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Biennial ISSFAL Meeting Updates Fatty Acid Research

Every other year the International Society for the Study of Fatty Acids and Lipids (ISSFAL) convenes for the presentation of current research in the field. This year’s meeting in Maastricht, Netherlands, focused on lipids in metabolic health and disease with a series of presentations on conditions such as the metabolic syndrome and obesity, brain function and mental health, fatty acid metabolism and lipid signaling, maternal and infant health, neuro-inflammation and nutrigenomics among others.

Evidence is growing to link dietary omega-3 fatty acids with lower insulin resistance, less fat deposition and improved glucose tolerance in the metabolic syndrome. Other participants reported that animals fed less linoleic acid than is found in western diets and then switched to a western diet had 27% less fat, fewer fat cells and lower blood triglycerides than control animals. It was suggested that increased consumption of omega-3 fatty acids might prevent the metabolic abnormalities associated with the metabolic syndrome, but that once diabetes developed, the effect of omega-3 fatty acids was much less.

Several presentations argued that omega-3 fatty acids, especially the long-chain ones eicosapentaenoic and docosahexaenoic acids (EPA, DHA, respectively) might improve cognitive function in older adults. Evidence from one study observed that older adults with mildly impaired cognition had less EPA, but more omega-6 docosapentaenoic acid, in their red blood cells. The latter was correlated with poorer performance on two verbal tests and auditory learning. Higher levels of omega-6 docosapentaenoic acid usually occur only in omega-3 fatty acid insufficiency.

A provocative presentation raised the possibility that insufficient dietary DHA in the US military might be linked to higher soldier deaths from suicide than from combat. If established, such findings would support current evidence that low DHA status is a risk factor for suicide.

This issue of the PUFA Newsletter returns to the question of whether omega-3 fatty acids are associated with lower total mortality and asks whether the question is relevant. Another article confirms reports of the low intakes of DHA in US toddlers, but reports significantly fewer cases of bronchiolitis among infants with the highest levels of DHA in their red blood cells.

Two papers identify new clinical conditions associated with omega-3 fatty acids. One suggests that premenopausal women with higher intakes of omega-3 fatty acids have a significantly lower chance of developing endometriosis. Another links higher long-chain omega-3s with a lower risk of age-related hearing loss. In another article, authors from the University of Oklahoma describe mutation in a retinal protein that results in a rare type of macular degeneration. This abnormality results in a failure to make very-long-chain polyunsaturated fatty acids via chain-lengthening. The investigators showed that this mutation is not involved in the synthesis of DHA, which also requires chain elongation. What are these very-long-chain fatty acids doing in the retina?
Cardiovascular Health

Fish or Marine Omega-3 Intake Linked to Reduced Heart Failure or Coronary Syndrome

Two more observational studies in large populations have confirmed previous reports that middle-aged and older adults with the highest intakes of fish or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) are significantly less likely to develop heart failure or acute coronary syndrome. In heart failure, cardiac pumping is so impaired that there is insufficient blood to meet the body’s needs. Acute coronary syndrome is an umbrella category that includes patients who suffer a myocardial infarction or have unstable angina pectoris, chest pain caused by too little blood flow. Both conditions significantly increase the risk of cardiovascular mortality.

In the first study reported by Emily Levitan and colleagues in the U.S. and Sweden, 36,234 women 48 to 83 years of age, with no history of heart failure, myocardial infarction or diabetes were monitored for 9 years through the Swedish cause-of-death registers. Primary outcomes were hospitalizations or deaths from heart failure identified by codes from the International Classification of Disease. Five categories of fish consumption were assessed from a food frequency questionnaire administered at baseline from which the intakes of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were estimated. The investigators focused their attention on the consumption of fatty fish like herring, mackerel, salmon, whitefish and char, which are more commonly consumed in Sweden compared with other regions and countries. Relative risks of heart failure hospitalization or morality were calculated using those who never consumed fatty fish or the first quintile of marine omega-3 fatty acid intakes as the reference.

During the follow-up period, there were 596 hospitalizations for heart failure and 55 deaths, equivalent to a rate of 20 cases per 10,000 person-years. There was a progressive decrease in the relative risk of heart failure or death with increasing fatty fish consumption up to 2 servings a week, which was associated with a statistically significant 30 percent risk reduction in analysis adjusted for multiple confounding variables (Figure).

The authors suggested a potential threshold of 400 mg of n-3 LC-PUFAs per day, based on cubic spline analysis. This is higher than the threshold of 250 mg of EPA plus DHA per day determined in a meta-analysis of prospective cohort trials and coronary heart disease mortality by Mozaffarian and Rimm.

Other studies reported lower rates of heart failure with tuna consumption in elderly US men and women and with total fish and total n-3 LC-PUFA consumption in Japanese men and women. Another US study found no association between total fish intake and risk of heart failure with fish or marine omega-3 fatty acid intake in men and women 48 to 83 years of age.

When the relationship between heart failure or death was analyzed in terms of n-3 LC-PUFA consumption, there was a significant inverse trend with increasing n-3 LC-PUFA intake, accounting for a 25% reduction in risk in the highest intake group. The median intake of n-3 LC-PUFAs in this quintile was 570 mg/day. Notable among these results was the absence of any additional risk reduction with the consumption of fatty fish 3 or more times a week compared with twice a week. However, the lowest chance of heart failure or death (RR = 0.75, 95% CI, 0.58-0.96) was observed in those with the highest intake of n-3 LC-PUFAs.

Figure. Relative risks of heart failure or death with fish or marine omega-3 fatty acid intake in men and women 48 to 83 years of age.

Consuming fatty fish or the long-chain omega-3s in them twice a week was associated with a 25% lower chance of developing heart failure compared with individuals who ate no fish.

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failure in US adults, but did not distinguish among different types of fish consumed.

The relationships between fish or fatty fish consumption and heart failure or acute coronary syndrome are inconsistent. A recent report found that neither fish nor n-3 LC-PUFA consumption was associated with reduced heart failure in older adults. Others reported that n-3 LC-PUFA treatment resulted in modest improvements in lifespan among patients with chronic heart failure. On balance, fatty fish or n-3 LC-PUFA consumption favors a lower chance of heart failure in observational studies, but the differences among patients, lifestyle factors and family history along with the relative insensitivity of dietary intake estimates, especially for low fish consumption, make it difficult to detect statistically significant associations.

The associations between fish or n-3 LC-PUFA intakes and acute coronary syndrome report are more consistent. One study from Norway used the omega-3 index, a measure of EPA plus DHA in red blood cells, to evaluate the recurrence of myocardial infarction in patients hospitalized with acute coronary syndrome. Over a 2-year period, the investigators found no significant reduction in all-cause or cardiac death with increasing omega-3 index values. In contrast, others have reported a significant inverse association between the omega-3 index and acute coronary syndrome in patients with depressive symptoms. The same laboratory reported that red blood cell linoleic acid was inversely related to acute coronary syndrome, but higher levels of trans oleic acid were associated with greater risk. A different study reported that long-term fish and fish oil consumption was associated with a lower risk of cardiovascular events in the 30-day period following an acute coronary event in acute coronary syndrome patients.

The intake of total fish or lean fish was not linked to the risk of acute coronary syndrome. The study sheds no light on the possible effect of fatty fish consumption on arrhythmias or sudden cardiac death because of the small number of cases of fatal myocardial infarction.

These recent studies support the advantage of consuming fatty fish or marine omega-3 fatty acids for the protection of heart health.


Weak Link Between Seafood Omega-3 Consumption and Coronary Calcification

The deposition of calcium in the coronary vessels is a biomarker for the risk and severity of coronary heart disease and a strong predictor of cardiovascular events including myocardial infarction. The amount of calcium deposits correlates with the amount of atherosclerosis. Experimental studies have suggested that omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs)...

Men over the age of 50 who ate at least 12 g/day of fatty fish were 30% less likely to develop acute coronary syndrome compared with men who did not eat fatty fish. Eating lean fish was not linked to a lower risk.

Doctors sometimes screen for the amount of calcium deposits in the coronary arteries. These are strongly linked to future cardiovascular events. The question is whether their formation is less in people who eat fatty fish.
may reduce arterial calcification, but no associations with n-3 LC-PUFA intakes and markers of atherosclerosis were observed in a Japanese study or a large US study.

This report on coronary calcium comes from the Rotterdam Coronary Calcification Study, part of the population-based, prospective, cohort study examining the risk factors for chronic diseases in participants aged 55 or older. This investigation focused on the relationship between the consumption of fish and n-3 LC-PUFAs and estimates of coronary calcification based on electron beam computed tomography (Figure). After exclusions for incomplete data and prevalent heart disease, 1,570 participants remained in the study. The average age of the participants was 64 years.

Food intake data were collected using a food frequency questionnaire at baseline with arterial scanning conducted an average of 7 years after the baseline examinations. Calcium scores were calculated according to Agatston’s method with a total score obtained for the entire epicardial coronary system. Three categories of coronary calcification severity were defined as none or minimal having a score ≤ 10, mild or moderate between 11 and 400, and severe scoring above 400. Fish consumption was separated into none (reference), 1 to 19 g/day or more than 19 g/day, about two-thirds of an ounce. The investigators estimated the risk of moderate or severe cardiac calcification based on proportional hazards regression, which they converted to a prevalence ratio by assigning a constant risk period to all participants. They used different models for their calculations based on an increasing number of confounding variables.

Participants’ fish consumption was low, with a median of 12 g daily, equivalent to less than one serving a week. The median intake of eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) was 97 mg/day, with about 30% of the participants consuming no fish. Calcium scores were highly skewed, with 39% of men and 18% of women having severe scores. Prevalence ratios for mild calcification, adjusted for the greatest number of confounding variables, decreased with greater fish consumption and were 13% lower in those consuming more than 19 grams of fish a day compared with non-fish eaters, \( P = 0.03 \). The trend was similar for severe calcification, but did not reach statistical significance. The consumption of EPA plus DHA was not significantly associated with any level of coronary calcification.

In sub-group analyses, the association between fish consumption and lower coronary calcification was more pronounced for women, with prevalence ratios for moderate or severe calcification of 0.81 and 0.77, respectively. No association was observed in men, or in either sex for EPA plus DHA intake and calcification score.

The authors cautioned that the variety of fish consumed by this population sample was probably limited, with cod being the most popularly consumed fish. Preparation by frying might also have reduced the chance of finding a significant association. The association between fish consumption and coronary calcification was weak and occurred mainly in women. It might also be attributable to non-lipid components in fish, such as selenium.


Is Total Mortality Lower with Higher Long-Chain Omega-3s and Coronary Heart Disease?
This question has not been resolved since the publication by Hooper and colleagues of a controversial systematic review of the evidence linking omega-3 polyunsaturated fatty acids (n-3 PUFAs) with cardiovascular events and total mortality. The review did not distinguish between shorter or long-chain (LC) omega-3 PUFAs. The authors concluded, “long or shorter chain omega-3 fats do not have a clear effect on total mortality [or] combined cardiovascular events.” Others had reached a similar conclusion for postmenopausal women and for men with acute myocardial infarction.
In contrast to these conclusions on all-cause mortality, many papers reported significantly reduced risks of fatal coronary heart disease and sudden cardiac death with greater n-3 LC-PUFA consumption in populations without heart disease or in those with heart failure or at high risk for myocardial infarction or sudden death.

The issue of whether or to what extent omega-3 fatty acids affect all-cause (total) mortality has returned in five recent papers. All address the question in populations with various forms of cardiovascular disease or at high risk of it. Weighing the evidence about this question is important for assessing the validity of certain claims for the health effects of eating fish or omega-3 fatty acids and for a larger understanding of the range of clinical conditions influenced by these nutrients.

The study by James Pottala and colleagues monitored an ethnically diverse population of 956 adults with clinically confirmed coronary artery disease who had not had an acute coronary syndrome (myocardial infarction or angina pectoris) in the preceding 6 months. Participants had their fatty acids, lipids and inflammatory markers measured in whole blood and were followed for a median of 6 years. In that time, 25 percent or 237 participants died. The investigators grouped participants by whether their long-chain omega-3 values for eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) were above or below the median value of 3.6 percent. Then they calculated hazard ratios for all-cause mortality, adjusting for an increasing number of confounding variables in their statistical models.

The reduction in the risk of death from any cause (39%, \(P < 0.0001\)) with higher levels of EPA and DHA was greatest in the model with the least number of adjustments for confounding variables and decreased to 26% (\(P < 0.05\)) with adjustment for the most variables. The authors concluded that these observations further support the evidence that lower levels of EPA and DHA confer greater risk for all causes of death in patients with coronary heart disease.

Mari Manger and her colleagues examined the relationship between fish or total long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) and the risk of major coronary events or mortality in 62-year-old Norwegian patients being treated for coronary artery disease. Most (85%) of the participants had stable angina. There were 2,412 participants who were followed for a median period of 57 months.

This population is known for its high fish and cod liver oil consumption. The calculated intakes of n-3 LC-PUFAs by quartiles ranged from 0.6 g/day in the lowest quartile to 2.6 g/day including both dietary and supplemental sources. Over nearly 5 years, there was no association between the intake of n-3 LC-PUFAs and all-cause mortality, coronary death, acute myocardial infarction or verified progression of coronary artery disease. Neither was there a dose-response relationship between outcomes and the amount of n-3 LC-PUFAs consumed. However, post hoc analysis of the hazard ratios for coronary events and n-3 LC-PUFA intakes suggested a significantly greater risk in the lowest quartile of n-3 LC-PUFA consumption (about 300 mg/day).

If one compares these results with the suggested threshold level of 250 to 500 mg/day for EPA plus DHA postulated by Mozaffarian and Rimm for the prevention of coronary death, Manger’s results lean toward the consumption of about 500 mg/day of n-3 LC-PUFAs for the prevention of coronary and all-cause mortality.

Gunnar Einvik studied a group of elderly Norwegian men aged 64 to 76 years at high risk of developing cardiovascular disease. The 563 men were randomized to consume 2.6 g/day of n-3 LC-PUFAs for 3 years. Total mortality in those consuming n-3 LC-PUFAs was compared to a similar group taking a corn oil placebo. In an analysis adjusted for multiple confounding variables, the hazard ratio for consuming n-3 LC-PUFAs was 0.53 (95% CI, 0.27 to 1.04), which was not statistically significant. In spite of the lack of statistical significance, with only 38 deaths, the reduced chance of mortality was substantial and suggestive of a significant effect on a larger scale.

In another small study of patients with acute myocardial infarction, investigators in Seoul, Korea, led by Sang-Hak Lee, enrolled 508 participants within the first 24 hours of the onset of symptoms. The mean age of participants was 63 years. Inclusion criteria were an elevated creatine kinase greater than twice the reference limit, with either chest pain lasting more than 30 minutes or an electrocardiogram showing new ST-T change (Figure) or new left bundle branch block. The investigators assessed the

Figure. Electrocardiogram showing elevated ST wave.
relationships between plasma phospholipid EPA and DHA and all-cause and cardiovascular mortality in the whole sample and separately in men and women. Patients were monitored for an average of 16 months during which time 36 died. Most (29) deaths were from cardiovascular causes.

At baseline, EPA and DHA levels did not differ between male and female patients. All-cause and cardiovascular mortality were significantly lower in patients in the highest tertile of EPA (> 1.65%), but neither classification of mortality was associated with plasma DHA levels. Low EPA was an independent predictor of all-cause mortality in women, but not in men. The study sample was 28% female. It should be noted that plasma phospholipid EPA and DHA values were considerably higher than those observed in western populations, except for northern Europe.

Finally, Kristian Filion and colleagues performed a meta-analysis of 25 randomized controlled trials on the effect of n-3 PUFAs (including one using alpha-linolenic acid) on total mortality in high-risk patients with cardiovascular disease. They included one study of patients with diabetes. Using the pooled data, the authors determined that n-3 PUFAs were not associated with a lower chance of all-cause mortality. However, they determined that the probability of some benefit remained high (0.93), even though larger, longer trials had smaller effects. Removal of the study using alpha-linolenic acid did not change the results. The authors noted that n-3 PUFAs might reduce other cardiovascular events, including cardiac and sudden death, although the confidence intervals were wide.

Collectively, do these latest reports add something useful for understanding the link between n-3 LC-PUFAs and all-cause mortality? Three reports observed a reduction in total mortality with higher intakes or status of n-3 LC-PUFAs in patients with cardiovascular disease, but one did not reach statistical significance. The other two found no association between the consumption of n-3 LC-PUFAs and total mortality. Maybe we should ask whether the question is relevant. One can assert that reduced risk for death from any cause could be the expected outcome for the effects of n-3 LC-PUFAs given their diverse effects throughout the body. If one of their chief effects is a reduction in cardiovascular mortality, the leading cause of death in most countries, wouldn’t one expect to see that reflected in total mortality? The answer will depend on the age of the population, whether they have heart disease already or are at high risk of it, and the proportion of all deaths from heart disease. For example, the proportion of deaths from heart disease varies from one quarter in middle-aged populations to one half in older populations with heart disease. The question sets up an interesting academic exercise, but negative findings can be used to doubt the overall contribution of these fatty acids to health.

Viewed collectively, three reports found no significant effect of n-3 LC-PUFAs on total mortality, but two reported significantly lower mortality with higher intakes of n-3 LC-PUFAs. This was likely due to a reduction in cardiovascular events.


Worth Noting


Fish consumption and long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) intakes are low in many western countries, particularly among women of child-bearing age and children. In the U.S., women aged 20 to 40 consume an average of 100 mg/day of n-3 LC-PUFAs, according to national survey data from 1999 to 2000. The same data indicated that children under the age of 6 and those aged 6 to 11 consume about 30 mg of these fatty acids daily. For Canadian children 4 to 8 years old, n-3 LC-PUFA and docosahexaenoic acid (DHA) intakes were estimated in one study to be about 119 and 54 mg/day, respectively. Another study estimated the DHA consumption of Canadian children at 37 mg/day. For U.S. children, DHA intake was estimated to be 20 mg/day.

A recent workshop report on the dietary intake of eicosapentaenoic acid (EPA) and DHA in children concluded that there are insufficient data on children’s intakes of these fatty acids to derive recommendations for n-3 LC-PUFA consumption. However, the report noted that the limited data available suggest that intakes of EPA and DHA in children tend to be lower than in adults and may also be lower than in infants who are breastfed or consume formula supplemented with DHA. There is no reason to assume that children’s need for these fatty acids is reduced during growth and development or that the absence of clinical deficiency symptoms means children are getting enough n-3 LC-PUFAs.

With this information in mind, Laura Minns and colleagues at the University of Kansas Medical Center, USA, and Mead Johnson Nutrition conducted a randomized controlled trial to determine the usual DHA intakes of 86 ethnically diverse, middle and upper income toddlers between the ages of 18 and 36 months and the effects of providing them with different amounts of DHA for 60 days. Children with weight-for-length measurements within the 10th to 90th percentiles on the CDC growth charts were randomly assigned to consume cow-milk-based toddler formula containing 0, 43 or 130 mg of DHA per 237 mL daily serving. The investigators measured red blood cell DHA concentrations in red blood cell and plasma phospholipids at enrolment and day 60 and monitored the children monthly for illnesses and adverse events. Medical records were obtained at the end of the study to verify illnesses.

At enrolment, the mean DHA intake of the children, obtained by parental 24-hour recall and a log of formula consumption, was 20.8 mg/day, but intakes were skewed. The median DHA intake was 13.3 mg/day. The baseline DHA content of red blood cells in the DHA-0 toddlers was higher than in the other two DHA groups, so the baseline values were included as a covariate when analyzing the changes in red blood cell DHA.

After 60 days of treatment, 82 toddlers remained in the study. There was a significant linear dose-response from baseline in the DHA content of red blood cells, red cell phospholipids and plasma phospholipids with increasing intakes of DHA. Formula consumption did not differ significantly among the treatment groups.

Regarding adverse events, the number of occurrences decreased with increasing consumption of DHA, with those in the DHA-130 group having significantly fewer adverse events. Group differences were detected only for respiratory illnesses, with significantly fewer illnesses in the DHA-130 group (17%) compared with the DHA-0 group (46%). The most frequently reported illness was cough. Other investigators have reported a lower incidence of illness and fewer illnesses per child in Thai school children who consumed a fish oil supplement in addition to having a diet rich in fish. Fewer cases of bronchiolitis were reported in LC-PUFA-supplemented infants at 5, 7 and 9 months of age.

This study confirms the low DHA intakes and status of US toddlers. This observation is worrisome because young toddlers in the U.S. and Canada have very low intakes of long-chain omega-3 fatty acids, especially DHA. A recent study reports that toddlers 18 to 36 months old avidly take up DHA when provided with more.
children continue to accumulate DHA in their brains for several years, even though brain growth slows. Brain DHA content is essential for brain structure and function, cell signaling, neurotransmission, the production of synapses and more. In young infants, breastfeeding and the addition of DHA to infant formula increase the consumption of n-3 LC-PUFAs. After weaning, few foods are available to sustain the consumption of long-chain essential fatty acids, particularly DHA. This study demonstrates that DHA-supplemented toddler formula can be effective in boosting DHA consumption in early childhood and may have the added benefit of reducing respiratory illnesses.


Does DHA Intake in Infancy Improve IQ Scores at Age 4?

It is generally accepted that infants, especially those born preterm, receiving docosahexaenoic acid (DHA) during infancy fare better on a variety of neurodevelopmental assessments, including visual acuity, compared with infants who do not receive DHA from unsupplemented infant formula. For many reasons, the literature on infant developmental outcomes is inconsistent, especially for term infants. Some studies have reported superior intellectual, cognitive or behavioral performance in young children born with higher DHA status, but differences may not be sustained in later years. Others have reported no difference between DHA-supplemented and unsupplemented infants at various ages. Again, the evidence is inconsistent, study designs and assessments vary widely, and unaccounted confounding variables can be a concern.

Breast milk and infant formula supplemented with DHA are the best sources of DHA after birth and most infant formulas now contain this fatty acid. Trials have usually compared neurodevelopmental outcomes in infants fed breast milk, DHA-fortified or unsupplemented formula.

In a recent observational study of children’s intelligence (IQ) at 4 years of age, investigators in the U.K. recruited 396 pregnant women between the ages of 20 and 34 from general medical practices in the Southampton area. The 268 mothers enrolled in the study freely elected whether to breastfeed or give formula to their infants, and each selected and recorded the type of formula used. The investigators calculated the total number of days each child received formula or breast milk until 6 months of age and grouped participants according to the time they received each type of feeding. DHA exposure was estimated from average milk volumes for each month of feeding and the DHA content of the different formulas. The DHA content of breast milk was based on a recent estimate for women in the U.K.

The investigators assessed the children’s IQ at age 4 at home using the Wechsler Pre-School and Primary Scale of Intelligence, 3rd edition. They used subtests of the Developmental Neuropsychological Assessment to evaluate attention, sensorimotor ability, memory and language use. They also collected socio-economic information from the mother and her partner. The final sample included 130 breastfed children, 65 fed a DHA-supplemented formula and 46 receiving an unsupplemented formula. The total DHA intakes for the 6-month period for each group were 12, 13 and 2 g, respectively, with a range of 0 to 24 g.

At age 4, children fed the DHA-supplemented formula for the first 6 months of life, but not the breastfed children, had significantly better full scale and verbal IQ scores compared with children who consumed unsupplemented formula. As DHA intake was similar in both of the former groups, why did their IQ scores differ?

At 4 years of age, none of the IQ measurements (full scale, verbal or performance) differed between the breast milk group and the unfortified formula group when the analysis was adjusted for maternal IQ, education, social class, receiving social benefits, age at birth and birthweight (Table). When the...
DHA-supplemented formula group was compared with the unfortified formula group, full scale and verbal IQ were significantly higher in those receiving the DHA-supplemented formula. None of the other neuropsychological or visual-perception assessments differed significantly among the groups. Further, there was no trend for improved scores with increasing DHA intake up to the age of 6 months, suggesting that DHA intakes did not affect the scores on full scale or verbal IQ, even though they were higher in the DHA-fortified group compared with the unfortified formula group. Instead, the authors suggested that confounding variables attenuated the IQ scores relative to the unadjusted analysis (Table).

This study reported ambiguous results on the relationship between DHA intake and childhood intelligence scores in 4-year-old children who were born at term. DHA consumption in breastfed and DHA-formula-fed infants for the first 6 months after birth was equivalent (12 and 13 g/6 mo, respectively), yet only the DHA-formula children had significantly higher full scale and verbal IQ scores in multivariate analysis compared with children who consumed unsupplemented formula. Had the investigators measured the DHA content of the participants’ breast milk, their estimate of the DHA intake in the breastfed group might have been more accurate. Adjustment for multiple confounding variables had a greater effect in reducing IQ scores in breastfed than DHA-formula infants. This may suggest that socioeconomic factors, particularly mothers’ education, social class and IQ, between mothers who breastfeed and those who use formula are more powerful determinants of childhood performance on IQ assessments than DHA intakes. As the authors suggest, if these factors are not adequately considered in infant feeding studies, previous reports may be misinterpreted because critical confounding variables were overlooked.


Long-Chain Omega-3s Associated with Lower Risk of Endometriosis

Trolling through the data from the Nurses’ Health Study, a prospective cohort study with 122,000 participants, begun in 1976, has netted an enormous catch of intriguing associations between diverse lifestyle factors and various health conditions. Many of these observations stimulated additional research, including controlled intervention studies and the search for mechanisms of action. A second cohort of 116,000 nurses was started in 1989 to examine the relationships between the use of oral contraceptives, diet and lifestyle factors and cancer. Participants in the Nurses Health Study-II were younger (25 to 42 years) than those in the original study (30 to 55 years).

A recent paper from the second cohort describes the relationship between total fat and fatty acid intakes and the risk of developing endometriosis. This painful condition is characterized by the presence of extra-uterine tissue in the pelvic cavity (Figure). It occurs in 1 in 10 women of child-bearing age and is confirmed by laparoscopy.
Few modifiable risk factors have been associated with the development of endometriosis, but one study reported a reduced risk of the condition with higher intakes of green vegetables and fruit. Higher risk was associated with the consumption of beef, other red meat or ham. Endometriosis has also been linked to organochlorine pesticide residues, p,p’-DDE, the main metabolite of DDT and PCBs, dioxin-like compounds. The U.S. banned the production of DDT and PCBs in 1972 and 1979, respectively, but they persist in the environment.

In this observational study, investigators from Harvard Medical School, USA, identified 1,766 women who reported a diagnosis of endometriosis, of which 1,199 were confirmed by laparoscopy. Of these, 228 reported an infertility evaluation and 970 cases had no past or current infertility. Exclusions from the study were those with implausible energy intakes, endometriosis or infertility prior to enrolment. Infertility is strongly correlated with endometriosis. Participants were premenopausal and had an intact uterus.

Dietary fat intake was assessed by food frequency questionnaire and cumulative averaged intakes were calculated from three data collection periods as described in detail in the paper. Fat intakes were divided into quintiles and exposures for each category were calculated as the number of cases divided by the person-time accumulated. Multivariable and incident rate ratios (RR) were calculated using time-varying Cox proportional hazards models with adjustment for potential confounding variables.

Two classes of fats were significantly associated with endometriosis in all multivariate statistical models. Consumption of long-chain omega-3 fatty acids (n-3 LC-PUFAs) significantly reduced the risk by about 22% in the highest intake group, while trans fatty acids increased the risk by about 50% in the highest consumption group (Figure). Total fat intake was not associated with the occurrence of endometriosis. Comparing the risk of endometriosis and n-3 LC-PUFA consumption in women with infertility and those who had never been infertile showed no differences between the groups. However, for women with endometriosis and infertility, the highest consumption of trans fatty acids increased the risk of endometriosis by 72%, whereas in women who had never been infertile, the risk was 48% greater.

The investigators noted also that palmitic acid, a 16-carbon saturated fatty acid found in animal products, some margarines and baked goods, was significantly associated with a 52% increased risk of endometriosis in those with the highest intakes of this fatty acid. Other saturated fatty acids and animal fats were not related to endometriosis risk.

The observation that consumption of n-3 LC-PUFAs might reduce the risk of endometriosis in premenopausal women invites research in randomized, controlled trials.
controlled trials. It is plausible that higher intakes of n-3 LC-PUFAs might reduce the pain associated with this condition because of their anti-inflammatory effects. Although one report on the use of essential fatty acids in premenstrual syndrome found no effect of these fatty acids, another reported a significant reduction in dysmenorrhea in adolescents who consumed n-3 LC-PUFAs. Danish researchers reported that higher n-3 LC-PUFA intakes were associated with milder menstrual symptoms. In a surgical model of endometriosis, a diet rich in eicosapentaenoic acid, an n-3 LC-PUFA, suppressed the thickening of the interstitium, an active site for inflammation. This intriguing report warrants additional investigation.


**Worth Noting**


### MENTAL HEALTH AND BEHAVIOR

**EPA Supplementation Linked to Improved Behaviors in ADHD Subgroups**

Attention deficit hyperactivity disorder (ADHD) is a heterogeneous, clinically recognized condition characterized by impulsivity, inattention and hyperactivity. Children with ADHD often have symptoms overlapping other disorders, such as coordination problems, dyslexia, social difficulties and cognitive deficits. ADHD occurs most commonly in children, but in a majority of cases some symptoms persist into adolescence and adulthood. A recent estimate of the prevalence of ADHD in the U.S. reported a lifetime occurrence of 9%, with the highest prevalence in childhood and males (about twice as frequent). The US National Health Interview Survey reported a prevalence of 8% in children aged 3 to 17 years.

Long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) and other PUFAs have been associated with the condition because of their importance in brain structure and function, processing of emotional responses, low concentrations in ADHD and limited success in treating individuals with the condition. However, not all studies have reported low PUFA status in ADHD or treatment effectiveness with n-3 LC-PUFAs. Several intervention studies have used a combination of n-3 and n-6 fatty acids. Studies using a relatively high proportion of eicosapentaenoic acid (EPA) have reported improvement in some symptoms, depending on the type of ADHD the children exhibited. Pure DHA was without effect in one study. ADHD behaviors may be predominantly combined hyperactive/impulsive, oppositional/defiant or inattentive and it is not known which type(s) of ADHD might be most responsive to treatment with EPA, n-3 LC-PUFAs or combined n-3 and n-6 fatty acids.

A recent double-blind, controlled study from Sweden explored whether children with clinically diagnosed combined type ADHD or subgroups of these children would respond to EPA supplementation. The investigators recruited children aged 7 to 12 years from 8 psychiatric and pediatric clinics in the country. Participants were free of other medical conditions, mental retardation, autism and epileptic seizures during the preceding 2 years. Various other clinical conditions, such as endocrine disorders, impaired hearing or vision and psychotic symptoms were also reasons for exclusion. Of the 109 eligible children, 17 dropped out, leaving 92 for intention-to-treat analysis. Additionally, 10 more had deficient follow-up data, leaving 82 participants with complete data.

One month before the first clinic visit, parents and teachers completed their respective Conners’ Rating Scales for the participants and parents, teachers and doctors confirmed the ADHD diagnosis using the...
ADHD-RS rating scale and clinical judgment. A week later (baseline) the investigators assessed the participants’ nonverbal reasoning ability using the Raven’s Progressive Matrices evaluation and measured hyperactivity with the Qb-test and infrared motion analysis. Participants gave a blood sample and received either the test supplement (500 mg EPA + 2.7 mg DHA + 10 mg Vitamin E mixed tocopherols) or the placebo capsules containing rape-seed oil and medium-chain triglycerides. Capsules were distributed every 5 weeks thereafter. Parents completed a 24-hour food frequency questionnaire.

At week 15, the investigators assessed the participants for ADHD and co-morbid symptoms, administered a neuropsychological assessment and the Qb-test and obtained another blood sample. The primary outcome was the combined scores from the Conners’ rating scales by parents and teachers. Secondary outcomes were the separate evaluation of the parents’ and teachers’ rating scales and 3 clinical domains in the rating scales: hyperactivity-impulsivity, inattention and cognitive problems, and oppositional behavior.

After 15 weeks’ treatment, there were no significant differences between the placebo and EPA groups in the combined parents’ and teachers’ rating scales nor in their individual ratings. In the teachers’ subscale for inattention or cognitive difficulties, children who consumed the EPA supplement exhibited significant improvement. When the investigators analyzed the results for the children who were classified at baseline as having oppositional behaviors, the effect of EPA treatment on the combined scores of parents’ and teachers’ ratings was statistically significant ($P = 0.03$). This result was largely attributable to scores on the teachers’ ratings. Among these children, 52% of those who consumed the EPA supplement exhibited a 25% improvement or more, according to the teachers’ ratings for oppositional behavior and inattention or cognitive problems. Those who responded to the EPA supplement also had lower serum phospholipid EPA and higher arachidonic acid concentrations at baseline compared with non-responders (Table).

Qb-test scores for hyperactivity in children who were below the median at baseline improved by 25% or more in 36% of children who consumed the EPA supplement compared with 18% in the placebo group. However, these improvements did not reach statistical significance. In 22 children who were both hyperactive and oppositional-defiant, 13 consumed EPA and 9 received the placebo. Eight of the EPA children showed a 25% or greater improvement in hyperactive and oppositional behavior, while only one in the placebo group improved. These responses were significant, $P = 0.03$.

In this study, subgroups of children with ADHD who had either oppositional or hyperactive-impulsive behaviors responded to the consumption of EPA with significant reductions in their symptoms as assessed by their teachers. These findings support those of

<table>
<thead>
<tr>
<th>Serum or membrane</th>
<th>Non ODD* phospholipids at baseline</th>
<th>ODD</th>
<th>$P$</th>
<th>ODD-responsive after 15 wk EPA</th>
<th>ODD-non-responsive to EPA</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5 (EPA)</td>
<td>1.3 (0.8)</td>
<td>1.3 (0.5)</td>
<td>NS</td>
<td>1.0 (0.2)</td>
<td>1.6 (0.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>22:6 (DHA)</td>
<td>4.1 (1.0)</td>
<td>4.2 (1.2)</td>
<td>NS</td>
<td>4.0 (1.2)</td>
<td>4.8 (1.5)</td>
<td>NS</td>
</tr>
<tr>
<td>20:4 (ARA)</td>
<td>8.7 (1.2)</td>
<td>8.5 (1.6)</td>
<td>NS</td>
<td>9.0 (2.2)</td>
<td>8.2 (1.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Red blood cells</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5 (EPA)</td>
<td>0.9 (0.3)</td>
<td>1.0 (0.3)</td>
<td>NS</td>
<td>0.8 (0.2)</td>
<td>1.2 (0.4)</td>
<td>0.1</td>
</tr>
<tr>
<td>22:6 (DHA)</td>
<td>5.7 (0.9)</td>
<td>5.8 (1.1)</td>
<td>NS</td>
<td>5.6 (1.0)</td>
<td>6.2 (1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>20:4 (ARA)</td>
<td>15.1 (1.3)</td>
<td>14.4 (1.0)</td>
<td>NS</td>
<td>14.9 (1.1)</td>
<td>13.6 (0.8)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*ODD, opposition defiant disorder
Richardson and colleagues and Johnson’s research group who used a combination of fatty acids in particular subgroups of children with ADHD. Those with inattention or oppositional behavior are more likely to respond to EPA or n-3 LC-PUFAs, while those with hyperactivity may be less responsive. The study also observed that teachers’ ratings are more likely to detect behavioral changes than those from parents. Many problem behaviors occur in the classroom, so this observation was not surprising. In this study, children with oppositional behaviors who responded to EPA had significantly lower levels of EPA in their serum phospholipids compared with non-responders, although the number of children was very small. The authors also noted that the dose of EPA, 500 mg/day, was relatively small, but sufficient to be useful in subgroups of the participants.


DHA Supplementation in Healthy 9-Year-Olds Barely Affects Cognition and Behavior

The relationships between the polyunsaturated fatty acid (PUFA) status in children and their cognitive and behavioral development are under active investigation. Promising findings suggest that supplementation with either or both omega-6 (n-6) and omega-3 (n-3) PUFAs might improve learning and behavior, especially in children with disturbances in these areas. But findings continue to be inconsistent, in part because of differences in study populations, overlapping symptoms and disorders, different assessment tools and outcome measures, wide variation among individuals, genetic influences, age differences, treatments and doses and so on. Nevertheless, as illustrated in the preceding article on EPA supplementation in children with attention deficit hyperactivity disorder (ADHD), some children may benefit from PUFA supplements, especially long-chain (LC) n-3 PUFAs.

Some investigators have reported lower n-3 LC-PUFAs in children with ADHD. Most western diets, especially for children, provide only small amounts (30 mg/day) of these fatty acids. Children in poorer families may be at even greater risk of low n-3 PUFA consumption because their diets may include less fish. Little information is available on the fatty acid status of healthy children consuming typical diets and how it might relate to their socioeconomic status, learning and behavior. This study, conducted in Wales, U.K., aimed to address these questions in healthy children aged 8 to 10 years.

The investigators used fatty acid concentrations in cheek cells to determine the children’s fatty acid status and assessed their learning and behavior characteristics using standardized cognitive tests and questionnaires from parents and teachers. Cheek cell fatty acids have been previously shown to reflect dietary intake, plasma and red blood cell levels of essential fatty acids and have the advantages of being non-intrusive and easy to collect.

Of the 422 eligible children recruited, 11 had insufficient cell numbers for fatty acid measurements, leaving 411 participants whose average age was 9 years. Children were ineligible if their IQ was less than 70 or they had taken n-3 PUFA or gamma-linolenic acid supplements within the 6 months prior to enrollment. The investigators administered the following cognitive tests: Kaufman Brief Intelligence Test, Working Memory Test Battery for Children, Wechsler Individual Achievement Test-II, Test of Everyday Attention for Children: Creature Counting, and the Matching Familiar Figures Task. Parent and teacher ratings of behavior were obtained using the Swanson, Nolan and Pelham rating scale for ADHD, the Strengths and Difficulties Questionnaire and Developmental Coordination Disorder Questionnaire. Socioeconomic and demographic data were collected at baseline.

Cheek cell n-3 PUFAs were lower in children of lower socioeconomic status for total n-3 PUFAs, alpha-linolenic acid and eicosapentaenoic acid (EPA). Linoleic acid and total n-6 PUFAs were also negatively correlated with socioeconomic status, except for docosapentaenoic acid (DPAn-6), a fatty acid more frequently observed in n-3 PUFA deficiency.
In the assessments of cognition, docosahexaenoic acid (DHA) concentrations were significantly related to non-verbal IQ scores, whereas alpha-linolenic acid and total n-3 PUFAs were associated with more errors and fewer first correct responses on the Matching Familiar Figures Test. Digit recall standard scores were positively related to levels of gamma-linolenic acid and DHA/EPA ratio. Reading and spelling were unrelated to cheek cell PUFAs. Teacher behavior assessments showed negative relationships for inattention and hyperactivity/impulsivity with levels of alpha-linolenic acid and total n-3 PUFAs in the Swanson assessment for ADHD, but were positively related to the levels of DPAn-6.

Overall, this extensive cross-sectional exploration of the relationships between cheek cell fatty acid status, cognition and behavior revealed many statistically significant relationships, not all of which may be important in identifying key PUFAs that may be involved in children’s development. Interestingly, the authors found no significant relationships between arachidonic acid or DHA levels and cognition or behavior. These are key LC-PUFAs in the brain and have been linked in some studies to developmental outcomes in infancy and childhood.

The study confirms in healthy school children the observation in adults that those in lower socioeconomic status households are more likely to have lower intakes of total and long-chain n-3 PUFAs. The difference in the socioeconomic groups was illustrated by the significantly higher ratio of total n-6/n-3 PUFAs in children from the lower income levels. Such children also had significantly higher cheek cell levels of DPAn-6, an indicator of very low n-3 PUFA consumption. The only difference between males and females was the higher level of DPAn-6 in girls (0.04% vs 0.02% total buccal cell PUFAs).

The second part of this study investigated the effect of n-3 LC-PUFA supplementation (mainly DHA) in healthy children selected as described above. From 450 initial participants, 348 completed the first study phase of 16 weeks. Children were randomly assigned to consume 2 capsules per day providing a total of 400 mg of DHA, 56 mg of EPA plus vitamins A, C, and E or a placebo of olive oil plus vitamins. Phase 2 (not reported) will entail a one-way crossover of the placebo children to the n-3 LC-PUFA treatment for an additional 8 weeks in an open label design. Similar assessments of cognition and behavior as previously described were used with the addition of the computerized Penmanship Evaluation Tool to assess the handwriting process. This evaluation has been associated with attention difficulties and motor control in children with developmental coordination disorder.

### Table. Mean (SD) cheek cell fatty acids (% total fatty acids) at baseline and after 16 weeks of n-3 LC-PUFA supplementation (mainly DHA) in healthy 8 to 10 year old children in intention-to-treat analysis. Per-protocol analysis showed significant differences in the same comparisons.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Group</th>
<th>Baseline</th>
<th>Week 16</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:4 (ARA)</td>
<td>PUFA</td>
<td>0.83 ± 0.43</td>
<td>0.80 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.84 ± 0.83</td>
<td>0.83 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>n-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5 (EPA)</td>
<td>PUFA</td>
<td>0.05 ± 0.10</td>
<td>0.10 ± 0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.06 ± 0.07</td>
<td>0.07 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>22:6 (DHA)</td>
<td>PUFA</td>
<td>0.08 ± 0.09</td>
<td>0.34 ± 0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.08 ± 0.18</td>
<td>0.18 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Total n-6</td>
<td>PUFA</td>
<td>9.17 ± 2.81</td>
<td>7.43 ± 3.65</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>8.95 ± 7.19</td>
<td>7.19 ± 3.76</td>
<td></td>
</tr>
<tr>
<td>Total n-3</td>
<td>PUFA</td>
<td>0.72 ± 0.44</td>
<td>0.66 ± 0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>0.75 ± 0.49</td>
<td>0.48 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>PUFA</td>
<td>18.63 ± 19.64</td>
<td>3.46 ± 9.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>16.46 ± 18.23</td>
<td>118.23 ± 10.47</td>
<td></td>
</tr>
</tbody>
</table>
The statistical analyses included both intention-to-treat and per-protocol analysis of covariance.

After 16 weeks’ supplementation with n-3 LC-PUFAs there were significant increases in cheek cell EPA and DHA in the treatment group and DHA in the placebo group as well (Table). There was no change in total n-3 PUFAs in the supplemented groups, but these fatty acids fell significantly in the placebo group compared with baseline levels. EPA might be expected to increase as a result of retroconversion of DHA, but its increase in the placebo group remains unexplained. The changes in the placebo group were not nearly as large as those in the supplemented children.

Cognitive assessments showed no effect of n-3 LC-PUFA supplementation in any measure in the intention-to-treat analysis. Among compliant participants (per protocol analysis), supplementation was associated with an improved number of first correct responses, visual perception and impulsivity. The handwriting data showed no differences between groups at 16 weeks after controlling for baseline performance.

Teachers’ ratings on the strengths and difficulties questionnaire revealed a significant decrease in the total difficulties score between the two groups after controlling for baseline performance, with the decrease in the placebo group being significant. With the parents’ ratings, only the prosocial scores between baseline and week 16 differed significantly between the two groups, a difference (worsening) occurring mainly in the placebo group.

Results from this study help fill a knowledge gap about n-3 LC-PUFA supplementation. These differences were observed only in the per protocol analysis. Parents’ ratings of their children’s prosocial behavior declined in the placebo group over the 16-week study in per protocol analysis, but did not change significantly in the supplemented group, hinting that n-3 LC-PUFAs might have helped prevent a deterioration in prosocial behavior. Thus, n-3 LC-PUFA supplementation consisting mainly of DHA had little effect on cognitive or behavioral measures in a large sample of healthy children.


DHA Deficiency in Major Depressive and Bipolar Disorders Revealed in Red Blood Cells

Many lines of evidence point to the involvement of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in various psychiatric disorders, including major depressive and bipolar disorders and aggressive, violent or suicidal behavior. Consumption of fish or n-3 LC-PUFAs is lower in patients with major depressive or bipolar disorder and in pregnant women with depressive symptoms. In prospective studies, the long-term risk of developing depressive symptoms is greater in populations with low seafood consumption.

Lower concentrations of n-3 LC-PUFAs and some other fatty acids have been observed in plasma or red blood cells of patients with major depressive or bipolar disorder. These reports prompted intervention studies with n-3 LC-PUFAs to explore their treatment potential. A meta-analysis and review of the efficacy of n-3 LC-PUFAs in patients with depressive symptoms concluded that these fatty acids have significant anti-depressive effects, although both reviews noted methodological concerns. In bipolar disorder, findings have been inconsistent and
the study quality variable, but some studies reported improved symptoms in patients who consumed eicosapentaenoic acid (EPA) supplements. Consumption of n-3 LC-PUFAs has not been associated with adverse effects.

Intervention studies with n-3 LC-PUFAs have used purified EPA, docosahexaenoic acid (DHA), a combination of these fatty acids or fish oil, which also contains docosapentaenoic acid (22:5n-3). DHA has mostly been ineffective, while symptom improvements have been observed with EPA or a combination of EPA plus DHA where EPA exceeds the content of DHA. Thus, a review of the most effective n-3 LC-PUFAs for mood disorders concluded that EPA was more effective than DHA in treating depressive illness.

Additional evidence that n-3 LC-PUFAs are linked to these disorders has come from post-mortem brain analyses. The orbitofrontal cortex of individuals who died with major depressive or bipolar disorder contained significantly less DHA compared with the brains of control patients. Others have reported reductions in brain DHA and increased arachidonic acid in the anterior cingulate cortex of individuals with major depressive disorder. However, not all post-mortem analyses have observed such decreases. Of particular interest is the recent report that red blood cell DHA content was positively associated with functional activity in the prefrontal cortex in healthy school-aged boys.

Because there are few ways to evaluate brain fatty acid composition directly in living patients, researchers have generally relied on measurements in red blood cells to estimate changes in brain composition. In infants, post-mortem analysis showed a direct correlation between PUFA levels in red blood cells and the brain. Animal and human studies have also shown that red blood cell DHA is highly correlated with brain DHA. How close the relationship is in adults has not been well studied.

Extending the current evidence relating n-3 LC-PUFAs to mood disorders, Robert McNamara’s group at the University of Cincinnati Medical College, USA, evaluated the red blood cell fatty acid profiles in patients with a history of major depressive or bipolar disorder and their associations with symptom severity. Participants currently in remission and not taking anti-depressant or mood stabilizing medications 2 weeks prior to blood collection were recruited from the Psychiatric Clinical Research Center at the University of Illinois, Chicago. Healthy control participants matched for sex and age, with no history of psychiatric illness, were recruited from the greater Chicago area. There were 20 patients with major depressive disorder, 20 with bipolar disorder and 20 healthy controls in the study. Their mean ages were 35, 31 and 36 years, respectively.

The investigators measured symptom severity using the Hamilton Depression Rating Scale and Clinician-Administered Rating Scale for Mania for depressive and manic symptoms, respectively. Data were available for 55 of the original participants and the analyses were corrected for multiple comparisons. Analysis of variance was used to examine the effect of group and sex on red blood cell fatty acids. Linear regression analyses were performed to determine the relationships between fatty acids, fatty acid ratios and symptom severity scores.

The most striking observation in the fatty acid profiles was the significant reduction in DHA in both sets of patients compared with the healthy controls (Figure). The sum of red blood cell EPA and DHA (Omega-3 Index) was also significantly lower in patients compared with controls. In specific comparisons of each patient group with the controls, DHA reductions were also

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**Medication-free patients with a history of major depressive or bipolar disorder had significant deficits in their red blood cell DHA content compared with healthy controls. No other PUFAs were disturbed. Patients also had Omega-3 Index values below 4%, an indicator of high risk of cardiovascular mortality.**

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**Figure. Red blood cell PUFAs in unmedicated patients with a history of major depressive disorder or bipolar disorder compared with healthy controls.**

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significant, with a 20% reduction in DHA in patients with major depressive disorder and a 32% reduction in those with bipolar disorder. Although arachidonic acid levels did not differ among the groups, possibly because the participants were not taking medications, the ratios of arachidonic acid to DHA and to EPA plus DHA were also significantly higher in the patient groups compared with the controls. These effects were due to the reductions in DHA, not to changes in EPA or arachidonic acid.

Gender and age were not significantly related to the PUFA concentrations in any group. Likewise, symptom severity in either group of patients was unrelated to red blood cell fatty acid composition after correction for multiple comparisons.

The investigators noted that an indicator of delta-6 desaturase activity, the ratio of 20:3n-6 to 18:2n-6, correlated inversely with red blood cell DHA and was significantly elevated in patients with either clinical condition. These observations suggest that the DHA deficits observed in the patient groups were not wholly attributable to reductions in delta-6 desaturase activity. A similar conclusion was reached for elongase activity based on the ratio of 22:4n-6 to 20:4n-6. In addition, the concentration of 22:5n-6, a fatty acid usually increased only in n-3 LC-PUFA deficiency, was not elevated in the patient groups, suggesting that in these conditions, peroxisome function is impaired.

Of additional interest was the observation that the Omega-3 Index in the patient groups was below 4%, the level considered to mark a significant risk for death from coronary heart disease. Those with major depressive disorder had an Omega-3 Index of 3.8% and those with bipolar disorder had an average value of 3.3%, while the average value for controls was 4.8%. These observations suggest that patients with these disorders are at significantly higher risk of cardiovascular mortality.

It may seem paradoxical that intervention studies have reported the effectiveness of EPA treatment in these disorders, but not DHA. Some reports have noted improvements in symptoms with the combined intervention of EPA and DHA or the consumption of cod liver or fish oil. Abundant evidence suggests that deficits in brain DHA and pyramidal neuron atrophy are involved in affective disorders as discussed in the second paper by McNamara below. However, EPA has anti-inflammatory properties in brain, and in animals affects the release of neurotransmitters, improves memory and has neuroprotective properties in aging. It is likely that EPA and DHA and their metabolites affect different processes in brain function in these disorders. As details of the underlying pathology in these depressive conditions emerge, how EPA and DHA may be involved in affective disorders is likely to become clearer.

McNamara RK, Jandacek R, Rider T, Tso P, Dwivedi Y, Pandey GN. Selective deficits in erythrocyte docosahexaenoic acid composition in adult patients with bipolar disorder and major depressive disorder. J Affect Disord 2010;


Worth Noting


Visual Function

Mutant Retinal Gene Linked to Rare Macular Dystrophy and Very-Long-Chain PUFAs

Degeneration of the macula occurs frequently in older adults, particularly women. The macula is a small yellow spot near the center of the retina, which is responsible for sight in the central visual field. About half the cases of age-related macular degeneration (AMD) are linked to a variant of the complement factor H gene. Over the last decade, three different mutations in the ELOVL4 gene have been shown to be responsible for another type of macular degeneration, Stargardt-like macular dystrophy.
This relatively rare condition develops in childhood, adolescence or early adulthood, usually in individuals with a family history of the condition.

The ELOVL4 gene encodes for a protein that is normally targeted to the endoplasmic reticulum, the site of fatty acid biosynthesis. The ELOVL4 protein together with other resident proteins of the endoplasmic reticulum is involved in the biosynthesis of very-long-chain (VLC) fatty acids having 28 to 40 carbons (Figure). VLC-PUFAs are synthesized in retina, testis, sperm and brain and are found in retinal sphingolipids.

VLC-PUFAs are reduced or absent in people with Stargardt-like macular dystrophy. This is because the mutations in the ELOVL4 gene result in an ELVOL4 protein that can no longer be targeted to the endoplasmic reticulum. Hence, it was proposed that misrouting of the ELOVL4 protein was responsible for the absence or reduction of VLC-PUFAs in retina, which in turn may result in the death of photoreceptor cells in this genetic disorder. In contrast to Stargardt-like macular dystrophy, people with peroxisomal disorders have increased VLC-PUFAs.

Previous work by these investigators demonstrated that cardiomyocytes modified to contain the ELOVL4 gene and cultured with EPA or docosapentaenoic acid (22:5n-3) converted these fatty acids to VLC-PUFAs containing 24 and 26 carbons. These were further elongated to n-3 VLC-PUFAs containing 28 to 38 carbons, with 34:5n-3 and 36:5n-3 predominating. In the present report, the investigators verified that the 661W cells made the ELOVL4 protein and could elongate the n-3 PUFAs having 20 to 24 carbons to 24:5n-3. Cells incubated with docosapentaenoic acid produced 24:5n-3. Levels of DHA did not differ from controls regardless of the concentration of either precursor in the medium.

Next, the investigators created a knock-down version of the 661W cells, which silences the ELOVL4 gene. Even without the ELOVL4 protein, the cells were able to elongate alpha-linolenic acid to EPA (20:5n-3), docosapentaenoic acid (22:5n-3) and tetracosapentaenoic acid (24:5n-3) as determined by high-pressure liquid chromatography. When isotope-labeled alpha-linolenic acid was used in these experiments, there was no increase in radioactivity in the precursor pool nor reduced radioactivity in the products, indicating that ELOVL4 was not involved in producing these elongation products. It has been established that DHA (22:6n-3) is produced from the desaturation of 24:5(n-3) to 24:6(n-3) in the endoplasmic reticulum followed by beta-oxidation in the peroxisome.

Additional evidence that ELVOL4 protein is not involved in DHA synthesis is the observation that retinal DHA is not reduced in Stargardt-like macular dystrophy. In transgenic mice expressing one of the Stargardt-like macular dystrophy mutations, increasing the DHA content of the photoreceptor membranes did not prevent the retinal degeneration characteristic of the disease. In the present studies, silencing the ELOVL4 gene had no effect on the elongation of alpha-linolenic acid or docosapentaenoic acid to EPA or 24:5n-3, supporting the view that the ELVOL4 protein is not involved in the synthesis of DHA in retina.

The ELOVL4 gene might be involved in the synthesis of docosahexaenoic acid (DHA) a long-chain omega-3 PUFA (n-3 LC-PUFA) concentrated in the retina. As described in a publication by Martin-Paul Agbaga and colleagues at the University of Oklahoma, USA, the investigators tested this hypothesis by silencing the expression of ELOVL4 in cone photoreceptor cells derived from the cell line 661W. The authors also presented findings from additional studies with these and other cell lines at the May 2010 meeting of the International Society for the Study of Fatty Acids and Lipids in Maastricht, Netherlands. This work showed that eicosapentaenoic acid (EPA) was the preferential precursor for the synthesis of VLC-PUFAs by ELOVL4 in photoreceptor cells.

Figure. Example of a VLC-PUFA showing the polyunsaturated methyl end (left) and the saturated carboxyl end (right) of the chain. Reproduced with permission from J Lipid Res 2010;51:1624-1642.
photorceptor cells. Instead, the protein is involved in the synthesis of VLC-PUFAs having 28- to 40-carbon atoms, fatty acids important in the retina, brain and sperm. In a mouse model of Stargardt-like macular dystrophy, the absence of the ELOVL4 protein was accompanied by a selective deficiency of the 28- to 36-carbon VLC-PUFAs. While DHA is vital for photorceptor cell function, its production from precursors, little of which occurs in humans, appears independent of the ELOVL4 protein.


Worth Noting


Age-related hearing loss is 5 times more common in men than women, yet its cause is unknown. Its development has been linked to nutrition and heart disease. Two recent studies looked at whether hearing loss was related to the intake of long-chain omega-3 fatty acids.

Hearing loss has also been associated with diabetes and cardiovascular disease. A recent case study report from Italy described a significantly increased risk of sudden sensorial hearing loss with hypercholesterolemia, low serum levels of nervonic acid (24:1n-9), a fatty acid involved in the biosynthesis of myelin, and low levels of coenzyme Q10. Other nutrition related links with hearing loss include poor vitamin B12 and folate status. It was also reported that folic acid supplementation slowed the decline in mean hearing threshold at low frequencies in older adults.

Prevalence of hearing loss in US adults aged 20 to 69 years ranged from 6 to 8 percent for the period 1976 to 2006, but was estimated at 16% for the 2003 to 2004 survey period. It is more than five times more likely in men than women.
Two recent papers examined the relationship between fish consumption or long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) intakes in existing population or clinical trial cohorts in older adults. One study in the Netherlands involved 819 men and postmenopausal women aged 50 to 70 years who were participants in a randomized controlled trial on the effects of folic acid supplementation on hearing, carotid intima-media thickness and cognitive performance. Participants were excluded for any of the following: middle ear dysfunction or unilateral hearing loss, consumption of vitamin B supplements, vitamin B12 levels <200 pmol/L, renal or thyroid diseases, insufficient blood obtained at sampling. There were 720 eligible participants whose average age at baseline was 60 years. Hearing was evaluated at baseline and 3 years later.

Dullemeijer and colleagues performed pure tone audiometric assessments using an audiometer (Madsen Voyager 522), circum-aural earphones and a handheld response button system. The testing was based on the Hughson and Westlake method as previously described. The outcomes measures are the mean pure-tone air conduction hearing thresholds in the low (0.5-kHz to 2-kHz) and high (4-kHz to 8-kHz) frequencies. Participants with a difference of 20 dB (decibels) or more in mean air conduction hearing thresholds between the right and left ear were excluded. Fatty acids were measured in fasting plasma cholesteryl esters. Total n-3 LC-PUFAs included eicosapentaenoic, docosapentaenoic and docosahexaenoic acids.

After 3 years, hearing thresholds for low and high frequencies increased by 1.4 dB and 4.8 dB, respectively. Participants in the highest quartile of n-3 LC-PUFAs had significantly less hearing loss in the low frequencies compared with those in the lowest quartile of intake. There were no significant differences in the changes in high frequency thresholds among the n-3 LC-PUFA groups. When the results from the participants taking folic acid supplements were analyzed separately from the placebo group, the effect of n-3 LC-PUFAs in slowing hearing loss in the low frequency range remained significant for the placebo group, but not those receiving folic acid. Changes in hearing thresholds for low and high frequencies were not related to plasma saturated fatty acid concentrations.

The investigators noted that the difference in hearing loss between the lowest and highest quartiles of n-3 LC-PUFA intakes was small, a loss of -0.8, whereas those in the 60 to 70 year category experienced a difference of -1.5 dB between the highest and lowest quartiles of plasma n-3 LC-PUFAs.

The second study recruited participants 49 years of age and older from the ongoing Blue Mountains Eye Study, New South Wales, Australia. Survivors from the first 5 years of the study plus nearly 1,200 new registrants and were identified and received hearing measurements. Surviving participants who were assessed 10 years after the baseline enrollment were also included. Hearing loss data were obtained in 2,956 participants and after exclusions for a history of hearing loss and incomplete audiologic data, 2,442 participants remained.

The design of the study enabled the investigators to determine whether there was a relationship between hearing loss and fish consumption at baseline and whether there was an association between fish or n-3 LC-PUFA or total n-3 PUFA intakes and hearing loss after 5 years' followup. The investigators averaged the frequencies measured at 0.5, 1.0, 2.0 and 4.0 kHz to obtain an average score. They defined hearing loss as any difference greater than 25 dB in hearing thresholds between the baseline and 5-year measurements. Scores used the difference between the 0.5 kHz and 4.0 kHz values in those without hearing loss at baseline in the better of two ears.

At baseline, 32 percent of participants had hearing loss. These were more likely to be male, older, exposed to workplace noise and have had a stroke or type 2 diabetes. At enrollment, those with the highest total n-3 PUFA intake, but not those with the highest intakes of n-3 LC-PUFAs or fish, were significantly less likely to have hearing loss (11% lower risk) in multivariable analysis.

After 5 years, there was no significant longitudinal association between the consumption of n-3 PUFAs and hearing loss. However, there was a significant 24% decrease in incident hearing loss when calculated for each standard deviation increase in energy-adjusted n-3 LC-PUFA consumption. No other fatty acids, including total n-3 PUFAs, alpha-linolenic acid and total n-6 PUFAs, were associated with the risk of hearing loss. Increased frequency of eating fish was also significantly

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and inversely associated with hearing loss at 5 years (42%) in multivariate-adjusted analysis.

The investigators also explored whether fish or n-3 LC-PUFA consumption was related to the progression of hearing loss in participants who had an increase in hearing threshold of more than 5 dB from baseline 5 years. Of 386 participants with baseline and 5-year measurements, 47% exhibited hearing loss of more than 5 dB. In multivariate analysis, eating fish more than once but less than twice a week was associated with a significant 47% lower risk of progressive hearing loss compared with those who didn’t eat fish (Figure). Those who ate fish twice or more a week had a 20% lower risk of progressive hearing loss that was not significant.

With somewhat different study designs and methods, participant ages and criteria for hearing loss, both studies reported a significant inverse association between the consumption of n-3 LC-PUFAs and hearing loss. Both studies observed a greater incidence of hearing loss in men compared with women. Only the second study observed a lower incidence of hearing loss at baseline in participants with greater intakes of n-3 LC-PUFAs. Those with hearing loss were significantly older than those without hearing loss, an observation noted in both studies. However, both studies reported a significantly lower risk of hearing loss over time (3 and 5 years in the first and second studies, respectively) with higher intakes of n-3 LC-PUFAs, though the statistical analysis differed between the studies. In the second study, greater fish consumption, ranging from 1 to 2 meals a week, was associated with a 42% lower chance of developing hearing loss over 5 years.

Both groups of investigators noted that vascular factors might be involved in hearing loss, especially in the lower frequencies. The investigators noted that only a single artery supplies the cochlea of the inner ear where hearing loss occurs. Impaired blood flow could contribute to the loss or disruption of hair cells in the cochlea resulting in hearing loss. In an analogous manner, retinopathy, which is characterized by microvascular abnormalities in the retina, was associated with hearing loss in women, but not in men. In both conditions, n-3 LC-PUFAs might improve the microvascular circulation.
