals, the up-ending of business-as-usual, and the celebration of the sun's return from the depths of the Winter Solstice. (Readers in the southern hemisphere have six months to wait.) Even now the din remains and the "Lord of Misrule" has failed to abdicate. May 2007 clear a path to peace on our turbulent planet.

This issue leads with guest authors Les Cleland and Michael James from the Royal Adelaide Hospital, Australia, who describe the use of liquid fish oil in treating rheumatoid arthritis. The lower toxicity of omega-3 polyunsaturated fatty acids (PUFAs) compared with non-steroidal anti-inflammatory drugs, their effectiveness in reducing arthritis symptoms and the lower risk of cardiovascular disease fortify their case.

Whether omega-3 PUFAs have up-ended conventional thinking about diet and heart disease can be debated, but two thoughtful reviews of the benefits versus the risks of fish consumption agree: the advantages outweigh the possible disadvantages. Despite worries about the possible risks from environmental contaminants, the Institute of Medicine found the evidence insufficient for drawing conclusions. These appraisals appear in the cardiovascular section.

Several articles address different aspects of brain function and behavior. In children, suggestive evidence is presented that long-chain omega-3 PUFAs are reduced in young adults with attention deficit hyperactivity disorder. This finding is consistent with several other studies. A pilot study in autistic children, who may have reduced concentrations of omega-3 PUFAs, reported that the consumption of fish oil was associated with improvements in three of five behaviors characteristic of the condition. The sample size was too small for statistical significance, but in a field with few data, these results provide a glimmer that seems worth pursuing.

Encouraging results were reported in the first randomized intervention trial in patients with Alzheimer's disease. For patients with only mild symptoms, consumption of long-chain omega-3 PUFAs was linked to significantly slower mental decline. Confirmation of a lower risk of Alzheimer's with higher blood concentrations of DHA, a long-chain omega-3 PUFA, was reported in participants from the Framingham heart study.

Best wishes for enjoyable reading, joyful holidays and long pauses in life's tango.

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GUEST ARTICLE

Fish Oil for Anti-Inflammatory Effect: A Practical Approach to Symptom Control with Reduced Risk

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The Evidence Base

There is a substantial body of information regarding the anti-inflammatory effects of fish oil in appropriate doses. The evidence encompasses relevant biochemistry, epidemiology, animal studies and clinical trials. The opus of randomized control trials includes 15 studies in rheumatoid arthritis, in which worthwhile effects were shown.[1,2] Disease modifying effects of fish oil have been shown also in IgA nephropathy where there was reduced deterioration in renal function, including reduced progression to end stage renal failure, and in Crohn's disease where there was a lower relapse rate.[3,4]

The Problem of NSAID Toxicity

The non-steroidal anti-inflammatory drugs (NSAIDs) exert a direct analgesic effect that has become central in the management of arthritis for more than 50 years, despite lack of evidence for a beneficial effect with regard to long-term disease outcomes. The pain relief derived from the use of these agents would justify their use without question, if their effects on health, otherwise, were neutral. Unfortunately, these agents, as a class, are associated with substantially increased risks for serious upper gastro-intestinal (GI) events, such as major hemorrhage and perforation, and for serious cardiovascular (CV) events. The extent of the latter risk has only been appreciated since evidence of increased CV events emerged in long-term studies with new NSAIDs, known generically as coxibs. These had been touted for safety based on selectivity for inhibition of the inducible isozyme of cyclooxygenase, COX-2. Unfortunately, this benefit proved illusory, as reduction in GI adverse events is offset by increased risk for serious CV events. Evidence for increased CV risk with the coxibs led to scrutiny of older NSAIDs, which, based on earlier perceptions of likely risk, had not been assessed from this aspect. Numerous database analyses and case control studies have confirmed that increased CV risk is a class effect of NSAIDs, as is the upper GI risk. Furthermore, while GI risk with highly selective COX-2 inhibitors is reduced relative to some less selective NSAIDs, GI risk exists to some degree with use of all NSAIDs, including the coxibs.

Appreciation of unavoidable serious health risks during symptomatic treatment with NSAIDs led some health authorities to recommend limiting the use of NSAIDs to the smallest dose and shortest time needed to control symptoms.[5] This outcome can be assisted by fish oil. In anti-inflammatory doses, fish oil has been shown, after a period of latency of about two months, to reduce discretionary use of NSAIDs for symptomatic relief in rheumatoid arthritis (RA). Clinic experience and biochemical effects suggest a similar NSAID-sparing effect is likely in more prevalent osteoarthritis. Furthermore, within the Western context, fish oil has not been associated with health risks and has a number of health benefits. Also, fish oil is not contra-indicated by concurrent medications and can be used safely with aspirin and with warfarin.[6] Thus, it is logical that fish oil, subject to tolerance, should be a component of any strategy to limit NSAID use for symptomatic treatment of arthritis.

Fish Oil and the Cardiovascular Risk Associated with Inflammatory Disease

The cardiovascular benefits of fish oil are well recognized and occur through multiple mechanisms. In RA, a common inflammatory disease, there is an approximately 2-fold increased mortality, due largely to increased CV events.[7] It is notable that 'traditional' CV risk factors account for only about 30% of this increased mortality. While chronic systemic inflammation is also likely to contribute, approximately 80% of RA patients in a large database of North American practices were found to receive ongoing treatment with NSAIDs.[8] Since database analyses suggest commonly prescribed NSAIDs increase CV risk by about 50%, one can expect NSAID use to be a significant contributor to CV events and mortality in RA. Fish oil taken at anti-inflammatory doses by patients with early RA favorably altered plasma triglyceride and HDL profiles, decreased inflammatory mediator production and markers of systemic inflammation, and decreased NSAID use.[9] Thus, fish oil could decrease cardiovascular risk associated with RA by multiple mechanisms.

Practical Issues in Using Fish Oil as an NSAID Substitute in RA

Having been involved in one of the early randomized controlled trials of fish oil in RA in the mid 1980s, the Rheumatology Unit at the Royal Adelaide Hospital

addressed barriers to routine application of this approach in the 1990s. The main obstacles are:

Cost: The health products industry, like the pharmaceutical industry, gears prices to what the market will bear rather than reasonable profit relative to production costs. The price of fish oil in capsules aligns with a general affordability for one or two capsules per day, the dose at which most users self-medicate. Anti-inflammatory doses of fish oil are an order of magnitude higher, making use of fish oil for anti-inflammatory effect unaffordable for many potential users.

Suitable formulations: Trials of fish oil that have shown anti-inflammatory effects have generally used daily doses of 10 to 20 1000 mg tablets of fish body oil. Fish body oil typically contains EPA 18% and DHA 12% by weight. Thus a dose of 3 to 6 g long-chain n-3 fatty acids (n-3 LC-PUFAs) is delivered. The number of capsules required is daunting and release of fish oil in the stomach is delayed, which may accentuate reflux and repeated taste. Also, the large number of capsules required entails a significant gelatin load, which can cause dyspepsia.

Accordingly, use of bottled fish oil was explored as a means of delivering anti-inflammatory doses of n-3 LC-PUFAs.

History of Use of Bottled Fish Oil for Anti-inflammatory Effect

Initially, a commercially available, devitaminized cod liver oil was used to establish the practicalities of giving fish oil layered on juice, swallowed without stirring and followed by sipping a juice only chaser, then food. This proved to be a broadly applicable, sustainable and affordable treatment strategy. When this product was withdrawn, standard bottled cod liver oil was used. While this product contained useful amounts of vitamin D_3 and both vitamin D and vitamin A may have anti-inflammatory effects, concerns have been raised regarding the negative effects of higher intakes of vitamin A on bone mineral density and fracture risk. While this effect has not been shown with cod liver oil, which contains potentially countermanding vitamin D, the possible negative effects of vitamin A were avoided by changing to a fish body oil of the sort typically found in fish oil capsules. However, whereas formulations of cod liver oil have been available as a bottled liquid for more than two centuries, fish body oil formulations are much more recent and, almost universally, involve encapsulation of the fish oil in gelatin. Certainly, at the time of our decision to switch from cod liver oil to fish body oil, no suitable bottled fish oil product was commercially available in Australia. Initial experience with bottled fish oil was based on bottling in quanta of 500 mL from a bulk purchase of fish oil (200L drum).

After successful testing in the clinic and several cycles of onsite bottling at the Royal Adelaide Hospital, arrangements were made with Melrose Laboratory to produce 500 ml bottles of fish oil for general commercial distribution. To the best of our knowledge, this is the only product available that provides sufficient bottled fish oil for readily affordable long-term use as an anti-inflammatory agent. One bottle provides a 5-week supply of fish oil for ingestion at our recommended anti-inflammatory dose of 15 ml daily, for a cost of approximately 40 cents per day.

Long Term Clinical Experience

In addition to general application as an NSAID-sparing agent in the Rheumatology Clinic, we have evaluated long-term use of fish oil in a cohort of patients with recent onset RA, who are treated according to a standardized treatment strategy with adjustments in medication according to explicit rules contingent on residual disease activity and intolerances. In this cohort, fish oil has been continued long-term by a majority of subjects. As well as being associated with significantly reduced NSAID use and favorable CV risk profiles compared to those who do not take fish oil, there was better disease suppression with fish oil use.[9] The study has thus established the affordability, feasibility and effectiveness of long-term use of fish oil in RA. Data in osteoarthritis are lacking and a study is commencing to address this deficiency.

Conclusion

In summary, fish oil is a safe, acceptable alternative to NSAIDs for arthritis. NSAIDs have the advantage of prompt action, but for long-term use, fish oil has clear advantages in terms of less risks and collateral health benefits.

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CARDIOVASCULAR HEALTH

New Reports Conclude Benefits of Fish Consumption Outweigh Risks

Controversy about the health benefits of fish consumption versus the potential risks from contaminants in seafood is sustained by several forces. Despite wide agreement that consuming fish or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) is associated with significant reductions in risk of cardiovascular mortality and sudden death, doubts exist about data quality, selected outcomes or the applicability of findings to countries beyond the study population. Previous quantitative benefit-risk assessments of fish consumption and heart disease mortality, stroke and child cognitive development concluded that the benefits of fish consumption outweigh the risks. Nevertheless, some consumers see it differently. Fear of contaminants and popular advice contradict recommendations from government agencies and health organizations. Consumers are more aware of potential dangers than benefits. Fish consumption in some US women declined after a national mercury advisory, largely perceived as a warning.

Emphasis must be placed on adequate consumption–12 ounces/week–of other [i.e., low mercury] fish and shellfish to provide reasonable amounts of DHA and avoid further decreases in already low seafood intake among women.

Two more of fish consumption and various health outcomes attempted to clarify and measure these relationships. One report issued by the Institute of Medicine (IOM), a component of the National Academies in the United States, and the other an independent academic analysis concluded that seafood consumption is associated with reduced cardiovascular deaths. Both encouraged pregnant women to consume adequate amounts of fish and shellfish to obtain docosahexaenoic acid (DHA) and pointed out that women can minimize their exposure to methylmercury by avoiding fish species known to have high levels of contaminants.

Avoiding or reducing fish consumption because of fears about contaminants has its own risks. Dariush Mozaffarian and Eric Rimm of the Harvard Medical and Public Health Schools, respectively, Boston, USA, noted that avoiding modest fish consumption because of confusion about risks and benefits could result in thousands of excess (i.e., avoidable) deaths from heart disease and suboptimal neurodevelopment in children. "Emphasis must be placed on adequate consumption—12 ounces/week—of other [i.e., low mercury] fish and shellfish to provide reasonable amounts of DHA and avoid further decreases in already low seafood intake among women," they asserted. Twelve ounces is equivalent to 336 grams.

The available evidence to assess risks [from contaminants] to the US population is incomplete and useful to limited extent.

Risks to cardiovascular health and mortality from avoiding fish have been well characterized, but neurodevelopmental risks to the fetus from exposure to low levels of methylmercury are uncertain, inconsistent, subtle and perhaps confounded. The IOM noted, "The available evidence to assess risks [from contaminants] to the US population is incomplete and useful to limited extent." More than any other factor, uncertainty and contradictory claims about neurodevelopmental risks from low level exposure to methylmercury fuels controversy and confusion. Prudence dictates caution regarding risks to infant neurodevelopment from seafood-borne contaminants; thus, pregnant and nursing women are advised to avoid fish species known to be high in methylmercury.

Scant attention has been given to the protective nutrients such as selenium and DHA found mainly in seafood. The paradox is that seafood is also the primary source of selenium, an element that sequesters mercury and helps protect against its toxicity. DHA, an essential nutrient for brain development and function, is most abundant in seafood, especially fatty fish species. The possible presence of organic contaminants may further complicate the assessment of methylmercury risks.

The analysis presented by Mozaffarian and Rimm combined data relating fish or fish oil intake and the risk of coronary heart disease mortality from prospective cohort studies and randomized clinical trials. They evaluated the findings using nonparametric statistics to fit a curve through the data points in a way similar to forcing a flexible elastic rod through the points. They scaled the reference group relative risk according to levels of eicosapentaenoic acid (EPA) and DHA consumed, because reference intakes varied from 150 to over 2,000 mg/day.

Their results (Figure) showed that consumption of 250 mg/day of EPA and DHA was associated with a significant 36% reduction in mortality risk. In this analysis, intakes of EPA and DHA above this amount conferred no additional reduction in mortality risk. In their view, this "threshold effect" would explain the greatly reduced mortality of the Japanese, whose median EPA and DHA intake is 900 mg/day and whose death rates from heart disease are 87% lower

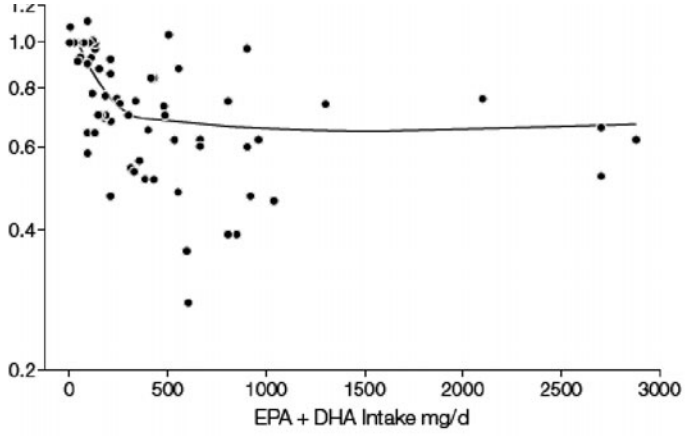

Figure. Relationship between intake of fish or fish oil and relative risks of death from coronary heart disease in pooled analysis of prospective cohort studies and randomized trials. *Image © 2006 American Medical Association, reproduced with permission from* JAMA *2006;296:1885.*

than in western populations. However, other observations suggest that the Japanese do not develop heart disease at the rates seen in western countries, thus changes in their cardiovascular mortality attributable to high fish intakes would be expected to be very small.

If one omits the four observations of populations consuming an average of 2 g/day or more of EPA and DHA, the relative risk or coronary mortality would continue to decline with EPA and DHA intakes up to about 1 g /day. As the authors also suggested that maximum anti-arrhythmic effects would require an intake of about 750 mg/day, the interpretation of a threshold effect of about 250 mg/day could be skewed by observations in populations having less heart disease and therefore different mortality risks than in most western countries.

Mozaffarian and Rimm examined the effect of n-3 LC-PUFAs on total mortality, observing that the magnitude of the effect would be proportional to the contribution of heart disease to total mortality. When randomized and double-blind placebo-controlled trials were combined, they estimated that fish or fish oil consumption would reduce total mortality by 17%, a figure comparable to the estimated effect of statins (15%) on all-cause mortality.

Heterogeneous and insufficient data prevented the authors from quantifying the benefits of DHA consumption in neurodevelopmental outcomes. However, the authors concurred with the conclusions of many studies that maternal intake of DHA is beneficial for early neurodevelopment.

With regard to cancer risks from polychlorinated biphenyls (PCBs) and dioxins, Mozaffarian and Rimm reported that the 24 excess cancer deaths per 100,000 individuals over a 70-year lifespan, potentially attributable to eating farmed salmon (8 for wild salmon), would be more than offset by the reduction in 7,125 deaths from coronary heart disease. Put differently, the benefits in reduced heart mortality outweighed the cancer risks by 100 to 370 times for farmed salmon and by 300 to more than 1,000 times for wild salmon. The margin of benefits increases further if one reduces the 10-fold safety factor built into cancer risk estimates derived from animal experiments and other differences in these comparisons.

The IOM noted that current US seafood consumption (14 g/day for individuals aged 2 and over) is below levels recommended by several groups. The committee found "increased seafood consumption is associated with a decreased risk of cardiovascular deaths and events," but concluded that evidence is insufficient to assess whether these effects are mediated through EPA and DHA consumption, decreased saturated fat intake or other correlates of eating seafood. Seafood contains many other nutrients that provide health benefits or may facilitate the action of EPA and DHA. But the latter assessment fails to account for the beneficial effects observed in many studies using fish oil supplements and purified n-3 LC-PUFAs, which have minimal effect on saturated fat consumption. The committee also found the evidence inconsistent for protection against further cardiovascular events in people with a history of myocardial infarction.

Pregnant and nursing women can safely consume 12 ounces (336 g) of seafood/week, but should avoid large predatory fish and limit albacore tuna consumption to 6 ounces (168 g) per week.

Considering maternal and infant health, the IOM endorsed the current government advisory that pregnant and nursing women can safely consume 12 ounces (336 g) of seafood/week, but should avoid large predatory fish and limit albacore tuna consumption to 6 ounces (168 g) per week. The IOM committee stated that the uncertainties about health risks from seafood contaminants to the general population and the evidence to assess those risks precluded any conclusions.

Of the two reports, the IOM's conclusions added little to the discussion on the potential health effects of methylmercury and organic pollutants found in widely varying amounts in different fish species. While

expressing caution about the effects of n-3 LC-PUFAs, the IOM provided no convincing alternative explanation for the health effects reported in dozens of studies using fish oil or purified EPA and DHA. By failing to embrace data supporting n-3 LC-PUFA mechanisms of action in cardiovascular health unrelated to blood lipid levels, such as anti-arrhythmic and anti-inflammatory effects, the IOM report provided a limited picture of fish consumption in cardiovascular health. To its credit, the report identified many knowledge gaps and offered strong suggestions for overcoming these deficiencies.

Both reports concluded that the benefits of regular seafood consumption outweigh the risks and that too little is known about the risks of low level exposure to methylmercury. The effect of protective nutrients, such as selenium, on methylmercury exposure warrants more extensive investigation. Mozaffarian and Rimm advance the discussion by estimating a threshold level of EPA and DHA intake that would significantly reduce the chance of cardiovascular mortality. They provide numbers to weigh the cardiovascular benefits against the theoretical risk of additional cases of cancer and assess the hazards of avoiding fish. Their assessment considers costs and ways to meet recommended intakes, potential benefits of n-3 LC-PUFA consumption above the threshold for mortality reduction and other issues relevant to increased fish and n-3 LC-PUFA consumption.

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DHA Biomarker Reflects Slower Progression of Coronary Artery Disease

There is strong evidence that consumption of n-3 LC-PUFAs significantly reduces cardiovascular mortality and sudden death, but whether they can slow or reverse the progression of atherosclerotic plaques is uncertain.

Much of the discussion about the cardiovascular benefits associated with the consumption of fish oils or their long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) focuses on lowering the likelihood of dying from coronary heart disease. There is strong evidence that consumption of these fatty acids significantly reduces cardiovascular mortality, myocardial infarction and sudden cardiac death, particularly in people who already have the disease. Regular fish or n-3 LC-PUFA consumption favorably modifies cardiovascular risk factors, may lessen the severity of the metabolic syndrome–a condition linked to increased cardiovascular risk, affects nuclear receptors such as peroxisome proliferator-activated receptors that regulate the expression of genes involved in lipid metabolism and inflammation of the vascular endothelium, and may benefit other chronic and degenerative diseases.

Development of Atherosclerotic Plaques

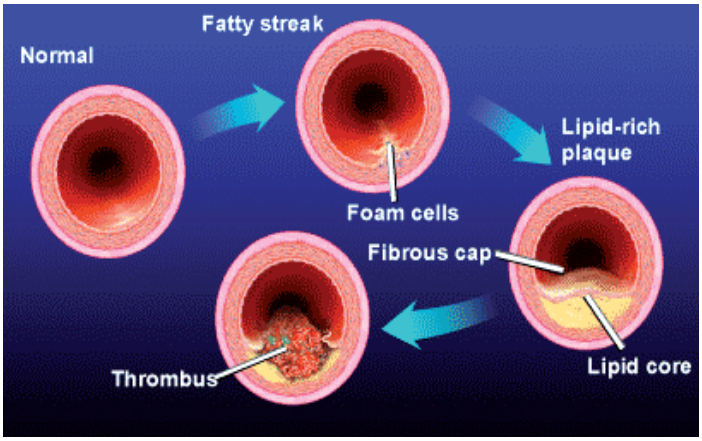

Figure. Stages of arterial plaque formation.
Image reproduced from the Diabetes Roundtable.

Many adverse coronary events are a consequence of the slow accumulation and deterioration of deposits in arterial walls known as atherosclerotic plaques (Figure). Whether n-3 LC-PUFAs can slow or reverse the progression of these lesions is uncertain, although there are reports that they retard pathological changes in carotid arteries. Higher fish consumption was associated with smaller decreases in coronary artery diameter and smaller increases in percent stenosis in postmenopausal women, but findings with high-dose n-3 LC-PUFA supplementation were no different from control in patients with coronary heart disease. In another report, carotid artery plaques containing greater concentrations of n-3 LC-PUFAs had altered morphology and fatty acid composition consistent with increased plaque stability and reduced inflammation compared with plaques having less n-3 LC-PUFAs. These limited data suggest a possible benefit of n-3 LC-PUFAs on atherosclerotic disease progression, but require considerable substantiation before the drums can roll.

Dr. Arja Erkkila and colleagues at the University of Kuopio, Finland, extended their previous investigation of atherosclerosis progression by examining biomarker data from a prospective cohort of postmenopausal women with coronary artery atherosclerosis. In the original study, 228 postmenopausal women with one or more coronary artery stenosis of at least 30% of the

luminal diameter, who were younger than 80 years of age and not taking estrogen replacement treatment, were randomized to receive 0.625 mg estrogen, 0.625 mg estrogen plus 2.5 mg progesterone, or placebo for a mean duration of 3.2 years. Neither intervention had any significant effect on the progression of atherosclerosis. Plasma lipid fractions were analyzed for alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA were most abundant in the phospholipid fraction, whereas ALA was abundant in the triglyceride fraction. Measurements of coronary artery diameter and percent stenosis were determined from blinded coronary angiograms assessed at baseline and after an average of 3.2 years.

Participants were grouped according to the median concentrations of n-3 PUFAs in their triglycerides and phospholipids. The following discusses mainly the findings from the phospholipid data. By far the most abundant n-3 PUFA in plasma phospholipids was DHA at 2.6 mol%, 5 times the concentration of EPA at 0.5 mol%. Changes in the minimum coronary artery diameter over 3.2 years relative to the median phospholipid EPA and DHA concentrations are shown in the Table.

Table. Changes in minimum coronary artery diameter over 3.2 years according to lower or higher than the median phospholipid EPA and DHA concentrations

Change in mean minimum coronary artery diameter, mm			
n-3 LC-PUFA	Low <Median	High >Median	*P*
EPA			
Baseline	1.93 ± 0.03	1.92 ± 0.03	0.77
Loss in diameter*	0.09 ± 0.02	0.06 ± 0.02	0.10
DHA			
Baseline	1.94 ± 0.03	1.90 ± 0.03	0.28
Loss in diameter*	0.10 ± 0.02	0.04 ± 0.02	0.007

*Adjusted for age, location of coronary segment, body mass index, smoking, cholesterol-lowering medication, hormone replacement therapy use, diabetes, education, clinic, time of follow-up, revascularization procedures and alcohol intake.

There was no difference in loss or narrowing of the artery diameter in terms of phospholipid EPA concentration, but loss in diameter was significantly less in those with phospholipid DHA concentrations above the median (*P*=0.007). Similar findings were obtained for percent stenosis, where the change in patients with DHA concentrations above the median (1.35 ± 0.76%) was significantly less than in those with DHA concentrations below the median (3.75 ± 0.74%). A third indication of reduced disease progression was the significantly smaller number of new lesions in women with DHA concentrations above the median (2.0 vs 4.2 % of measured segments). Note that significantly less artery narrowing and stenosis were also observed for median triglyceride DHA concentrations.

Loss in arterial diameter was significantly less in patients with phospholipid DHA concentrations above the median compared with those whose concentrations were below the median. Similar findings were obtained for stenosis.

These results add to the growing chapter of evidence suggesting that n-3 LC-PUFAs reduce arterial disease progression after it is established. Although it is not clear how these fatty acids slow the onset of new lesions and the growth of existing ones, there are several plausible mechanisms, including changes in membrane properties, cell signaling, moderation of inflammation and altered eicosanoid metabolism. The study marks an important step for the relevance and validity of biomarkers in assessing and monitoring the progress of atherosclerosis and reduces the uncertainty associated with dietary assessments. Moreover, these findings achieved statistically significant differences in artery narrowing and new lesions in a relatively short time, 3 years, in an older population.

Erkkila AT, Matthan NR, Herrington DM, Lichtenstein AH. Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. J Lipid Res *2006;47:2814-2819.*

MATERNAL & INFANT HEALTH

Some Maternal LC-PUFAs Affect Infant Neurologic Condition

In spite of many studies on the neurodevelopment of healthy term infants in relation to the long-chain polyunsaturated fatty acid (LC-PUFA) status of the mother during pregnancy or the infant at birth and through childhood, it is unclear whether there are lasting effects of low LC-PUFA status in fetal development or the first few months of life. Higher docosahexaenoic acid (DHA) status has been associated with better visual acuity, higher global development scores, more mature infant sleep patterns and improvements in other cognitive or neurodevelopmental measures, but data are inconsistent. Provision of n-3 LC-PUFAs to healthy term infants for 2 months was associated with reduced occurrence of mildly abnormal general movements, but some neurological effects seen at 3 months of age were not observed at 18 months. Thus, the clinical

significance of early mild developmental effects that may be transient remains uncertain.

More mature infant sleep patterns and other neurodevelopmental measures have been associated with higher DHA status, but some neurological effects may be transient.

This report by Hylco Bouwstra and colleagues at the University of Groningen, The Netherlands, extends the neurodevelopment studies by this group. Healthy term infants either breast-fed or fed formula with or without supplemental LC-PUFAs for 2 months were continued on unsupplemented (control) formula until 6 months of age. At 18 months of age, the infants received neurologic assessments according to the Hempel and Bayley Scales of Infant Development. The Hempel Scales examine motor functions such as grasping, sitting, crawling and standing along with the quality of motor behavior, muscle tone, reflexes and cranial nerve function. Each child was classified as neurologically normal, showing signs of minor neurological dysfunction or definitely abnormal. Scores were further summarized over a range of relevant outcomes to obtain an optimality score. The Bayley Scales reflect mental and psychomotor development including problem solving, discrimination, language and social skills. Assessment scores were related to umbilical fatty acid concentrations at birth.

Children with minor neurological dysfunction had significantly lower concentrations of eicosanoic acid (20:0) and higher total trans *fatty acids in the umbilical vein, suggesting the importance of prenatal exposure to these fatty acids.*

Of the 474 infants initially enrolled in the study, umbilical cord tissue was available for 317 infants (67% of the sample). None of the study infants showed a definitely abnormal neurologic condition and 15 (5%) had minor neurological dysfunction. Children with minor neurological dysfunction had significantly lower concentrations of 20:0 (eicosanoic or arachidic acid) in the umbilical vein and artery and significantly higher concentrations of *trans*, *trans* linoleic acid in the umbilical vein compared with neurologically normal children. Higher total *trans* fatty acids and all individual *trans* fatty acids except for *trans* 18:1n-9 or n-7 were significantly associated with lower neurologic optimality score at 18 months in multivariate analysis. The significant negative association between neurologic optimality scores and *trans* fatty acids in the umbilical vein emphasize the importance of limiting prenatal exposure to these fatty acids.

In the overall analysis, PUFA supplementation was unrelated to neurological outcomes. However, in univariate analysis, umbilical vein arachidonic acid concentration was positively associated with neurological optimality score, but the effect disappeared when other variables were considered. Infants in the lowest quartile of umbilical vein DHA content had significantly lower neurological optimality scores than infants in the upper 3 quartiles. These findings indicate that higher content of *trans* fatty acids and lower concentrations of DHA in umbilical vein were associated with suboptimal neurologic conditions at 18 months. These associations were observed with the Hempel assessment, but not with the Bayley scales for psychomotor or mental development, suggesting that the former may be a more sensitive tool for assessing subtle effects of maternal nutrition on infant neurodevelopment.

Higher content of trans *fatty acids and lower concentrations of DHA in maternal fatty acid transfer to the fetus were associated with suboptimal neurologic conditions at 18 months.*

These findings are in general agreement with previous observations on the importance of arachidonic acid and DHA for optimum neurologic development in infancy. However, the relative importance of each fatty acid differed over the course of time. DHA and arachidonic acid contents in the umbilical vein were associated with more optimal neurologic condition at 10 to 14 days after birth, but at 3 months of age, only umbilical arachidonic acid was related to better neurologic status. At 18 months, only neonatal DHA was related to more optimal neurologic status. Changes in the associations of these fatty acids with time could explain some of the inconsistencies in other studies. Further, these studies suggest that prenatal LC-PUFA availability may be more important than postnatal LC-PUFA availability as there were no differences between infants fed unsupplemented or LC-PUFA-enriched formula.

The other intriguing observation from this study is the association between *trans* fatty acid concentration in umbilical vein and suboptimal neurologic condition. *Trans* fatty acids alter cell membrane properties in ways similar to saturated fatty acids, but apparently they are not incorporated into animal neuronal membranes. *Trans* fatty acids are avidly taken up by

placental membranes. The significance of reduced eicosanoic acid to neurologic condition is unknown.

The authors suggested that the Hempel neurologic assessment may be a more sensitive measure of infant motor development than the widely used Bayley psychomotor scales and provided additional evidence that some neurodevelopmental effects may be transient.

The authors suggested that the Hempel neurologic assessment may be a more sensitive measure of infant motor development than the widely used Bayley psychomotor scales, but more direct comparisons between the two assessments will be needed to confirm this interpretation. These findings confirm the importance of adequate maternal DHA and AA for optimum infant neurodevelopment, but provide no evidence that postnatal LC-PUFA supplementation benefits the neurodevelopmental outcomes examined. Included with previous reports on these infants at younger ages, this study provides additional evidence for the importance of LC-PUFAs and age in neurologic measurements and the fact that some neurodevelopmental effects may be transient.

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Does DHA Affect Placental Function?

Increased need for micronutrients and long-chain polyunsaturated fatty acids (LC-PUFAs) in pregnancy is recognized, but not always met. Supplements and food fortification have helped reduce shortfalls in folic acid consumption, but intakes of omega-3 (n-3) LC-PUFAs may be below recommended intakes, unless women consume fatty fish or fish oil supplements. Many, if not most, women in North America are unaware of the importance of docosahexaenoic acid (DHA) an n-3 LC-PUFA, during pregnancy and lactation for optimum brain development of the fetus and infant. Some physicians have called for recommendations encouraging women to consume purified fish oil during pregnancy and lactation.

Healthy pregnancy outcomes depend on healthy placental function. In early pregnancy, the placenta undergoes cell proliferation with low rates of apoptosis or cell death. Placental apoptosis increases in the third trimester, shortly before delivery, and in intrauterine growth restriction.

Folic acid and DHA, important nutrients for healthy pregnancy outcomes have not been investigated for their effects on placental growth.

Folate deficiency has been associated with increased placental apoptosis *in vitro*, whereas DHA increases apoptosis in some cells, but not others. The effects of these nutrients on human placental growth have not been investigated.

Mario Klingler and colleagues at Ludwig-Maximilians-University of Munich, Germany, examined the effect of fish oil and folic acid supplementation on 154 healthy pregnant women, 18 to 40 years of age, who were randomly assigned to consume either DHA (500 mg) plus eicosapentaenoic acid (EPA, 150 mg), folic acid (400 µg 5-methyltetrahydrofolate), both supplements, or a placebo without n-3 LC-PUFAs or folic acid. Women were given the supplements from week 20 of pregnancy through delivery. Placentas were obtained within 15 minutes of delivery and samples from different predefined locations were obtained and pooled for determination of fatty acid and folate concentrations. Cell proliferation was assessed by measuring proliferation cell nuclear antigen. Other measurements included cytokeratin 18 neoepitope, a protein found only in apoptotic cells and *p*53 (tumor protein *p*53) a transcription factor or "master watchman" that regulates the cell cycle. Complete data on fatty acids and cellular apoptosis and proliferation were available from 55 samples.
Consumption of fish oil increased placental DHA and EPA concentrations significantly above concentrations in the placebo and folic acid only groups. Similarly, supplemental folic acid increased the folate concentration in maternal and infant plasma above that in the placebo group. Intriguingly, plasma folate was significantly greater in the plasma of women consuming both fish oil and folate or fish oil alone, compared with folic acid supplementation alone (Figure). The effect of fish oil alone was not observed in the infants' plasma folate concentrations. Interaction between dietary DHA and folate status has been reported previously, but in that instance, folate deficiency was associated with depleted DHA and folate feeding increased red blood cell DHA. In this study, feeding fish oil alone increased maternal plasma folate concentrations.

Women who were supplemented with both fish oil and folic acid had significantly increased placental cell proliferation, mainly in the trophoblast cells. Women receiving either supplement alone did not differ in cell proliferation from the placebo group. There were no other differences among the groups in apoptosis or other markers of the cell cycle. Neither

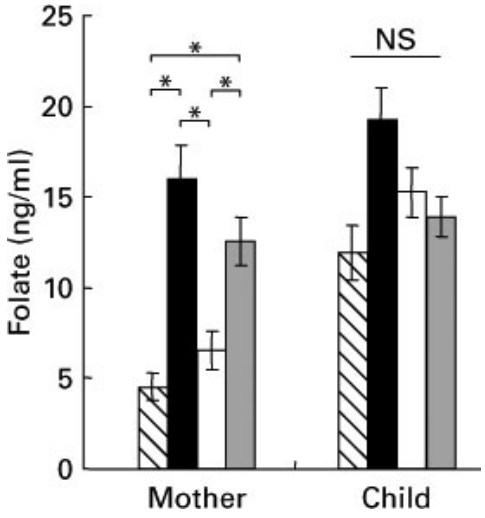

Figure. Effect of supplementation with placebo (hatched) fish oil plus folic acid (black), folic acid (clear) or fish oil (shaded) or on maternal and child plasma folate concentrations. *Reproduced from the* Br J Nutr *2006;96:182-190 with permission from the authors and the Nutrition Society.*

DHA nor folate supplementation affected the length of gestation, infant birthweight or length, or placental weight, as has been reported in other studies.

The implications of the effect of fish oil plus folate on placental trophoblast cell proliferation are unknown. The independent effect of fish oil in increasing maternal plasma folate concentration warrants further investigation, especially as folate and DHA responses have been linked in at least one other study. The connection between neural tube defects and folate deficiency and increased risk of cerebral palsy with marginal DHA intake further implicates interactions between these nutrients.

Klingler M, Blaschitz A, Campoy C, Cano A, Molloy AM, Scott JM, Dohr G, Demmelmair H, Koletzko B, Desoye G.The effect of docosahexaenoic acid and folic acid supplementation on placental apoptosis and proliferation. Br J Nutr *2006;96:182-190.*

MENTAL HEALTH

Mildly Impaired Cognition in Italian Elderly Less Likely with Dietary PUFAs

Prevalence of mild cognitive impairment without dementia increases with age and affects as many as 30% of Canadians over the age of 65. In a study of US African Americans, prevalence of mild cognitive impairment rose from 19% in those aged 65 to 74 years to 38% in seniors older than 85. A survey in the U.K. reported a prevalence of 18% in the elderly 75 years of age or older. Progression of mild cognitive impairment to dementia is affected by vascular risk factors, such as increased cerebral ventricular volume. Several factors related to the progression of atherosclerosis, such as serum cholesterol concentration, lipid peroxidation, oxidative stress and inflammation, appear to be linked to neurodegenerative decline. Low blood concentrations of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) have been associated with increased risk of both conditions.

Results from the Italian Longitudinal Study on Aging provide additional data on the relationship between dietary fatty acids and the risk of cognitive impairment and its rate of progression in a population sample from southern Italy consuming a Mediterranean diet. Three samples of participants aged 65 to 84 years were selected at different times; 278 participants were followed for 2.6 years from 1992-1993. Of these, 186 completed a second survey in 1995-96 and a third group of 95 non-demented participants was retested in 2000-01. Participants were evaluated with the mini mental state examination for global cognitive function and the Babcock story recall for immediate and delayed recall. Dietary intakes were assessed at baseline with a semi-quantitative food frequency questionnaire and 13-hour fasting blood samples were obtained.

The only dietary variables associated with lower risk of mild cognitive impairment were total PUFAs, monounsaturated fatty acids and energy consumption.

Cognitive status was determined using multiple criteria: absence of dementia, normal general cognitive function, objective evidence of memory impairment in the lowest 10th percentile of age- and education-adjusted Babcock recall scores, and independence in daily living as defined by 6 criteria. During the median first follow-up time of 2.6 years or 622 person-years, there were 18 new evaluations of mild cognitive impairment. The only dietary variable associated with risk of mild cognitive impairment was a borderline non-significant inverse relationship with total PUFA consumption. The crude hazard ratio for increased PUFA consumption was 0.65 (95%CI=0.43-0.98, *P*=0.04). Upon adjustment for multiple confounders, this association was not statistically significant.

When the sample of 95 participants was evaluated after 8.5 years of follow-up, risk of mild cognitive impairment was negatively associated with monounsaturated and PUFA intakes, as well as with total energy consumption. The interaction of these variables with time in 4-year

increments was statistically significant after adjustment for sex, age, education, comorbidities, body mass index, mental examination score and baseline energy consumption. It is noteworthy that this population consumed 29% energy from fat, 17.6% energy from monounsaturates and 3% energy from PUFAs.

These studies introduce an assessment of cognitive function that goes beyond the standardized Mini Mental State Examination to include practical assessment of skills needed for independent daily living e.g., bathing, dressing, toileting, transferring from bed to chair, continence and feeding plus home management skills such as use of the telephone, light housework, meal preparation, managing money and medications. A broader scope for assessing cognitive function in older people might facilitate the assessment of change over time. As the authors noted, the small sample size limited the power of the study and may have introduced bias.

Other studies in larger population samples have reported a reduced risk of cognitive impairment or lower rate of cognitive decline with higher fish intake, but plasma n-3 PUFA status was higher in Canadian patients with dementia than in nondemented controls. Increased risk of impaired cognition has been associated with higher consumption of saturated fatty acids and linoleic acid. These contrasting reports suggest that measures of total PUFA intake may be too imprecise to assess the apparently contradictory effect of different types of PUFAs on cognitive function in the elderly. Results from randomized controlled trials, such as that being undertaken in the U.K., are much needed.

Solfrizzi V, Colacicco AM, D'Introno A, Capurso C, Torres F, Rizzo C, Capurso A, Panza F. Dietary intake of unsaturated fatty acids and age-related cognitive decline: a 8.5-year follow-up of the Italian Longitudinal Study on Aging. Neurobiol Aging *2006;27:1694-1704.*

Solfrizzi V, Colacicco AM, Introno DA, Capurso C, Del-Parigi A, Capurso SA, Argentieri G, Capurso A, Panza F. Dietary fatty acids intakes and rate of mild cognitive impairment. The Italian Longitudinal Study on Aging. Exp Gerontol *2006;41:619-627.*

CLINICAL CONDITIONS

Attention Deficit Hyperactivity Disorder (ADHD)

Reduced DHA in Young Adults with ADHD Linked to Behavioral Symptoms

Attention deficit hyperactivity disorder (ADHD) is a condition characterized by impulsivity, lack of attention and overactivity. It occurs in children and adults and can interfere with school, work, family relationships and social interactions with peers. Other conditions, such as dyslexia, behavioral and motor difficulties and more frequent health problems may accompany the disorder. Estimates of its childhood prevalence range from 2% to 18%. Up to 70% of children with ADHD retain its symptoms as adults. Development of ADHD is affected by genetic factors involved in catecholamine and serotonin neurotransmission and is readily inherited.

Children and adults with ADHD have significantly lower concentrations of long-chain polyunsaturated fatty acids (LC-PUFAs) in their plasma and red blood cell phospholipids, particularly of arachidonic acid and docosahexaenoic acid (DHA). However, these children do not appear to have essential fatty acid deficiency, although some 40% exhibit excessive thirst and dry skin classically associated with essential fatty acid and omega-3 PUFA deficiency. Provision of LC-PUFAs from both omega-6 (n-6) and omega-3 (n-3) families and varying amounts of the n-3 LC-PUFAs DHA and eicosapentaenoic acid (EPA) have yielded mixed results. Some studies reported no improvements and others observed improvements in some, but not all, symptoms in children with various activity and learning disorders.

Children and adults with ADHD have significantly lower concentrations of long-chain polyunsaturated fatty acids, particularly of arachidonic and docosahexaenoic acids.

In the study reported here, Dr. Caryl Antalis and colleagues at Purdue University, Indiana, USA, selected a sample of 35 young adult college students with clinically diagnosed ADHD and compared their characteristics, fatty acid profiles, dietary intakes, skin/thirst symptoms and other biochemical parameters with a matched group of 112 students without ADHD at the same campus. ADHD was assessed by structured interview, Conners' Adult ADHD Rating Scales and the Wechsler Adult Intelligence Scale. Control participants, who were matched for sex, body mass index and smoking, also completed the Conners' Scales assessment. Twelve participants from each group provided a blood sample, 24-hour urine collection and 3-day diet record.

Students with ADHD were predominantly male (63%), Caucasian, older (ADHD, 24.3 yr vs control, 22.3 yr, P=0.05) and were more likely to be smokers (35% vs 14%) than controls. Scores for thirst and skin symptoms were significantly higher among ADHD participants (score 4.5 ± 2.3 ADHD vs 3.6 ± 2.5 controls, P=0.04). Fatty acids in plasma and red cell phospholipids differed

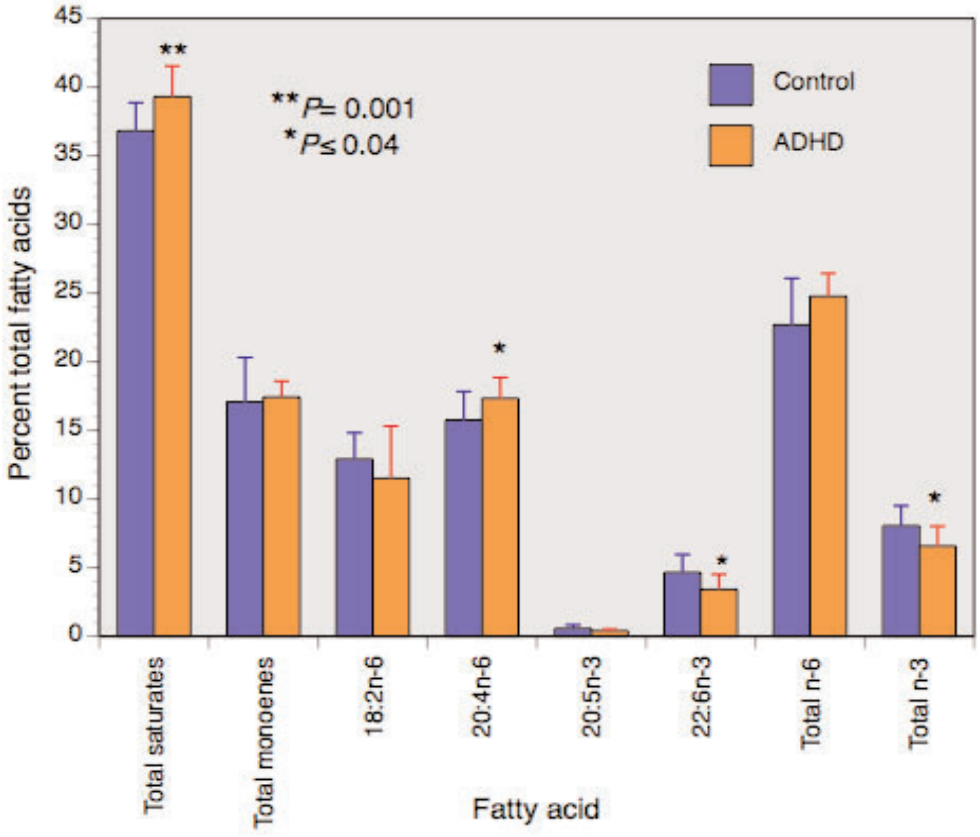

Figure. Fattyacids in red blood cells of young adults with ADHD or matched controls

considerably, as is generally observed. Total monounsaturated and n-3 PUFA concentrations were higher in red cells than in plasma phospholipids, but total n-6 PUFAs were nearly twice as high in plasma as in red cell phospholipids. Highlights of the red blood cell fatty acids are shown in the Figure. Arachidonic acid (20:4n-6) and total saturates were significantly higher in the red blood cell phospholipids of the ADHD participants compared with controls, but total n-3 LC-PUFAs and DHA (22:6n-3) were significantly lower in the ADHD participants. There were no significant differences in linoleic acid or EPA concentrations between the two groups. Markers of oxidative stress, including urinary F_2-isoprostanes, did not differ between the two groups except that ADHD participants had significantly higher plasma ascorbic acid concentrations than controls. Significant inverse correlations were found between red blood cell DHA and inattention and hyperactivity, suggesting that lower DHA status was associated with more severe behavioral symptoms. Nutrient intake was similar in both groups except that ADHD participants consumed significantly more saturated fat and were more likely to take multivitamin supplements. These differences would explain the increased saturates and ascorbic acid values observed in the ADHD group. Dietary records revealed no differences between the groups in LC-PUFA consumption.

Reduced concentrations of LC-PUFAs, including both arachidonic acid and DHA have been reported consistently, but in this study, only DHA concentrations were significantly reduced in the ADHD participants, while red blood cell arachidonic acid concentrations were significantly elevated. This observation suggests that conversion of linoleic to arachidonic acid was unimpaired. The association between DHA and behavioral symptoms further suggests the involvement of DHA in ADHD, but with a small sample size, interpretation of these findings is limited.

Antalis CJ, Stevens LJ, Campbell M, Pazdro R, Ericson K, Burgess JR. Omega-3 fatty acid status in attention-deficit/hyperactivity disorder. Prostaglandins, Leukot Essent Fatty Acids *2006;75:299-308.*

Fatty Acid Desaturase Genes, PUFAs and ADHD

The development of attention deficit/hyperactivity disorder (ADHD) has a strong genetic component as demonstrated in studies of families, twins and adoption. Considerable research has focused on polymorphisms–variations in the sequence of DNA–of genes involved in neurotransmission, particularly for the norepinephrine, dopamine and serotonin pathways. The significance of individual polymorphisms is controversial and several candidate genes have been associated with altered neurotransmission in ADHD in many, but

not all, studies. Several medications used to treat ADHD affect these neurotransmitters.

The development of attention deficit hyperactivity disorder (ADHD) has a strong genetic component involving variations in inherited traits (polymorphisms) for neurotransmission.

Fatty acid abnormalities, such as reduced concentrations of long-chain polyunsaturated fatty acids (LC-PUFAs), occur in ADHD. These have been linked to altered neurotransmission, behavioral abnormalities and omega-3 (n-3) LC-PUFA deficiency in animal studies as recently reviewed by Dr. Sylvie Chalon. These connections are emerging in human research too. However, studies on the effects of supplemental LC-PUFAs have provided mixed results and differ considerably in the population studied, dose, concomitant treatment, duration and study design. Some genetic abnormality in LC-PUFA metabolism is possible.

Dr. Keeley Brookes and colleagues at the Institute of Psychiatry, London, U.K., reasoned that individuals with ADHD might have polymorphisms in their fatty acid regulatory genes that may accelerate the oxidation of LC-PUFAs.

Dr. Keeley Brookes and colleagues at the Institute of Psychiatry, London, U.K., reasoned that individuals with ADHD might have polymorphisms in their fatty acid regulatory genes that may accelerate the oxidation of LC-PUFAs. The investigators looked specifically for single nucleotide polymorphisms in 3 key fatty acid desaturase enzymes in a population of children with ADHD and ethnically matched control subjects. They also collected information on maternal use of alcohol during pregnancy, because alcohol affects the dopamine transporter gene, fatty acid synthesis and oxidation, and may increase the risk of ADHD in the offspring.

The investigators used a DNA pooling approach for initial screening of ADHD and control samples previously validated on the basis of allele frequency differences. DNA was obtained from cheek swabs of children aged 5 to 15 years who were clinically diagnosed with ADHD combined subtype following Diagnostic and Statistical Manual of Mental Disorders-IV criteria with additional evaluation cross-checks. Four DNA pools were constructed with 90 samples in each, 2 ADHD pools, and 1 male and 1 female control pool. Pools were analyzed for allele frequency and individual genotyping carried out for selected markers with the greatest frequency differences among the pools. Yes/no screening questions were used to assess prenatal use of alcohol and tobacco. Genotype data were used to generate case-control differences and test for association.

There are 3 human fatty acid desaturase genes with 45 known single nucleotide polymorphisms, of which 29 were clearly reproducible in the case-control gene pools. In the fatty acid desaturase gene-1 (FADS-1), 3 polymorphisms had frequency differences between the probands and controls greater than 5%. The investigators selected the two largest differences. For FADS-2, one polymorphism differed by more than 5% between the probands and the control and none was greater than 5% for the third FADS-3 gene. In total, 3 single nucleotide markers were individually genotyped. The allele (version of a gene) frequencies in the probands and controls are shown below.

The results of genetic screening for variants in the 3 human fatty acid desaturase genes in samples of children with ADHD and healthy matched controls revealed a strong association between ADHD and increased frequency in the single nucleotide polymorphism rs498793 of the fatty acid desaturase-2 gene compared with controls. This gene codes for

Table. Frequency of fatty acid desaturase alleles in individual genotypes from ADHD and control samples

	ADHD		CONTROL			
Marker	Allele 1	Allele 2	Allele 1	Allele 2	*P*	Odds Ratio (95% CI)
FADS-1						
rs174545	.688	.311	.707	.293	.59	0.91 (.64-1.3)
rs 174548	.712	.288	.713	.287	.9	1.02 (.71-1.46)
FADS-2						
rs498793	.653	.347	.540	.460	.004	1.6 (1.15-2.23)

the delta-6 desaturase enzyme that is the rate-limiting step in the conversion of 18-carbon PUFAs to their long-chain derivatives. It is also involved in the conversion of the n-3 LC-PUFA eicosapentaenoic acid to docosahexaenoic acid.

The investigators noted that the functional effects of the rs498793 polymorphism are unknown. If this alteration led to reduced production or activity of the enzyme, it would imply reduced formation of LC-PUFAs, with accumulation of precursors needing to be oxidized. This could explain the biochemical observations of reduced LC-PUFAs and increased n-3 PUFA oxidation associated with ADHD. Other genetic evidence is consistent with the potential involvement of the FADS-2 gene in ADHD. This report will stimulate additional research into gene-linked alterations of LC-PUFA metabolism in ADHD.

Brookes KJ, Chen W, Xu X, Taylor E, Asherson P. Association of fatty acid desaturase genes with attention-deficit/hyperactivity disorder. Biol Psychiatr *2006;60:1054-1061.*

Chalon S. Omega-3 fatty acids and monoamine neurotransmission. Prostaglandins, Leukot Essent Fatty Acids *2006;75:259-269.*

Autism

Hints that Omega-3 PUFAs May Improve Symptoms in Autism

Autism, and its milder version Asperger syndrome, cover a range of pervasive developmental disorders–hence the term autistic spectrum disorder–which are observed as impaired communication skills, poor social interactions, restricted repetitive behaviors and sleep disturbances. The condition may be present from birth and is four times more common in boys than girls. Autistic children often have serious behavioral problems and self-injurious behavior that undermine their development. Medication has achieved limited improvements, often with unacceptable side effects.

Like children with attention deficit hyperactivity disorder, autistic children have significantly lower levels of docosahexaenoic acid (DHA), a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) in their plasma phospholipids, according to some studies, but not all. There is one report of increased n-3 PUFAs, increased oxidative stress and impaired methylation in autistic children that implicates PUFA metabolism. Others have reported increased lipid peroxidation and reduced levels of major antioxidant proteins in autistic children, observations consistent with increased oxidative stress.

Reports of improved behaviors with n-3 LC-PUFA supplementation in conditions with some symptom overlap with autism prompted Dr. Paul Amminger and colleagues at the Medical University of Vienna, Austria, to conduct a pilot study of supplementation with 1.5 g/day of n-3 LC-PUFAs in 13 children with autism. Twelve children completed the study. Children (all males) ranged from 5 to 17 years of age, with an average age of 10.5 years. All met clinical criteria for autistic disorder and were free of other medical and psychiatric disorders requiring medication. Children receiving psychotropic medications were excluded, but those stable on anticonvulsant medication and without seizures for 6 months were included.

Some studies suggest that autistic children have significantly lower n-3 LC-PUFAs in their plasma phospholipids compared with healthy children.

Children were randomized to consume either fish oil or placebo capsules for 6 weeks. Supplements were provided as 7 capsules/day and contained 1.54 g/day total eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids in the ratio of 1.2 to 1 or coconut oil placebo. The flavor of the placebo capsules was masked with 1 mg fish oil. Behavioral symptoms were assessed at baseline and 6 weeks with the Aberrant Behavior Checklist rating scale, which provided scores for irritability, social withdrawal, stereotypy (repetitive unvaried movements), hyperactivity and inappropriate speech. The assessment scores for both treatment and placebo groups are shown below.

Table. Behavior assessment scores for autistic children after 6 weeks of fish oil or placebo supplementation

	FISH OIL (n=7)			PLACEBO (n=5)		
Behavior	Baseline	6 Weeks	Change	Baseline	6 Weeks	Change
Irritability	29.3 ± 9.2	24.6 ± 8.7	4.7 ± 3.5	26.4 ± 5.7	21.8 ± 2.8	4.6 ± 7.5
Social withdrawal	24.4 ± 12.0	18.9 ± 13.3	5.6 ± 8.1	25.6 ± 4.4	21.0 ± 2.0	4.6 ± 5.6
Stereotypy	14.4 ± 5.1	13.0 ± 5.2	1.4 ± 2.2	7.8 ± 6.4	8.8 ± 4.1	-1.0 ± 3.4
Hyperactivity	33.3 ± 4.8	29.3 ± 5.7	4.0 ± 2.4	24.6 ± 5.5	27.6 ± 5.9	-3.0 ± 9.9
Inappropriate speech	8.3 ± 4.0	7.6 ± 4.0	0.7 ± 3.0	9.0 ± 1.6	9.4 ± 2.9	-0.4 ± 2.9

At the end of 6 weeks, behavior scores in both groups of children changed from baseline values, with lower (improved) scores for each parameter in the fish oil group. Scores for stereotypy and hyperactivity in the placebo group deteriorated over the study period, but improved in the fish oil group. The two groups had similar scores for all behaviors at baseline except in hyperactivity, which was higher in the fish oil group (33.3 vs 24.6). However, none of the changes between the groups reached statistical significance, undoubtedly because of the small sample size. These observations suggest that some behaviors in autistic children, notably hyperactivity, may improve with fish oil consumption. Thus, the study provides some support for further investigating the effects of n-3 LC-PUFAs in autism. Considering the adverse side effects of several current medications for autism and the relatively mild and short-lived effects (mild to moderate stomach upset, diarrhea) of taking numerous (7) fish oil capsules, the potential of n-3 LC-PUFAs should not be overlooked. More concentrated or liquid n-3 LC-PUFA preparations might make compliance easier to achieve.

Amminger GP, Berger GE, Schafer MR, Klier C, Friedrich MH, Feucht M. Omega-3 fatty acids supplementation in children with autism: A double-blind randomized, placebo-controlled pilot study. Biol Psychiatry *2006; In press.*

Alzheimer's Disease

DHA-Rich Omega-3 PUFAs Slow Cognitive Decline in Mild Alzheimer's Disease

Low concentrations of DHA increase the risk of developing Alzheimer's disease. In animals, DHA promotes neuronal cell survival and protects against some pathological changes in the condition. Is DHA an effective treatment for the disease?

Epidemiological evidence suggests that low concentrations of docosahexaenoic acid (DHA), a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA), increase the risk of developing Alzheimer's disease, a debilitating loss of memory and cognition. Brains of Alzheimer's patients have less DHA than those of similarly aged patients without the condition. DHA is the precursor of neuroprotectin D1 in the human brain, a substance that promotes neuronal cell survival and reduces cell apoptosis. In animal models, DHA protects against pathological changes in the dendrites of neurons and reduces beta-amyloid protein formation. It is also involved in brain glucose transport and utilization as well as the regulation of gene expression. Thus, treatment with eicosapentaenoic acid (EPA) or DHA or both might be expected to ameliorate the condition or slow its progress. A pilot study suggested that 3 months' treatment with eicosapentaenoic acid had little effect on cognitive measures in Alzheimer's patients. Until now, however, there have been no reports of randomized controlled trials in which Alzheimer's patients received treatment with EPA or DHA.

Dr. Yvonne Freund-Levi and colleagues at the Karolinska Institute, Stockholm, Sweden, evaluated the effects of 6 months' daily treatment with a high-DHA supplement or placebo in 204 patients (average age 73 years) clinically diagnosed with Alzheimer's disease and living in their own homes. All patients were taking acetylcholine esterase inhibitors and 174 completed the 1-year study. The supplements provided 1.7 g DHA and 0.6 g eicosapentaenoic acid (EPA) or 2.4 g linoleic acid as a placebo. After 6 months, all participants received the n-3 LC-PUFA supplement for another 6 months. Global function was assessed with the Clinical Dementia Rating Scale and cognitive functions with the Mini-Mental State Examination and the modified cognitive portion of the Alzheimer Disease Assessment Scale administered at baseline, 6 and 12 months. Blood samples were collected at the same times.

Alzheimer's patients with very mild disease receiving DHA-rich n-3 LC-PUFAs had a significantly slower rate of disease progression at 6 months compared with the placebo group.

At the end of 6 and 12 months, there were no significant differences between the n-3 LC-PUFA and placebo groups in either test of cognitive function. In subgroup analysis, patients were grouped according to having a Mini-Mental score above or below the median of 24 points and compared. A higher score indicates less severe disease. Again, at 6 months, there was no difference between the placebo and n-3 LC-PUFA groups, but in patients with more advanced disease,

those receiving n-3 LC-PUFAs tended to decline more rapidly than those receiving the placebo (difference not statistically significant). However, patients in the n-3 LC-PUFA group with very mild Alzheimer's disease, i.e., 32 patients with Mini-Mental scores of 27 or greater, had a significantly slower rate of disease progression at 6 months compared with the placebo group (placebo, -2.6 points vs n-3 LC-PUFA, -0.5, P=0.01). When the placebo group received the n-3 LC-PUFAs, their previous decline in Mini-Mental scores stopped.

There were also significant differences after 6 months when specific components of the Mini-Mental Examination were compared in the two groups. The placebo group scores in "delayed word recall" and "attention" declined significantly in the first 6 months, then stabilized from 6 to 12 months after they received n-3 LC-PUFAs, whereas scores in the n-3 LC-PUFA group did not change over the course of the study. Similarly, when assessed by the Alzheimer Disease Assessment Scale, "delayed word recall" declined significantly in the placebo-treated mild disease group in the first 6 months, but not in the n-3 LC-PUFA group. There were no differences between the two groups in overall or attention scores by this evaluation.

Applying *post hoc* subgroup analysis to the patients with the most advanced disease, who appeared to decline more rapidly than patients with mild symptoms, the investigators observed no differences between the placebo and n-3 LC-PUFA groups in either assessment scores or any components of these evaluation measures.

If there is a critical period of disease development before the clinical symptoms of Alzheimer's disease appear, then early exposure to n-3 LC-PUFAs could provide defensive anti-inflammatory and neuroprotective effects against the disease.

What do these suggestive findings mean? In their discussion, the authors noted that positive effects of n-3 LC-PUFAs in Alzheimer's patients with the mildest symptoms would be consistent with epidemiologic studies suggesting a preventive rather than a therapeutic effect of fish consumption or n-3 LC-PUFA intake. Memory decline in patients with mild symptoms treated with placebo halted once the patients received n-3 LC-PUFAs. If there is a critical period of disease development before the clinical symptoms of Alzheimer's disease appear, or significant risk reduction from long-term use of anti-inflammatory substances, as suggested from studies with non-steroidal anti-inflammatory agents, then early exposure to n-3 LC-PUFAs could provide defensive anti-inflammatory and neuroprotective effects against the disease. More controlled studies, begun before the onset of cognitive decline in older people, will be needed to clarify how n-3 LC-PUFAs affect risk and progression of Alzheimer's disease.

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Higher Plasma DHA Linked to Reduced Chance of Alzheimer's Disease

The likelihood of developing some type of dementia—Alzheimer's disease, vascular and Parkinson's dementia—increases exponentially the longer we live. About 70% of all cases of dementia are attributable to Alzheimer's disease, a condition that begins with memory loss and declining cognitive function. Several nutrition-related factors and conditions increase the risk of developing dementia or Alzheimer's disease in aging populations. For example, elevated plasma concentrations of homocysteine, an amino acid, result from insufficient intake of the B vitamins folic acid, vitamin B_6 and vitamin B_{12} and are linked to Alzheimer's disease. Low fish consumption is associated with a greater risk of Alzheimer's disease and may hasten its progression. Other diet-related conditions associated with increased risk of Alzheimer's include elevated cholesterol concentrations in some, but not all studies, cardiovascular disease and the metabolic syndrome and their respective risk factors. Data from studies in cultured cells and animal models indicate the involvement of docosahexaenoic acid (DHA), a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA), in several protective brain cell functions. Until recently, data on biomarkers of DHA status in Alzheimer's patients were scarce. In one report, serum cholesteryl ester-DHA levels were significantly lower in Alzheimer's patients compared with controls and were progressively reduced with the severity of the disease. Another study reported reduced total n-3 LC-PUFAs, DHA and eicosapentaenoic acid in plasma phosphatidylethanolamine in Alzheimer's patients, mild cognitive impairment and other dementias compared with controls.

Several nutrition-related conditions increase the risk of developing dementia or Alzheimer's disease, including elevated plasma homocysteine concentrations, low B vitamin intake, low fish consumption, elevated cholesterol, cardiovascular disease and the metabolic syndrome.

In this report, Dr. Ernst Schaefer and colleagues at the Nutrition Research Center on Aging, Tufts University, Boston, Mass., USA, examined the plasma phospholipid DHA concentrations in a cohort of 899 participants from the Framingham Heart Study, who were monitored for the development of stroke and dementia following their 20th biennial examination. All participants were free of dementia when their baseline plasma samples were obtained. Their average age at baseline was 76 years and 36% were men. There were dietary intake records for 488 participants (54% of participants). All participants were given the Mini-Mental State Examination biennially.

Participants who scored 3 or more points below their most recent mental examination were further assessed for dementia and those whose symptoms were longer than 6 months and whose scores for dementia severity were 1 or higher on the Clinical Dementia Rating Scale were considered incident cases. Participants for whom definite dementia could not be established were assessed annually. The average duration of follow-up was 9 years, during which time 99 cases of dementia–71 diagnosed as Alzheimer's disease–were identified.

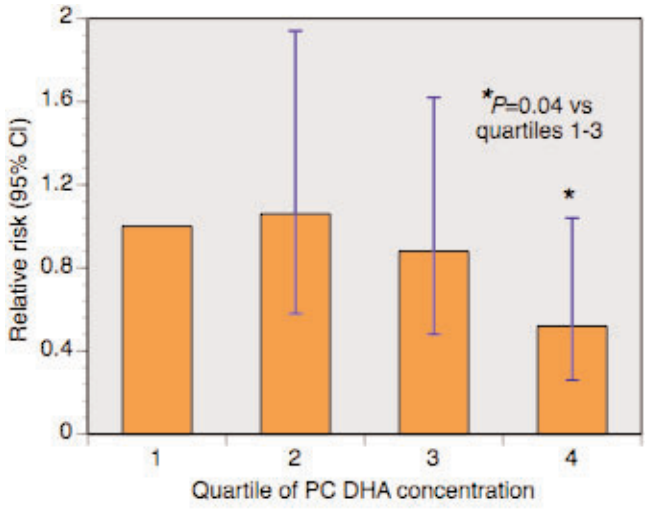

Figure. Relative risk of dementia by quartile of DHA concentration in plasma phospatidylcholine in ederly Framingham study participants.

The investigators calculated the relative risk of developing any dementia for each quartile of baseline plasma phosphatidylcholine (PC, a specific phospholipid) DHA concentration, using log-transformed data and adjusting for age, sex, apolipoprotein E ε4 genotype, homocysteine concentration and education level. Mean plasma PC-DHA levels were 3.5% for all participants and >4.2% for those in the 4th (highest) quartile. The adjusted relative risks by quartile of PC-DHA are shown in the Figure. The chance of developing any dementia in the highest quartile of DHA concentration was half (47% lower risk) that in the lowest quartile of PC-DHA and significantly lower than the three quartiles combined (RR=0.53, CI=0.29-0.97, *P*=0.04). Risk of Alzheimer's disease was 40% lower in the highest DHA quartile, but was not significantly different from the combined other quartiles. With 60 Alzheimer's cases out of 755 participants, there were likely too few to detect a significant effect. No other phospholipid fatty acid was associated with risk of dementia.

Participants in the highest quartile of DHA in their plasma phospholipid had half the chance of developing any dementia as all other participants in the lowest 3 quartiles combined.

The investigators also calculated the relative risks for dementia in terms of fish and DHA consumption, both of which were significantly related to PC-DHA concentrations. Dietary data were available only for a subset of participants (54%). Average fish consumption was 2 servings/week among participants, ranging from 1.3 to 2.9 servings/week in the lowest to highest quartiles. However, risk of dementia or Alzheimer's disease was not significantly associated with fish or DHA consumption. These observations suggest that the PC-DHA biomarker of fish and DHA intake was a more sensitive measure of DHA status than estimated dietary consumption.

If confirmed in future studies, these observations suggest that plasma phospholipid PC-DHA concentrations could be a useful predictor for the likelihood of developing dementia. It would be useful to know whether the relationship reported here holds for whole red blood cell fatty acid composition. It is of particular interest that Michael Selley, Angiogen Pharmaceuticals, Australia, recently reported significantly reduced red blood cell phospholipid PC-DHA in Alzheimer's disease patients compared with healthy controls. Further, red cell PC was significantly decreased, while phosphatidylethanolamine was increased in these patients. These changes were linked to impaired homocysteine metabolism and its effect on inhibiting the conversion of phosphatidylethanolamine to PC in the liver. A reduction in this conversion would reduce mobilization of DHA from liver into plasma and peripheral tissues that occurs mainly via PC-phospholipid. Such events would connect the risks of atherosclerosis, stroke and neurodegenerative conditions.

Schaefer EJ, Bongard V, Beiser AS, Lamon-Fava S, Robins SJ, Au R, Tucker KL, Kyle DJ, Wilson PW, Wolf PA. Plasma phosphatidylcholine docosahexaenoic acid content and risk of dementia and Alzheimer disease: the Framingham Heart Study. Arch Neurol *2006;63:1545-1550.*

Selley ML. A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease. A metabolic link between S-adenosylhomocysteine and polyunsaturated fatty acid metabolism in Alzheimer's disease. Neurobiol Aging *2006;24:903-907.*

Type 2 Diabetes

Meta-Analysis: Fish Oil Lowers Diastolic Blood Pressure, Raises Factor VII in Type 2 Diabetics

Because the effects of n-3 PUFAs on hemostasis appear to be modest and inconsistent, this aspect of n-3 PUFA metabolism receives comparatively little attention.

Blood clot formation is a key component of thrombosis, a leading cause of heart attack and stroke. Several studies have shown that the consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) reduces the tendency toward blood clotting and may hasten the dissolution of clots once they form. Some of the anti-coagulant effects of these fatty acids may be mediated through reduced thrombin generation, lower fibrinogen and interactions with other clotting factors, independent of vitamin K mechanisms. n-3 LC-PUFAs also lower the production of prothrombotic thromboxanes and prostaglandins, reducing the tendency for blood clotting. However, several studies have failed to detect effects of n-3 LC-PUFAs on hemostatic factors in healthy, hyperlipidemic or diabetic individuals. Because the effects of n-3 PUFAs on thrombosis appear to be modest and inconsistent, this aspect of n-3 PUFA metabolism receives comparatively little attention.

Obesity, hypertension and hyperlipidemia frequently accompany type 2 diabetes and contribute to the 2- to 4-fold increased cardiovascular risk observed in this condition. Fish oils rich in n-3 LC-PUFAs are associated with improved lipid profiles and lower blood pressure in diabetic patients with these characteristics. Epidemiologic evidence suggests that fish or n-3 LC-PUFA consumption reduces risk of cardiovascular disease and its progression in type 2 diabetes, but data from intervention trials are scarce.

Dr. Janine Hartweg and colleagues at the University of Oxford performed a systematic review and analysis of the current information about n-3 LC-PUFAs (marine-derived) and hematological and thrombogenic factors in people with type 2 diabetes. Their review included 12 randomized, placebo-controlled trials that were evaluated for quality by considering the method of randomization, blinding or objective measurements, loss to follow-up, and systematic difference in care between intervention groups. Outcomes included blood pressure, fibrinogen, heart rate, factor VII, C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), platelet function, von Willebrand factor and endothelial function, adhesion molecules and selectins.

From 189 papers considered for inclusion, 12 met the inclusion criteria, providing results on 56 conventional and emerging risk markers in 847 participants with type 2 diabetes. Nine of the 12 papers (297 participants) measured the same outcomes and permitted pooling of 5 risk markers: plasma fibrinogen, systolic and diastolic blood pressure, heart rate and Factor VII. In these studies, the mean dose was 4.3 g/day (range 0.9 to 10 g/day) and average trial duration was 8.5 weeks. The median number of patients was 40.

The pooled analysis suggests that supplementation with n-3 LC-PUFAs derived from marine oil significantly reduces diastolic blood pressure by about 2 mm Hg. The effects of n-3 LC-PUFAs on systolic blood pressure, plasma fibrinogen and heart rate favored treatment, but did not reach statistical significance. Two other analyses of fish oil and blood pressure concluded that fish oil lowers blood pressure, with a greater reduction in individuals with higher elevations in pressure and larger doses of fish oil. These analyses were not restricted to diabetic participants, but a subgroup analysis of diabetic patients in one study reported a significant reduction in pressure.

The pooled analysis suggests that supplementation with n-3 LC-PUFAs derived from marine oil significantly reduces diastolic blood pressure by about 2 mm Hg, but raises Factor VII.

In Hartweg's meta-analysis, heart rate was not significantly reduced by fish oil consumption based on 2 studies. In contrast, a recent meta-analysis of controlled trials of fish oil and heart rate not restricted to diabetic patients reported a significantly reduced heart rate of 1.6 beats/min with fish oil treatment. The reduction was greater in participants whose baseline rate was at least 69 beats/min.

The other significant effect of n-3 LC-PUFAs in Hartweg's study was increased Factor VII, an effect favoring blood clotting. It is uncertain whether this change by itself, based on 2 trials, has a net adverse effect on blood clotting, as favourable changes in other clotting factors, e.g., reduced prothrombin factor X might offset this change.

For many variables examined there were too few trials with too few participants to obtain pooled estimates. In discussing the various limitations of their and others' analyses, the authors pointed out that small improvements in clinical measures not reaching statistical significance might still have important effects on a population-wide basis. This thorough data collection and analysis clarify the research gaps and some reasons why conclusions in this field have been elusive. Although

patients with type 2 diabetes have significantly increased risk of heart disease, they may not respond similarly to non-diabetic patients with high cardiovascular risk. As this analysis demonstrates, data on patients with type 2 diabetes are insufficient to answer important clinical questions. Does this high-risk group respond to n-3 LC-PUFAs similarly to non-diabetic patients at high risk of heart disease? The rapidly increasing worldwide prevalence of type 2 diabetes warrants an answer. There is an urgent need for potentially beneficial ways to prevent and ameliorate this condition and we should know if increased consumption of n-3 LC-PUFAs is one of them.

Hartweg J, Farmer AJ, Holman RR, Neil HAW. Meta-analysis of the effects of n-3 polyunsaturated fatty acids on haematological and thrombogenic factors in type 2 diabetes. Diabetologia *2006; In press.*

FRONTIERS

Transgenic *Fat*-1 Mouse Protected Against Induced Colon Inflammation

In theory, long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) might be useful in the treatment or amelioration of inflammatory bowel diseases because of their multiple anti-inflammatory effects. In practice, results are inconclusive, but have been associated with reduced corticosteroid requirements, less body pain, and prolonged remission of Crohn's disease in children. Administration of n-3 LC-PUFAs improved the balance between n-6 and n-3 PUFAs, reduced some mediators of inflammation, but aggravated the disease in various studies. In contrast to the inconsistent findings in human studies, those conducted in animal models of the disease have reported more positive results with n-3 LC-PUFAs. Further complicating the picture is the report that n-3 LC-PUFAs are more concentrated not less in peripheral blood mononuclear cells of patients with Crohn's disease. Given the high ratio of n-6 to n-3 PUFAs in western diets and the generally pro-inflammatory effects of n-6 PUFAs, better understanding of the involvement of dietary LC-PUFAs in inflammatory bowel diseases is needed. Recently identified n-3-LC-PUFA-derived anti-inflammatory lipoxins and resolvins suggest additional ways in which n-3 LC-PUFAs may ease the symptoms and progress of these diseases.

In contrast to the inconsistent findings in human studies, those conducted in animal models of inflammatory bowel disease have reported more positive results with n-3 LC-PUFAs.

A unique animal model for examining inflammatory processes in these diseases is the transgenic *fat-1* mouse, which converts n-6 to n-3 PUFAs. Normally, mammals cannot perform this conversion. Developed by Jing Kang and colleagues at the Massachusetts General Hospital, Boston, USA, the *fat-1* mouse has lower concentrations of n-6 PUFAs in most tissues and significantly higher n-3 PUFA concentrations, including the LC-forms. In this report, concentrations of arachidonic acid were similar in the colons of transgenic and wild-type mice (Table). With the exception of skeletal muscle, docosahexaenoic acid (DHA) is the predominant n-3 LC-PUFA in *fat-1* mice. The Table shows the LC-PUFA concentrations in colons of wild-type and *fat-1* mice fed a 10% safflower oil diet.

Colitis was induced in wild-type and transgenic mice using 3% dextran sulphate sodium in sterile drinking water for 5 days followed by 3 days without treatment. Typical symptoms of the disease were observed in both groups of mice, but were significantly less severe in *fat-1* mice compared with wild-type mice. *fat-1* mice lost significantly less body weight (about 5%) from day 5 through day 8 compared with wild-type mice (about 17%). Colon shortening was 15% in the *fat-1* mice versus 35% in the wild-type animals (Figure). Likewise, severity and thickness of the inflammatory infiltrate were significantly less in *fat-1* mice (Figure).

Table. LC-PUFAs (% total fatty acids) in colons from wild-type and *fat-1* transgenic mice

LC-PUFA	Wild-type	*fat-1*
n-6		
AA, 20:4	12.7 ± 2.9	12.5 ± 1.9
DTA, 22:4	3.0 ± 0.3	2.2 ± 0.4
DPA, 22:5	3.0 ± 1.1	0.6 ± 0.2
Total n-6	18.6 ± 4.9	15.2 ± 2.2
n-3		
EPA, 20:5	0.1 ± 0.0	2.0 ± 0.5
DPA, 22:5	0.1 ± 0.2	2.5 ± 0.6
DHA, 22:6	0.4 ± 0.1	4.7 ± 0.4
Total n-3	0.6 ± 0.2	9.9 ± 1.2

The *fat-1* mice began recovering from the dextran treatment on the second day after the treatment stopped, whereas the wild-type mice continued to lose weight and deteriorate. Transgenic mice synthesized anti-inflammatory mediators resolvins E1 and D3 neuroprotectin D1, prostaglandin E_3 and leukotriene B_5 in physiologically active amounts, but wild-type mice did not. The groups did not differ in concentrations of prostaglandin E_2 and leukotriene B_4 and the precursor of lipoxin A4, 15-hydroxyeicosatetraenoic acid.

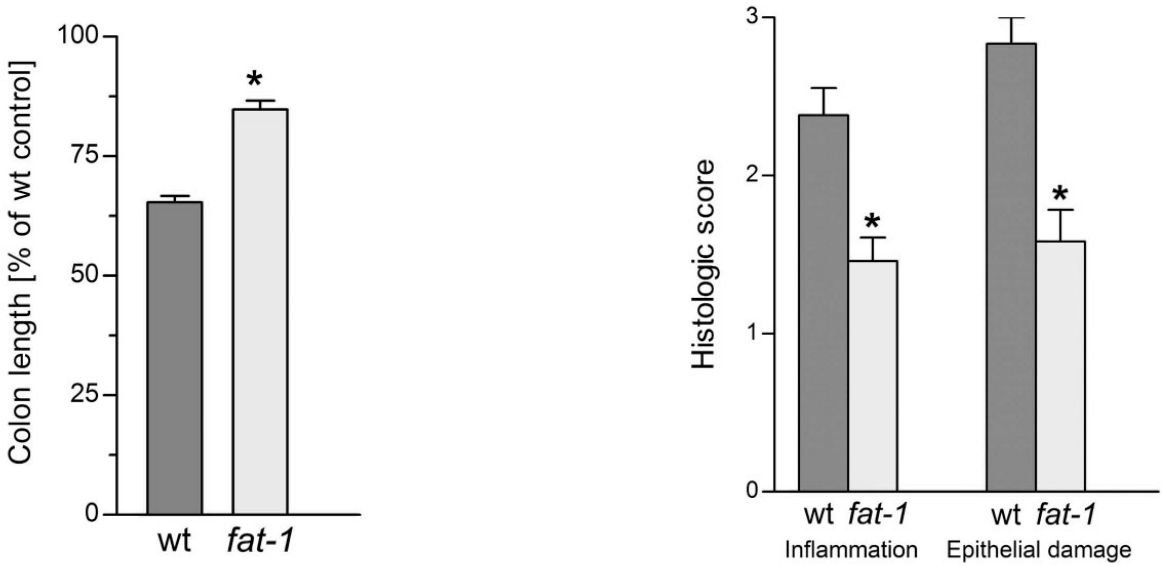

Figure. Colon shortening (left) and colon histology (right) in wild-type (dark bars) and transgenic *fat-1* mice after dextran treatment. *$P<0.01$.

Changes in gene expression in *fat-1* but not in wild-type mice were consistent with decreased inflammatory activity. For example, expression of genes for pro-inflammatory substances NF-κβ, TNFα, NO synthase and IL-1β were all dampened in the *fat-1* mice, while several genes involved in the repair and maintenance of intestinal mucosa and epithelial integrity were enhanced.

Taken together, these results provide clear evidence of more favorable outcomes in chemically induced inflammatory bowel disease in animals having significantly higher n-3 LC-PUFA concentrations. Interestingly, concentrations of arachidonic acid, prostaglandin E_2 and leukotriene B4 were unchanged in the transgenic mice compared with wild-type mice. It has previously been reported that human peripheral blood mononuclear cells from patients with Crohn's disease have lower concentrations of arachidonic acid and lower rates of production of prostaglandin E_2 and interferon-gamma compared with controls. This would suggest that mechanisms involving cellular integrity, the regulation of inflammatory processes and the production of guardian substances such as resolvins and protectins might be key in resisting inflammatory bowel diseases. Higher concentrations of n-3 LC-PUFAs would facilitate these protective mechanisms.

Transgenic mice synthesized anti-inflammatory mediators in physiologically active amounts, but wild-type mice did not. Changes in gene expression in fat-1 *but not in wild-type mice were consistent with decreased inflammatory activity.*

These investigators suggested that cytokine production is a key factor in the development and progress of inflammatory bowel diseases and their finding of reduced inflammatory cytokines TNF-α and IL-1β is consistent with this view. Long-term supplementation with high levels of n-3 LC-PUFAs reduced several cytokines by more than 60% in patients with colorectal cancer and procto-colitis. Both suppression of adverse inflammatory effects and enhancement of protective measures are fostered by n-3 LC-PUFAs. The authors discuss other balancing mechanisms involving n-3 LC-PUFAs that might contribute to enhanced colon protection in a complex physiologic environment. Whether the remarkable alterations in the distribution of n-6 and n-3 PUFAs observed in the *fat-1* mouse could be obtained by dietary means remains an open question.

Hudert CA, Weylandt KH, Lu Y, Wang J, Hong S, Dignass A, Serhan CN, Kang JX. Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis. Proc Natl Acad Sci USA *2006;103:11276-11281.*

Reduced Melanoma in Transgenic *Fat*-1 Mice with Higher Omega-3 and Lower Omega-6 PUFAs

Studies in human populations, in vitro *and* in vivo *have yielded mixed findings about n-3 LC-PUFAs in melanoma.*

The potential value of long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFAs) in reducing the risk, growth or spread of cancer has been recognized for many years, but studies in human populations have yielded mixed findings. A strong inverse association between total PUFA consumption and melanoma has been reported. The same study reported a 40% lower risk of melanoma with fish consumption of 15 g/day or higher, but in the small sample this association was not statistically significant. *In vitro* studies have reported reduced invasiveness of melanoma cells in the presence of eicosapentaenoic acid (EPA), inhibition of melanin production by alpha-linolenic and linoleic acids, inhibition of melanoma cell growth by docosahexaenoic acid (DHA) in some cell lines but not in others, but *in vivo*, EPA enhanced tumor growth and metastasis in mice inoculated with B16 melanoma cells. N-3 LC-PUFAs reduced cyclooxygenase-2 production in cultured 70W melanoma cells and reduced their invasiveness, whereas arachidonic acid had the opposite effect. Other studies have reported that DHA enhanced the effectiveness of other anti-tumor agents. Such discordant findings call for rigorous experimental designs using a variety of tumor lines and different levels of n-6 and n-3 PUFAs to sort out the relationships of these fatty acids in the development and spread of melanoma.

A novel approach was undertaken by Dr. Shuhua Xia and colleagues at the Massachusetts General Hospital in Boston, USA, who studied the responses of transgenic mice and wild-type mice implanted with the B16 melanoma cell line. The transgenic mice were genetically modified to express the *fat-1* gene, which allows them to convert n-6 PUFAs to n-3 PUFAs, a transformation mammals cannot ordinarily do. Thus, *fat-1* transgenic mice had lower tissue concentrations of n-6 PUFAs and higher concentrations of n-3 PUFAs, but arachidonic acid—although significantly lower in the *fat-1* mice—tended to be conserved. The transgenic mice were compared with wild-type littermates and all were fed the same 10% safflower oil diet.

Compared with wild-type mice, stromal tissue from *fat-1* mice had significantly reduced concentrations of arachidonic acid and docosapentaenoic acid (n-6) and significantly increased concentrations of all n-3 PUFAs. Linoleic acid, the predominant PUFA in stromal tissue, was similar in both wild-type and transgenic mice (27.5% vs 25.1%, respectively). In the tumors of both groups of mice, n-6 PUFAs were distributed approximately equally between linoleic and arachidonic acids, resulting in a 6-fold increase in tumor arachidonic acid concentration compared with the supporting stromal tissue. However, arachidonic acid concentrations were significantly lower in the tumors of *fat-1* compared with the wild-type mice (9.1% vs 12.8%, respectively, $P<0.05$). N-3 LC-PUFA concentrations, particularly of DHA, were significantly 2-fold higher in tumors of *fat-1* mice than in wild-type animals (1.3% vs 0.6%, respectively).

Three days after melanoma cells were implanted, all wild-type mice had a palpable tumor, but only 7 of the 10 fat-1 *mice had a palpable tumor by day 7 or 10.*

Seven and 15 days after the melanoma cells were implanted, tumors in the *fat-1* were less numerous and smaller than in the wild-type mice. By day 3, all wild-type mice had developed a palpable tumor, but only 7 of the 10 transgenic mice had a palpable tumor by day 7 or day 10. Tumor growth and volume in the *fat-1* mice was significantly slower and smaller than in the wild-type mice.

Parallel to the differences in n-6 and n-3 PUFA concentrations in the two types of mice, concentrations of prostaglandin E_2 (derived from arachidonic acid) were significantly higher in wild-type than in *fat-1* mice in stromal and tumor tissues (Figure). In tumor tissue, prostaglandin E_2 was approximately 3 times more abundant in wild type than in *fat-1* mice. In the *fat-1* mice, prostaglandin E_3 (derived from EPA) was by far the predominant prostaglandin in stroma and tumor tissues with almost none present in wild-type animals in either tissue (Figure).

The investigators also examined the tissue concentrations of PTEN, a phosphatase enzyme that suppresses tumors by down-regulating the AKT/PKB signaling pathway. Although this enzyme was barely detectable in the B16 melanoma cells, it was markedly increased in the melanoma tumors of the *fat-1* mice, suggesting its involvement in suppressing tumor growth and development in these animals. PTEN was minimally detected in the wild-type animals.

In another series of experiments, the researchers examined the effects of prostaglandins E_2 and E_3 on the growth and PTEN production of cultured B16 melanoma cells. Exposure to prostaglandin E_2 did not affect cell growth at any time, but prostaglandin E_3 significantly reduced growth by 30% to 40% at 48 and 72 hours. The investigators showed with flow cytometry that prostaglandin E_3 induced cell apoptosis. PTEN expression increased dramatically, as observed by Western blotting analysis. When arachidonic acid or EPA was added to

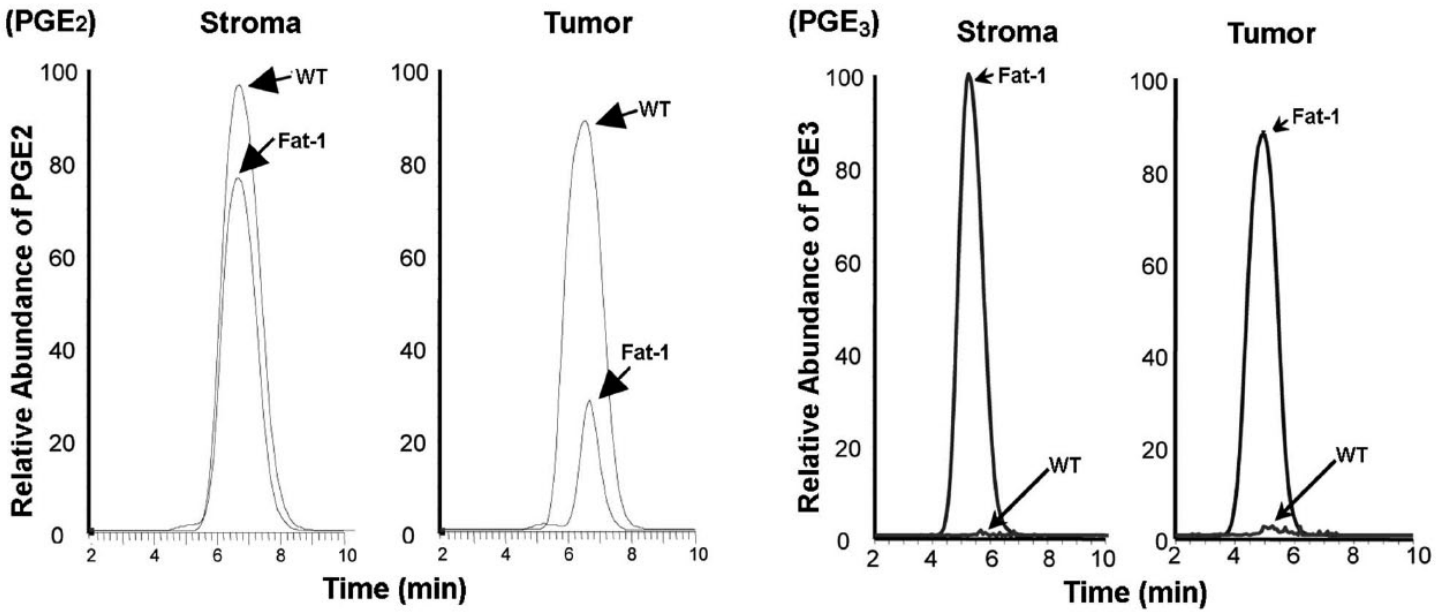

Figure. Prostaglandin E_2 (left) and E_3 (right) in stroma and tumors from wild-type and *fat-1* mice.

DHA was 4 times more abundant than EPA in both stromal and tumor tissue and may have anti-tumor effects in this animal model that were not examined.

the culture, only EPA markedly inhibited cell growth, an effect that was blocked by indomethacin, an inhibitor of cyclooxygenase, the enzyme that converts EPA to prostaglandin E_3. Thus, both prostaglandin E_3 and the PTEN pathway appear to be involved in the reduced tumor growth and development in the *fat-1* mouse. The authors noted the report that prostaglandin E_3 inhibited the proliferation of lung cancer cells *in vitro* and commented that PTEN mutations are common in various human tumors

It should be noted that the predominant n-3 LC-PUFA in the *fat-1* mouse, DHA, was 4 times more abundant than EPA in both stromal and tumor tissue. DHA has been reported to inhibit the growth of some melanoma cell lines. Nevertheless, EPA metabolites and other substances affected by these metabolites were associated with tumor suppression. It is possible that DHA might also have anti-tumor effects in this animal model that were not examined.

Another factor to keep in mind is that the B16 melanoma cells used in this study are derived from the C57B1/6 mouse strain and may have different characteristics from human melanoma lines, which themselves exhibit different metastatic behavior and responsiveness to n-3 LC-PUFAs. These experiments have created a large opportunity for additional research on the effects of n-3 LC-PUFAs in human melanomas and raise important questions about appropriate levels of n-6 PUFAs in these processes. With western diets providing 10 to 20 times or more n-6 as n-3 PUFAs we need a clearer understanding of the implications of this dietary pattern.

The encouraging findings from this research showed that in a transgenic model of reduced tissue n-6 LC-PUFAs and increased n-3 LC-PUFAs development of B16 melanoma tumors was significantly inhibited through mechanisms directly related to the metabolism of EPA. The advantage of this model is the simultaneous decrease in n-6 LC-PUFAs with a concomitant increase in n-3 LC-PUFAs. As the investigators pointed out, changes in both n-6 and n-3 LC-PUFAs may have a dual effect, reducing the production of n-6 LC-PUFA-derived cancer-promoting substances (e.g., prostaglandin E_2) and increasing the production of tumor suppressors, such as prostaglandin E_3 and PTEN. What it cannot reveal is whether supplementation with n-3 LC-PUFAs alone, or at various concentrations of n-6 LC-PUFAs, would have similar results. For applications in people, diet modification and supplementation are the available strategies.

Xia S, Lu Y, Wang J, He C, Hong S, Serhan CN, Kang JX. Melanoma growth is reduced in fat-1 transgenic mice: Impact of omega-6/omega-3 essential fatty acids. Proc Natl Acad Sci USA *2006;103:12499-12504.*