



PUFA NEWSLETTER

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Turning Over a New Leaf

This issue of the *PUFA Newsletter* covers many new studies on long-chain omega-3 polyunsaturated fatty acids published in the last quarter, including fascinating work on greater brain volumes in older women with the highest levels of red blood cell EPA and DHA. Perhaps most importantly, new research on cardiovascular health in a multi-ethnic cohort receiving only minimal cardiovascular care reported a 51% lower risk of cardiovascular events in participants with the highest levels of blood EPA and DHA. Changes in medical care have been a confounding factor in several recent studies that have failed to substantiate significant benefits of EPA and DHA consumption where the majority of patients were already receiving two or more cardiovascular drugs. These controversial studies generated numerous media headlines doubting the value of EPA and DHA in cardioprotective diets. This study and many others suggest it is too soon to dismiss the importance of n-3 LC-PUFAs in protecting heart health.

This issue also marks a new chapter in the *PUFA Newsletter's* life. In the past, it has been funded by grants from DSM Nutritional Products and previously from Roche Vitamins, but going forward, I am pleased to say that the Global Organization for EPA and DHA Omega-3s (GOED) will be assuming responsibility for both the *PUFA Newsletter* and Fats of Life website.

GOED is committed to advancing the understanding of how omega-3s affect health and will continue to ensure that the articles in the *PUFA Newsletter* are peer-reviewed by highly qualified lipid scientists. We are an organization funded by industry, but we are committed to a balanced assessment of the omega-3 PUFA science. Our goals will only be met in the long-run if we remain credible and continue the critical reporting of PUFA research that this newsletter has represented.

Joyce Nettleton and her team have diligently provided brilliant insights into the advances in omega-3 science for the past 11 years, but sadly, she has decided to focus more time on other endeavors. The good news for all of her fans (myself included) is that she is not disappearing

and will continue her science writing. Even better news for Joyce is that she hopes to spend more of her winters skiing and taking advantage of all that her home in Colorado offers. Readers of her editorials in the *PUFA Newsletter* know how fond she is of the slopes!

The role of omega-3s in human health is complex and I am amazed at how Joyce has been able to distill it all into easy-to-follow research summaries, without talking down to her audience. GOED

is a global organization and no matter where we go in the world, we have met people who bring up issues of the *PUFA Newsletter* or reference Joyce's writing. The resources she has built are truly unmatched in their distribution and a rare, useful commodity on the Internet. I promise to continue to strive to meet the incredible standard she has set going forward.

We are fortunate in finding a very capable successor to Joyce, Dr. Gerard Bannenberg. He earned his Ph.D. from the Karolinska Institutet in Stockholm, Sweden, and has worked in lipid biochemistry for most of his career. He did his post-doc work with Charles Serhan at Harvard, one of the most advanced thinkers in lipid science. Most recently, Gerard led the scientific work at Solutex, a Spanish-based manufacturer of EPA and DHA omega-3 oils. We are very excited to welcome him on board and look forward to many years of success.

Please feel free to share your feedback and suggestions with us!

Best regards,

Adam Ismail



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■ CARDIOVASCULAR HEALTH

Higher Omega-3 Intakes Linked to Lower Cardiovascular Events in Multiethnic Study

Amid controversy whether long-chain omega-3 PUFAs are linked to lower heart events and mortality, a multiethnic study of healthy adults reported a significantly lower risk of cardiovascular events with higher blood levels of omega-3s.

Recent clinical trials have reported **no additional benefit** on the risk of cardiovascular mortality or morbidity from the consumption of long-chain omega-3 PUFAs (n-3 LC-PUFAs), mainly EPA and DHA, which are found in fish and fish oil, krill or

algal supplements. These studies have mainly been conducted in patients with multiple cardiovascular health risks who were receiving statins and other state-of-the-art medical care. Under such circumstances, it becomes more difficult to demonstrate additional clinical benefit from small amounts of n-3 LC-PUFAs. The catch is, not all patients at risk of heart disease receive state-of-the-art medical care and may require larger amounts of n-3 LC-PUFAs to reduce their cardiovascular risks. In patients not receiving statins, the effect of n-3 LC-PUFA supplementation was associated with a **50% lower risk** of major cardiovascular events.

For these reasons and others, information from large observational studies in heterogeneous populations about the relationship between PUFA status and the risk of cardiovascular events (e.g., myocardial infarction, death from heart disease, stroke) is useful. It is even better when such studies examine tissue PUFA concentrations rather than dietary consumption estimates, which are prone to substantial measurement error.

Results from a study of 2,837 adults of multiethnic descent (Caucasian, African American, Hispanic and Chinese American) living in the U.S. whose health was monitored for up to 10 years have just been published and are described in this article. Participants in the study ranged from 45 to 84 years of age and were free of clinical cardiovascular disease at enrollment. On average, participants were 62 years of age. The investigators assessed participants' health status every 2 years and obtained fasting blood for plasma phospholipid fatty acid analysis at baseline. They also assessed dietary intake using a food frequency questionnaire. After excluding those who consumed fish oil supplements, there were 2,372 participants.

Not surprisingly in a U.S. population sample, seafood and n-3 LC-PUFA intakes were low, with the average daily intake of EPA, DPA and DHA amounting to 147 mg/day. However, consumption of these n-3 LC-PUFAs ranged between 103 and 203 mg/day between the lowest and highest quartiles, whereas linoleic acid (n-6) intakes were reciprocal, ranging between 10.5 and 7.9 g/day in these quartiles. In this cohort, approximately 16% of participants were taking lipid-lowering medications.

Circulating levels of EPA and DHA were inversely associated with a lower risk of cardiovascular incidence. Hazard ratios for the highest quartiles of EPA and DHA were 0.49 (95% CI, 0.30 – 0.79) and 0.39 (95% CI, 0.22 – 0.67), respectively. These hazard ratios are equivalent to risk reductions of 51 and 61%, respectively. There were no significant associations between plasma phospholipid measures of alpha-linolenic acid or arachidonic acid and incident cardiovascular disease. There was, however, a trend toward higher risk of cardiovascular disease with greater concentrations of linoleic acid, but the trend did not reach statistical significance (P = 0.08). Similar results were observed for the risk of coronary heart disease incidence, with significant risk reductions with phospholipid levels of EPA, DHA and total n-3 LC-PUFAs, but there were no associations with alpha-linolenic acid or linoleic acid.

Higher plasma phospholipid levels of EPA and DHA were associated with reduced risks of cardiovascular events of 51% or more. Those with higher EPA and DHA also had lower inflammatory markers.

Higher phospholipid levels of these n-3 LC-PUFAs were also associated with significant reductions in the inflammatory mediators, IL-6 and receptor-1 for TNF-, but not with CRP. EPA, DHA and total n-3 LC-PUFAs were not significantly associated with

lower triglyceride levels, but EPA and total n-3 LC-PUFAs were related to small but significant increases in low-density lipoprotein-cholesterol levels. Phospholipid alpha-linolenic acid levels were not associated with risk of cardiovascular or coronary heart diseases. Higher levels were inversely related to CRP levels, but not to other inflammatory markers measured.

These data confirm numerous previous studies in which higher tissue levels of n-3 LC-PUFAs were associated with significantly **lower risks** of cardiovascular and coronary heart disease and mortality in the general population not taking statins. Their association with significantly lower levels of inflammatory biomarkers

confirms [previous reports](#) of the anti-inflammatory effects of n-3 LC-PUFA in patients with cardiovascular disease. Inflammation is widely recognized as an important factor in cardiovascular disease and the ability of n-3 LC-PUFAs to [reduce inflammatory mediators](#) and [resolve inflammatory responses](#) suggests additional advantages of these PUFAs for [treating atherosclerosis](#) and cardiovascular disease.

This study extends the observations on the protective effects of higher blood levels of n-3 LC-PUFAs on cardiovascular and coronary heart disease events to span diverse race and ethnic groups with relatively low intakes of seafood and n-3 LC-PUFAs. It also confirms the weak associations with alpha-linolenic acid and heart disease mortality and, interestingly, reported no risk reduction with circulating or dietary intakes of linoleic acid. Thus, continued controversy is assured.

de Oliveira Otto MC, Wu JH, Baylin A, Vaidya D, Rich SS, Tsai MY, Jacobs DR Jr, Mozaffarian D. Circulating and dietary omega-3 and omega-6 polyunsaturated fatty acids and incidence of CVD in the Multi-Ethnic Study of Atherosclerosis. J Am Heart Assoc 2013;2:e000606. [PubMed] Open Access

Unravelling Contradictions in Atrial Fibrillation Studies: It's U

The effect of long-chain omega-3 PUFAs in patients at high risk of atrial fibrillation has varied from a significantly reduced risk to no effect to an increased risk of the condition. What is going on?

Atrial fibrillation is a type of arrhythmia that results from disorganized electrical signals in the upper chambers of the heart (atria). Instead of beating rhythmically, the atria quiver, allowing blood to pool. As a result, insufficient blood reaches the lower chambers, which pump the blood throughout the body. Atrial fibrillation may occur occasionally

or become a long-term recurring condition. Those who have experienced atrial fibrillation face increased risk of stroke, heart failure and recurrence as a complication of cardiac surgery. The development of postoperative atrial fibrillation significantly [increases the risk](#) of in-hospital and long-term mortality, stroke and [longer hospital stays](#) making its prevention a high priority in cardiac surgery.

The effects of long-chain omega-3 PUFAs (n-3 LC-PUFAs) on the risk of developing postoperative atrial fibrillation have been investigated in many studies of patients undergoing different types of cardiac surgery

with highly inconsistent results. Some studies reported a significant [reduction in the risk](#) of postoperative atrial fibrillation and others have found [no benefit](#). There are also observational reports of [greater risk](#) of atrial fibrillation with higher serum levels of EPA and DHA, particularly of EPA, in Japanese patients and in those consuming more than 4 servings of dark fish per week in the [Framingham study](#). Reasons for these disparities are not obvious, beyond differences in patient populations, dose of n-3 LC-PUFAs and duration of the supplementation prior to surgery. It is common to initiate supplementation only 2 to 5 days prior to surgery. However, maximum [incorporation of EPA and DHA](#) into myocardial phospholipids may take about 3 weeks for DHA and 6 weeks for EPA. This suggests there may be considerable overlap in the tissue levels between treated and control patients.

Combined data from 2 trials in which patients at high risk of atrial fibrillation were supplemented with EPA plus DHA prior to cardiac surgery were analyzed for the incidence of atrial fibrillation in relation to EPA and DHA status prior to surgery.

With these considerations in mind, Robert Metcalf and colleagues at the Royal Adelaide Hospital, Australia and the University of Iceland in Reykjavik, examined the relationships between red blood cell phospholipid EPA and DHA concentrations in patients with no history of atrial fibrillation and the risk of postoperative atrial

fibrillation or flutter during the first 6 days after cardiac surgery. This period represents the greatest risk of atrial fibrillation. They combined and analyzed data from 2 randomized clinical trials, one conducted in Adelaide, the other in Reykjavik. In both trials, patients were supplemented with EPA plus DHA prior to surgery.

The Australia trial included patients 18 years or older and the Iceland study restricted participants to those >40 years of age. Patients in Australia with previous atrial fibrillation, currently taking anti-arrhythmic drugs, consuming fish oil supplements or eating fish more than once a week were excluded. Supplements provided 2.2 and 4.5 g/day of EPA plus DHA in Reykjavik and Australia, respectively. The investigators focused on n-3 LC-PUFA status in red blood cells at the time of surgery, not the duration of treatment. Statistical analyses were adjusted for study site, age, surgical procedure (coronary artery bypass graft alone or with valve surgery), BMI, diabetes and statin use. The analysis included 355 patients.

The greatest risk of atrial fibrillation occurred in patients in the lowest and highest quintiles of EPA and DHA in their red blood cells. The lowest risk was observed in the intermediate quintiles of red blood cell EPA and DHA.

Atrial fibrillation occurred in 160 (45%) patients and was more frequent in those who were older, had had valve surgery and had a lower BMI. Multivariate analyses considering EPA and DHA concentrations as continuous variables showed no significant relationships with risk of atrial fibrillation. In comparing quintiles of

red blood cell EPA and DHA with risk of atrial fibrillation, EPA bore no significant relationship to risk, whether comparing the highest quintile with the lowest, or with the quintile having the lowest risk, which was either the second or fourth quintile. In contrast, risk of atrial fibrillation was more than 2 times greater in patients with the highest or the lowest levels of red blood cell phospholipid DHA, regardless of whether the comparison was based on the first quintile or the quintile with the lowest risk (Table). The data also showed that the pattern of risk with DHA level was U-shaped, with risk greatest for the lowest and highest quintiles and non-significant risks for the second and third quintiles.

When the risk of atrial fibrillation was determined using the sum of red blood cell EPA and DHA, the pattern was similarly U-shaped, with greater risk of the condition at either the lowest or highest concentrations of EPA + DHA. The risk for EPA + DHA was significantly greater in the analysis using the fourth quintile as the reference, OR for Q5 = 2.36 (95% CI, 1.15-4.85). The investigators also looked for a possible association with red blood

cell arachidonic acid, but found no relationship with risk in any multivariate analysis.

A recent Danish study also reported that the risk of incident atrial fibrillation followed a U-shaped pattern in association with dietary n-3 LC-PUFAs from marine fish. Rix and colleagues at the Aalborg University Hospital, Denmark, examined incident atrial fibrillation or flutter in adults aged 50 to 64 years of age who were free of cancer and living in the urban area of Copenhagen and Aarhus. A total of 57,053 persons were enrolled and monitored for a median of 13.6 years. There were 3,284 participants who experienced atrial fibrillation. Participants' consumption of different fish was estimated from a semi-quantitative food frequency questionnaire at baseline. Atrial fibrillation or flutter occurred with twice the frequency in men as women, 6.5 per 1000 person-years vs. 3.2 per 1000 person-years in men and women, respectively. The median consumption of n-3 LC-PUFAs was 630 mg/day, about 6 times as much as consumed in the U.S. In adjusted analysis,

Red blood cell and dietary consumption data both describe the lowest risk of atrial fibrillation in cardiac patients and older adults in the general population in association with moderate concentrations and intakes of EPA and DHA. High and low levels of either red blood cell or consumption were associated with greater risk.

the lowest risk of atrial fibrillation was observed in the third quintile of total n-3 LC-PUFA consumption (HR – 0.87 (95% CI, 0.78-0.98), with the greatest risks of approximately 1 observed in Q1 and Q5. Similar results were reported for EPA and DHA.

Table. Risk of atrial fibrillation (OR, 95% CI*) by quintiles of red blood cell phospholipid DHA in 355 patients undergoing cardiac surgery

	Quintiles				
	1	2	3	4	5
DHA, range, % total fatty acids	3.3 - 5.2	5.2 - 6.1	6.1 - 7.0	7.0 - 7.9	7.9 - 10.7
Cases of Afib, no. (%)	30 (42)	29 (41)	33 (46)	23 (32)	45 (63)
OR using Q1 as reference	1	0.75 (0.37 - 1.54)	0.83 (0.40 - 1.74)	0.42 (0.19 - 0.94)	1.04 (0.43 - 2.47)
OR using Q4 as reference	2.36 (1.07 - 5.24)	1.78 (0.84 - 3.76)	1.96 (0.95 - 4.06)	1	2.45 (1.16 - 5.17)

*Risk adjusted for study site, age, surgical procedure, BMI, diabetes and statin use.

Although these 2 studies are not directly comparable owing to the differences in assessments of n-3 LC-PUFAs (red blood cells vs. consumption) and populations (cardiac surgery patients vs. cancer-free, older adults in the general population), both studies reported that the lowest risk of atrial fibrillation was associated with moderate red blood cell or dietary levels closer to mid-range or median values. Both studies clarify some of the discrepancies among many studies that have examined n-3 LC-PUFAs and atrial fibrillation. But like Goldilocks searching for the “just right” breakfast, researchers and clinicians will need to define the range of dietary or red cell n-3 LC-PUFAs that is most closely associated with a significantly lower risk of atrial fibrillation, especially for cardiac surgery patients who face greater risk. Metcalf’s study suggests that a target range of red blood cell EPA+DHA of approximately 9.2 to 10.4% of total fatty acids is a good place to start. A randomized double-blind controlled trial using biomarkers would contribute to answering this question.

Metcalf RG, Skuladottir GV, Indridason OS, Sullivan TR, Bjorgvinsdottir L, Sanders P, Arnar DO, Gibson RA, Heidarsdottir R, Cleland LG, Palsson R, Farquharson AL, Young GD, James MJ. U-shaped relationship between tissue docosahexaenoic acid and atrial fibrillation following cardiac surgery. Eur J Clin Nutr 2014;68:114-118. [PubMed]

Rix TA, Joensen Am, Riahi S, Lundbye-Christensen S, Tjønneland A, Schmidt EB, Overvad K. A U-shaped association between consumption of marine n-3 fatty acids and development of atrial fibrillation/atrial flutter—a Danish cohort study. Europace 2014; Feb. 26. [PubMed]

Worth Noting

Tousoulis D, Plastiras A, Siasos G, Oikonomou E, Verveniatis A, Kokkou E, Maniatis K, Gouliopoulos N, Miliou A, Paraskevopoulos T, Stefanadis C. Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome. Atherosclerosis 2014;232:10-16. On file

Wachira JK, Larson MK, Harris WS. n-3 Fatty acids affect haemostasis but do not increase the risk of bleeding: clinical observations and mechanistic insights. Br J Nutr 2014;28:1-11. [PubMed]

Wu JH, Mozaffarian D. ω -3 Fatty acids, atherosclerosis progression and cardiovascular outcomes in recent trials: new pieces in a complex puzzle. Heart 2014;100:530-533. [PubMed]

Khawaja O, Maziarz M, Biggs ML, Longstreth WT Jr, Ix JH, Kizer Jr, Zieman S, Tracy RP, Mozaffarian D, Mukamal KJ,

Siscovick DS, Djoussé L. Plasma free fatty acids and risk of stroke in the Cardiovascular Health Study. Int J Stroke 2014; doi: 10.1111/ijss.12216. [PubMed]

Marangoni F, Novo G, Perna G, Perrone Filardi P, Pirelli S, Ceroti M, Querci A, Poli A. Omega-6 and omega-3 polyunsaturated fatty acid levels are reduced in whole blood of Italian patients with a recent myocardial infarction: the AGE-IM study. Atherosclerosis 2014;232:334-338. [PubMed]

Amiano P, Machon M, Dorronsoro M, Dolores Chirlaque M, Barricarte A, Sanchez M, Navarro C, Huerta JM, Molina-Montes E, Sánchez-Cantalejo E, Urtizberea M, Arriola L, Larrañaga N, Ardanaz E, Quirós JR, Moreno-Iribas C, González CA. Intake of total omega-3 fatty acids, eicosapentaenoic acid and docosahexaenoic acid and risk of coronary heart disease in the Spanish EPIC cohort study. Nutr Metab Cardiovasc Dis 2014;23:321-327. [PubMed]

■ MATERNAL AND INFANT HEALTH

Eating Fish in Pregnancy Linked to Higher Birthweight and Lower Risk of Preterm Birth

Fish consumption in pregnancy is linked to higher infant birthweight and longer gestation time in many, but not all, studies. Thus, it remains uncertain whether increased fish or long-chain omega-3 PUFA consumption can improve pregnancy outcomes.

Fish and shellfish consumption during pregnancy provides the fetus with several nutrients that may be scarce in maternal diets, such as selenium and long-chain omega-3 PUFAs (n-3 LC-PUFAs), which are essential for optimum brain growth and function. Some, but not all, prospec-

tive cohort studies have reported that mothers who consume seafood during pregnancy may deliver infants of higher birthweight. Some intervention studies with n-3 LC-PUFAs have reported higher birthweights among supplemented women, especially where maternal intakes of n-3 LC-PUFAs have been very low. However, many studies have observed little or no effect of additional n-3 LC-PUFA consumption on birthweight.

Low seafood consumption has been linked to a greater risk of preterm delivery and low birthweight. Some studies reported a significantly lower risk of preterm birth among women with higher intakes of n-3 LC-PUFAs, particularly for very early delivery among high-risk women, although reports are inconsistent.

A meta-analysis of supplementation among low-risk women suggested that the effect of n-3 LC-PUFA supplementation during pregnancy was small. A more recent meta-analysis of 3 randomized controlled trials of n-3 LC-PUFA supplementation in pregnancy reported that supplemented women had a 40% lower risk of delivering before 37 weeks' gestation ($P<0.05$) compared with unsupplemented mothers. Their infants weighed 71 g more ($P<0.05$) than the offspring of unsupplemented women and had a significantly higher gestational age of 4.5 days. Considering the multiple health risks confronting preterm infants, especially those born before 34 weeks' gestation, effective measures to reduce the risk of preterm birth could have large and positive effects on infant health. For this reason, resolution of the discrepancies in the data on maternal n-3 LC-PUFA status during pregnancy is important.

An analysis of data from 19 birth cohorts in Europe examined the relationship between fish consumption in pregnancy and infant birthweight, gestational age and risk of preterm delivery. Higher fish consumption was associated with benefits in all three outcomes.

A new report based on pooled and harmonized findings from 19 European birth cohort studies (12 countries) involving 151,880 mother-child pairs from the general population is a welcome addition to the literature on fish intake during pregnancy and infant growth outcomes. Eligible

studies provided data on maternal fish intake during pregnancy, birthweight and gestational age for births between 1996 and 2011. Data included only singleton, live births. Frequency of fish consumption was grouped into three categories, ≤ 1 time per week, >1 but <3 times per week and ≥ 3 times per week, based on tertiles of fish intake from food frequency questionnaires. Six cohorts had at least one category with $<5\%$ of participants that did not fit these categories and were excluded. Gestational age was estimated as the interval between the last menstrual period and delivery (72%), by ultrasound estimation (21%) or absent this information, from obstetrician estimation (7%).

Birthweights were defined as small-for-gestational-age if they were below the 10th percentile for the cohort-specific growth curves, stratified by gestational length and sex. Low birthweight was defined as $<2,500$ g and high birthweight as $>4,000$ g. Preterm was considered to be delivery before 37 weeks' gestation.

The statistical analysis was performed first by cohorts and second for cohort-specific effects using random and fixed-effects meta-analysis. Adjustment for confounding variables determined *a priori* included maternal age at delivery, height, prepregnancy BMI, education, smoking during pregnancy, parity and infant sex. For three cohorts, analyses excluded adjustment for maternal age, education, prepregnancy BMI or parity when data were lacking.

A summary of the main outcome variables across the 19 cohorts is presented in Table 1.

Table 1. Summary of pregnancy characteristics and outcome measures in 19 European birth cohorts

Outcome measure	Range of means	Country cohort low/high
Birthweight, kg	3.2 – 3.6	Greece/Denmark
Gestational age, wk	38.4 – 40.1	Greece/Denmark
Preterm births, %	2.8 – 10.5	Netherlands/Greece
Low birthweight, %	1.7 – 6.4	Belgium/Norway
Maternal age, yr	≥ 29	All cohorts
Prepregnancy BMI ≥ 25 , %	33	Portugal, Norway, U.K.
Smoking during pregnancy, %	7.7 -31	Netherlands/Spain
Fish intake, times/wk, median	0.4 – 4.5	Netherlands/Spain
Fatty fish intake, times/wk, median*	0.5	All cohorts

*Median fatty fish intake more than twice the median in Italy, Portugal, Spain and Poland

Table 2. Adjusted associations (β -coefficients, 95% CI) of fish and seafood intake during pregnancy with gestational age and preterm birth in 19 European birth cohorts

Fish consumption times/wk	Cohorts no.	Gestational age, days	Preterm birth, <37 wk gestation
Fish intake Frequency	19	-0.02 (-0.09 – 0.05)	1.00 (0.97 – 1.03)
>1 but <3	13	0.41 (0.25 – 0.57)	0.87 (0.82 – 0.92)
≥ 3	13	0.23 (0.05 – 0.41)	0.89 (0.84 – 0.96)
Fatty fish	13	0.14 (-0.31 – 0.03)	1.04 (0.98 – 1.10)
Lean fish	12	-0.02 (-0.12 – 0.08)	1.00 (0.96 – 1.05)
Other seafood	16	-0.03 (-0.18 – 0.12)	1.01 (0.96 – 1.07)

In adjusted analysis, more frequent fish consumption during pregnancy was associated with higher birthweight (β -coefficient of ≥ 3 times per week vs. between 1 and less than 3 times per week = 15.2 and 8.9, respectively). The consumption of fatty fish compared with lean fish was also more strongly related to birthweight (β -coefficients, 2.2 vs. 1.0, respectively). Compared with women who ate fish once or less often per week, infants of mothers who ate fish ≥ 3 times per week were 15 grams heavier. However, fish consumption was unrelated to the overall risk of having a low or high birthweight infant or small-for-gestational-age neonate.

Fish consumption was also associated with a small, but significantly greater gestational age, for both high and medium fish intakes, of 0.2 and 0.4 days, respectively, compared with women who rarely ate fish (Table 2). Similarly the risk of preterm delivery was significantly reduced with consuming fish between more than once but less than 3 times per week (RR = 0.9, 95% CI, 0.8 – 0.9) and with fish consumption greater than 3 times per week (RR = 0.9, 95% CI, 0.8 – 0.96). The type of fish consumed was unrelated to the risk of preterm delivery.

Overall, the rate of preterm births was relatively low (2.8 to 10.5%) compared to the rate of 12% in the U.S. in 2010, where ethnic diversity may be greater. The U.S. rate represents a 30% increase from the rate of preterm births observed from 1981 to 2006, indicating that the risk of preterm birth continues to be a substantial, possibly growing, public health challenge.

The investigators also observed increased birthweight (~40 g) in infants born to mothers who smoked during pregnancy when mothers with the greatest fish consumption (≥ 3 times per week) were compared with mothers who ate fish ≤ 1 times per week. A similar, but less marked,

difference (10 g) was observed among non-smoking mothers. Smoking is associated with significantly lower birthweight and gestational age. The possibility that higher fish consumption may lower these risks is an encouraging prospect.

Eating fish more often during pregnancy was associated with higher birthweights, a slightly longer gestation time and a small but lower risk of preterm delivery in over 151,000 women.

By combining similar data from 19 cohorts to obtain a very large database, these investigators found significant associations

between fish consumption and modestly higher infant birthweight, slightly longer gestational age and a small reduction in the risk of preterm delivery. More frequent fish consumption and greater intakes of fatty fish were most closely associated with birthweight, but were less important factors in gestational age and risk of preterm delivery. The present study cannot attribute its findings to n-3 LC-PUFAs found in fish, but suggests this possibility in the results of higher birthweights with fatty fish consumption.

Leventakou V, Roumeliotaki T, Martinez D, Barros H, Brantsaeter AL, Casas M, Charles MA, Cordier S, Eggesbo M, van Eijsden M, Forastiere F, Gebring U, Govarts E, Halldórsson TI, Hanke W, Haugen M, Heppel DH, Heude B, Inskip HM, Jaddoe VW, Jansen M, Kelleher C, Meltzer HM, Merletti F, Moltó-Puigmartí C, Mommers M, Murcia M, Oliveira A, Olsen SF, Pele F, Polanska K, Porta D, Richiardi L, Robinson SM, Stigum H, Ström M, Sunyer J, Thijs C, Viljoen K, Vrijkotte TG, Wijga AH, Kogevinas M, Vrijheid M, Chatzi L. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. Am J Clin Nutr 2014;99:506-516. [PubMed] Open Access

Attention and Working Memory of Term Infants Unrelated to Prenatal Maternal DHA-Supplementation

Whether maternal supplementation with DHA in pregnancy benefits the neurodevelopment of term infants is unresolved. There is evidence on both sides, but differences in term infants have been difficult to demonstrate.

One of the most important reasons to assure the adequate intake of long-chain omega-3 PUFAs (n-3 LC-PUFAs) during pregnancy and early infancy is the importance of these fatty acids, especially DHA, in the structural and functional development of the brain. Although DHA is found in cell membranes throughout the brain, it is especially concentrated in the hip-

pocampus, basal ganglia and frontal lobes, which are involved in cognition and executive functions. Animals and humans fed diets deficient in n-3 PUFAs during fetal and early postnatal life experience significantly reduced content of DHA in their cerebral cortex and retina compared with those fed adequate n-3 PUFAs. Suboptimal dietary and brain DHA contents have been linked to poorer performance on visual attention, cognitive and behavioral tests, although data are inconsistent, especially in term infants. Data from DHA-deficient animals have described various behavioral and cognitive deficits linked to lower DHA levels in the frontal cortex. In addition, n-3 PUFA-deficient animals display deficits in neuronal and behavioral plasticity that carry over into adulthood.

One of the challenges in evaluating cognitive and behavioral performance in humans is the selection of assessment measures. Tools that reflect global cognitive function may be insufficiently sensitive for capturing specific brain and executive functions in young children. For this reason, measurement of attention is one aspect that has gained acceptance as a reflection of frontal lobe and hippocampus function and has been linked to maternal DHA status.

A recent report describes the effects of maternal n-3 LC-PUFA supplementation in pregnancy and the performance of executive functions in the 2-year-old offspring. Participants in this analysis were part of a larger randomized controlled trial, DHA for Maternal and Infant Outcomes. The investigators recruited 185 participants when the offspring turned 2 years of age, selecting from those who had not been born preterm, with low birthweight or neurologic or

visual pathologies. Pregnant women were originally recruited into the study before 21 weeks' gestation and had consumed 800 mg of DHA with 100 mg of EPA per day or a blended oil placebo from enrollment until delivery. A total of 158 children completed the assessments. They were 27 months of age, on average, at the time of testing.

The primary outcomes were the children's looking times on measures of attention and the accuracy of locating a hidden figure on the working memory and inhibitory control assessment. Attention was evaluated using



3 tasks: single-object sustained attention, multiple object attention to one item and a distractibility task involving sustained attention to one object in the presence of a distracting stimulus. Each child's responses were video recorded. To assess working memory and inhibitory control, the investigators used a lentil-box version of the A-not-B task described by Spencer *et al.* and explained fully in the study publication.

The results of all the attention tests showed no differences between the offspring of treatment and control mothers. The investigators noted that the latency for distraction increased across the distractibility trials, suggesting a learning effect. This observation did not differ between the groups.

In the working memory assessment, the accuracy of finding the toy did not differ between the two groups. The investigators noted that control children were more

accurate in searching for the hidden toy during training trials than treatment children, but this difference did not appear during test conditions.

Toddlers born at term from mothers supplemented with 800 mg of DHA during the last half of pregnancy did not differ from control children in tests of attention or working memory when they were 27 months of age.

This study found no evidence that DHA levels in cord plasma were associated with

attention or working memory in 2-year-old children. The researchers observed some overlap of cord plasma

DHA between the treated and control groups, but in separate analysis, the associations for some assessments occurred in both groups. Lack of consistent effects suggests that these observations may have been due to chance. Overall, the lack of any relationship between cord blood DHA levels and attention or memory outcomes agrees with [other findings](#), including from [this cohort](#), using global assessments of neurodevelopment.

The investigators commented that even though cord plasma DHA levels in the supplemented mothers were higher than in the control women, term-born infants arrive with the full transfer of LC-PUFAs from the mother, which accelerates in the last trimester. Thus, the infants of both groups may have had sufficient DHA to provide for normal brain function. However, standard performance levels for the tests used have not been established. In well-nourished term infants, the provision of 800 mg of DHA per day in the last half of pregnancy was unrelated to the offspring's performance on attention and working memory tests.

Gould JF, Makrides M, Colombo J, Smithers LG. Randomized controlled trial of maternal omega-3 long-chain PUFA supplementation during pregnancy and early childhood development of attention, working memory, and inhibitory control. Am J Clin Nutr 2014;99:851-859. [PubMed] Open Access

Worth Noting

Ramakrishnan U, Imhoff-Kunsch B, Martorell R. Maternal nutrition interventions to improve maternal, newborn and child health outcomes. Nestle Nutr Inst Workshop Ser 2012;78:71-80. [PubMed]

Viljoen K, Vrijkotte TG, Wijga AH, Kogevinas M, Vrijheid M, Chatzi L. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. Am J Clin Nutr 2014;99:506-516. [PubMed] Open Access

Loomans EM, Van den Bergh BR, Schelling M, Vrijkotte TG, van Eijsden M. Maternal long-chain polyunsaturated fatty acid status during early pregnancy and children's risk of problem behavior at age 5-6 years. J Pediatr 2014;164:762-768. [PubMed]

Jones ML, Mark PJ, Waddell BJ. Maternal dietary omega-3 fatty acids and placental function [Review]. Reproduction 2014;147:R143-R152. [PubMed]

Giorlandino C, Giannarelli D. Effect of vaginally administered DHA fatty acids on pregnancy outcome in high risk pregnancies for preterm delivery: a double blinded randomised controlled trial. J Prenat Med 2013;7:42-45. [PubMed] Open Access

Kelsall CJ, Hoile SP, Irvine NA, Masoodi M, Torrens C, Lillycrop KA, Calder PC, Clough GF, Hanson MA, Burdge GC. Vascular dysfunction induced in offspring by maternal dietary fat involves altered arterial polyunsaturated fatty acid biosynthesis. PLoS One 7:e34492. doi:10.1371/journal.pone.0034492. [PubMed] Open Access

■ IMMUNE FUNCTION

DHA in Pregnancy Not Linked to Atopic Diseases in High-Risk Children at Age 3

There are many plausible reasons to think that increased consumption of long-chain omega-3 PUFAs (n-3 LC-PUFAs) during pregnancy or infancy may reduce the development of atopic allergic diseases, such as eczema, wheeze and rhinitis. These allergies in infants and children are characterized by the increased production of immunoglobulin E (IgE), a class of antibody capable of triggering strong inflammatory responses. Several randomized trials have reported [reduced severity](#) of atopic conditions in infants whose mothers consumed fish oil or fish during pregnancy, although results have been [inconsistent](#). Increased consumption of n-3 LC-PUFAs is not associated with a lower occurrence of atopic diseases, however.

Encouraging results associated with the consumption of long-chain omega-3 PUFAs by pregnant women whose offspring are at high risk of atopic diseases suggest that greater consumption of these fatty acids might delay the onset and reduce the symptoms of allergic diseases.

The well known anti-inflammatory properties of n-3 LC-PUFAs and their relatively low consumption in a background diet [high in omega-6](#) (n-6) PUFAs typical of most Western diets suggest that greater intakes of n-3 LC-PUFAs, especially during pregnancy, might reduce the development of atopic diseases in infants and children. The use and effectiveness of n-3 LC-PUFAs in the treatment of several chronic inflammatory diseases, including [rheumatoid arthritis](#) and chronic [inflammatory airway disease](#) bolster the reasons for continuing to explore n-3 LC-PUFAs in allergic diseases. A detailed review of the various [mechanisms](#) by which n-3 LC-PUFAs and their derivatives moderate inflammatory diseases has been published recently.

An extension of [previous research](#) on maternal n-3 LC-PUFA supplementation, mainly as DHA, in pregnancy

and the development of IgE-mediated allergic disease was reported by Debbie Palmer and colleagues at the Universities of Western Australia and Adelaide. Children whose mothers had been randomized to consume capsules containing 800 mg DHA plus 100 mg EPA or vegetable oil for the last half of pregnancy were monitored for the occurrence of IgE-associated eczema or atopic eczema and other atopic diseases at 1 and 3 years of age.

Toddlers in the study all had a parent or sibling with medically diagnosed allergic diseases. The researchers defined eczema according to medical review or a history of an itchy



rash with a chronic or fluctuating course (Illustration). Association with IgE was determined by skin prick sensitization to at least one of the tested allergens (whole hens' egg, cows' milk, wheat, tuna and peanut) and several aeroallergens (ryegrass, cat hair, mold, house dust mite and others). In this report, the same allergens were tested when the children were 3 years of age, with the addition of cashew nut, sesame seed and another species of house dust mite. Lack of availability of the cows' milk allergen resulted in the removal of this allergen from testing. A skin rash within 60 minutes of ingesting a food, with or without respiratory, gastrointestinal or cardiovascular symptoms, was used to determine IgE-associated food allergy. Asthma included 3 or more episodes of wheeze less than 6 weeks apart or daily use of asthma medication. The investigators defined allergic rhinitis as a history of sneezing or a runny/blocked nose accompanied by itchy-watery eyes in the absence of an upper respiratory tract infection.

Originally, 706 children were recruited for the study, of whom 587 were available for a skin prick test and medical review at 3 years of age. Of the children whose mothers consumed DHA in pregnancy, 17.3% were diagnosed with IgE-mediated allergic diseases compared with 22.6% in the control group. However, this difference did not reach statistical significance ($P=0.11$). Eczema was the most common allergic disease, affecting 16.3% of the children. The difference in eczema between the two groups, 13.8 vs 19.0% in the DHA and control groups, respectively, was not statistically significant ($P=0.10$). For the entire 3-year period, 4.6% of the children were diagnosed with at least one IgE-mediated food allergy, with egg being the most frequent allergy. There were no differences between the groups in the

occurrence of food allergies, respiratory disease or rhinitis. Compared with the diagnosis of allergic eczema at 1 year of age, more children received this diagnosis at age 3, 13.0 vs 9.2%, for ages 3 and 1 years, respectively. At both ages, the groups did not differ in the rate of sensitization to at least one allergen. These findings contrast with a recent report of an inverse relationship between cord blood and maternal plasma n-3 LC-PUFAs and the incidence of atopic eczema in 14-month-old Spanish infants.

Interestingly, the pattern of allergic sensitization changed dramatically over the 3-year period. In the first year of life, the predominant allergens were egg, peanut and cat hair, but at 3 years of age, sensitization to these allergens decreased significantly (except for cat hair),

while sensitization to airborne allergens such as mold, ryegrass pollen, house dust mite and cat hair was significantly higher.

Children at high risk of allergic diseases whose mothers consumed 800 mg DHA plus 100 mg EPA during the last half of pregnancy did not differ significantly from control children in the frequency of diagnosed IgE-mediated allergic diseases at the age of 3 years.

Findings from this follow-up study confirm the earlier results from these children and suggest that 800 mg of DHA supplementation during pregnancy was not associated with significantly reduced occurrence of IgE-mediated allergic

disease at the age of 3. At both 1 and 3 years of age, the difference in the occurrence of IgE-mediated allergic diseases between the DHA-treated and control groups was 5%, which was not statistically significant. From a public health perspective, this reduction in disease might affect large numbers of families and could have practical value. The investigators also reported a large shift in the pattern of allergic sensitivity away from food sensitivities toward airborne allergens from 1 to 3 years of age. Although these results in young children seem clear, other reports in adults with immune-based inflammatory diseases, such as rheumatoid arthritis, have demonstrated clinical benefits and reduced use of nonsteroidal anti-inflammatory drugs with large doses of n-3 LC-PUFAs. In other inflammatory diseases, such as inflammatory bowel disease, n-3 LC-PUFAs have not shown similar benefits. The particular allergic disease, dose and duration of n-3 LC-PUFA treatment, host factors and possibly differences between EPA and DHA in various allergic diseases might all contribute to these observations. One also hopes that

the emergence of specific [genetic determinants](#) of allergic diseases might provide additional insights into the hereditary component of these conditions. “Why” questions will continue.

Palmer DJ, Sullivan T, Gold MS, Prescott SL, Heddle R, Gibson RA, Makrides M. Randomized controlled trial of fish oil supplementation in pregnancy on childhood allergies. Allergy 2013;68:1370-1376. [PubMed]

Worth Noting

Montes R, Chisaguano AM, Castellote AI, Morales E, Sunyer J, López-Sabater MC. Fatty-acid composition of maternal and umbilical cord plasma and early childhood atopic eczema in a Spanish cohort,” published in the Eur J Clin Nutr 2013;67:658-663. [PubMed]

Magnusson J, Kull I, Rosenlund H, Håkansson N, Wolk A, Melén E, Wickman M, Bergström A. Fish consumption in infancy and development of allergic disease up to age 12 y. Am J Clin Nutr 2013;97:1324-1330. [PubMed] Open Access

Miles EA, Calder PC. Omega-6 and omega-3 polyunsaturated fatty acids and allergic diseases in infancy and childhood [Review]. Curr Pharm Des 2014;20:946-953.

■ BRAIN AND CNS

Greater Brain and Hippocampus Volume with Higher Red Blood Cell EPA + DHA in Older Women

If you want to peer into the brain, magnetic resonance imaging (MRI) is the instrument of choice. MRI is used for identifying and assessing brain changes in [healthy aging](#), normal [cognitive behavior](#), predicting [Alzheimer’s disease](#), studying [memory](#) and intellectual development, understanding diverse other brain

functions and much more. MRI studies have shown that [structural changes](#) in different brain regions occur throughout life, but the patterns differ with aging. Efforts to distinguish what happens in normal, healthy aging compared with pathological changes suggest considerable [overlap](#) between the two conditions. For example, [brain atrophy](#)

occurs in healthy aging, as observed in volume reductions in the entire cerebral cortex, especially in the temporal and prefrontal cortices, and cortical thinning. But some atrophy occurs in areas vulnerable to Alzheimer’s disease, while others happen in less susceptible regions. Atrophy [accelerates](#) with advancing age.

Changes in brain volume have emerged as important indicators of cognitive decline and dementia prior to the detection of clinical symptoms. Brain volume decreases with aging and diminution of whole brain volume has been detected at the [age of 30 years](#). The [rate](#) of decreasing brain volume is approximately twice as great in individuals with mild dementia as in nondemented older adults.

Loss of [fornix white matter](#) volume, but not hippocampal volume, was reported to predict impaired cognition. Many others have reported loss in [hippocampal volume](#) associated with declining cognition. In the offspring of the original Framingham Heart Study participants, those in the lowest quintile of [hippocampal volume](#) had a 4-fold greater risk of Alzheimer’s disease. Brain age scores derived from MRI imaging and brain atrophy have been used to [predict the conversion](#) of mild cognitive impairment to Alzheimer’s disease. [Cortical thinning](#), especially during early loss in cognition, along with reduced hippocampal volume were associated with early Alzheimer’s disease. Further, cognition [declines more quickly](#) in cognitively healthy older individuals with smaller hippocampal volumes.

Nutrition may also affect the risk of cognitive decline, Alzheimer’s disease and brain volumes. [Nutrient patterns](#) high in B vitamins and long-chain omega-3 PUFAs (n-3 LC-PUFAs) were associated with higher cognitive function and brain volume in a study of 87-year-old women. Low intakes of vitamin D or blood levels of its derivative 25-hydroxyvitamin D were associated with [poorer performance](#) on cognitive function tests and a [higher risk](#) of Alzheimer’s disease. Higher levels of vitamin D, DHA and physical activity were associated with [cortical sparing](#) in healthy adults 23 to 87 years of age. [Impaired glycemia](#) has also been linked to increase progression of mild cognitive impairment.

However, n-3 LC-PUFAs are the most extensively studied nutrients associated with impaired cognition and risk of Alzheimer’s disease. Focus on these fatty acids derives in part from DHA being the [most abundant PUFA in the brain](#). More than 20 years ago, [Soderberg](#) and colleagues reported that the brains of Alzheimer’s disease patients had significantly lower levels of arachidonic and docosahexaenoic acids. Since then, the [low consumption of fish](#) and n-3 LC-PUFAs has been associated

Changes in brain volume have emerged as important preclinical indicators of cognitive decline and dementia. Loss of brain volume is approximately twice as great in individuals with mild dementia compared with nondemented adults.

with a significantly higher risk of Alzheimer's disease, while those with the **highest concentrations of DHA** in their plasma phospholipids were reported to have half the risk of developing all-cause dementia.

Higher intakes of n-3 LC-PUFAs have been associated

Red blood cell EPA + DHA has been associated with higher brain volume in older women, while higher DHA was linked to better scores on visual memory, executive function and abstract thinking. This study examined the relationship between brain volumes in older nondemented women and red blood cell EPA and DHA.

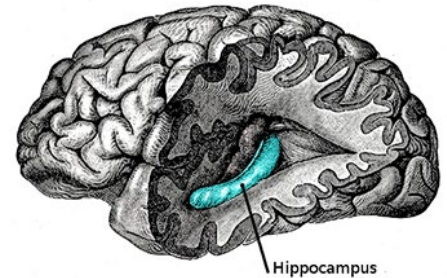
with greater **gray matter volume** in the right hippocampus and right amygdala. Red blood cell EPA and DHA concentrations were associated with white matter hyperintensity volumes and **total brain volume** in older women in the Framingham heart study who were free of dementia at the time of study. Participants with the lowest levels of red blood cell DHA

also had lower scores for visual memory, executive function and abstract thinking compared with those in the higher DHA quartiles. Others reported that higher **plasma EPA**, but not DHA, was associated with lower gray matter atrophy in the right hippocampal/parahippocampal area and right amygdala in cognitively healthy older adults. However, there are **discrepancies** between plasma and red blood cell fatty acids and those in the brains of individuals with mildly impaired cognition and Alzheimer's disease. It has also been suggested that **DHA homeostasis changes** in older adults in ways that might affect susceptibility to Alzheimer's disease.

To further explore and clarify the relationship between red blood cell n-3 LC-PUFAs and volumes in the whole brain and some specific regions, James Pottala and colleagues at the University of South Dakota examined brain volumes in a subset of participants in the Women's Health Initiative Memory Study. Participants from 14 U.S. research centers were free of dementia and averaged 78 years of age. The investigators assessed the volumes of the whole brain and the 4 lobes and the limbic, basal ganglia, corpus callosum and hippocampus regions, as well as in gray and white matter. Blood samples were taken at enrolment and MRI brain scans performed a median of 8 years thereafter. Exclusions from the final data set included degradation of blood samples, stroke or transient ischemic

attacks since screening, poor quality scans and missing data. Thus, 1,111 participants remained from the initial sample of 1,380 women.

Total brain volumes comprised 99% normal and 1% ischemic tissue and each type was analyzed separately. In bivariate analysis, ischemic brain volumes



were unrelated to DHA, EPA or omega-3 index (% red blood cell fatty acids as EPA + DHA). In covariate-adjusted analysis of total, normal and ischemic brain volumes, total brain volumes and normal tissue volumes were significantly associated with increasing concentrations of EPA + DHA. The absolute mean difference in the omega-3 index between Q1 and Q4 was associated with a 0.67% larger brain volume or approximately 5.8 cm³ after 8 years. These observations are similar to the findings in the Framingham study offspring women, where participants in the lowest quartile of the omega-3 index had a 0.49% smaller brain volume at the age of 67 years. There was a trend toward higher brain volumes with increasing DHA concentrations ($P=0.06$), but no association or trend in the brain volumes with EPA levels or in ischemic tissue.

Total brain volumes in these older women were significantly associated with increasing concentrations of EPA+DHA in red blood cells in adjusted analysis. Hippocampal gray matter volumes were significantly higher in the highest vs. the lowest quartile of red blood cell EPA+DHA.

When the investigators examined the brain volumes in 13 anatomical regions, including both white and gray matter, the estimated volume of gray matter in the hippocampus was significantly greater in those in the highest compared with the lowest quartiles of the omega-3 index (EPA + DHA). The

difference was 159 mm³ (Q1 vs. Q4, 95% CI, 0.01 – 0.31, $P = 0.03$). White matter in the corpus callosum was 66 mm³ greater for each SD increase in the omega-3 index, but the difference between Q1 and Q4 was not significant.

Finding no relationship between red blood cell EPA, DHA or the omega-3 index in ischemic tissue contrasts with results in the Framingham study where lower DHA levels were associated with greater white matter hyperintensity volumes as a percentage of the intracranial volume. In the Cardiovascular Health Study, 75-year-old women who consumed tuna or other fish ≥ 3 times per week were **less likely to incur subclinical infarcts** (ischemic lesions ≤ 3 mm diameter) or other white matter abnormalities compared with women who ate these fish $<$ once a month.

This observational study builds on a growing body of evidence suggesting that higher red blood cell concentrations of n-3 LC-PUFAs, which are associated with higher intakes of these PUFAs, indicate less rapid loss of brain volume and in turn, a slower loss of cognitive function in aging. That the volume of the hippocampus appears to be specially protected suggests that a link with less impaired cognitive function is likely. Considering the many functions of DHA in brain structure and activity, the neuroprotective effects of DHA in preserving cognitive function during aging appear increasingly compelling.

Pottala JV, Yaffe K, Robinson JG, Espeland MA, Wallace R, Harris WS. Higher RBC EPA + DHA corresponds with larger total brain and hippocampal volumes: WHIMS-MRI Study. Neurology 2014;82:435-442. [PubMed]

Desai A, Kevala K, Kim HY. Depletion of brain docosahexaenoic Acid impairs recovery from traumatic brain injury. PLoS One 2014;9:e86472. [PubMed] Open Access.

Worth Noting

Grayson DS, Kroenke CD, Neuringer M, Fair DA. Dietary omega-3 fatty acids modulate large-scale systems organization in the rhesus macaque brain. J Neurosci 2014;34:2065-2074. [PubMed]

Zhang M, Wang S, Mao L, Leak RK, Shi Y, Zhang W, Hu X, Sun B, Cao G, Gao Y, Xu Y, Chen J, Zhang F. Omega-3 fatty acids protect the brain against ischemic injury by activating Nrf2 and upregulating heme oxygenase 1. J Neurosci 2014;34:1903-1915. [PubMed]

MENTAL HEALTH AND COGNITION

EPA Significantly Reduces Depressive Episodes in Interferon-Alpha-Treated Hepatitis C Patients

Interferons are a class of cytokines with important immunoregulatory properties, including antiviral and

Interferon-alpha, a potent cytokine, is widely used to treat hepatitis C patients, but is associated with a high rate of depressive symptoms. A new study examined the effectiveness of pretreatment with EPA or DHA in reducing the incidence of depressive symptoms in these patients.

anticarcinogenic effects. They are especially important in the body's innate immune response to **viral infections** and are widely used in the primary and adjunct **treatment** of hepatitis B and C viruses, cancer, such as malignant **melanoma** and certain leukemias,

and in multiple sclerosis. However, their use is associated with several **side effects**, including **neuropsychiatric** and **depressive** symptoms, autoimmunity and **slower motor speed**. A systematic **review** of interferon-induced depression in patients with chronic hepatitis C reported that 1 in 4 patients developed a major depressive episode within 24 weeks of treatment. Neuropsychiatric side effects frequently cause patients to discontinue treatment. Antidepressant drug therapy carries its own set of adverse effects, which complicate the treatment of hepatitis C patients. As a result, clinicians have turned to alternative approaches to prevent or reduce depressive symptoms in interferon-alpha-treated patients. Enter long-chain omega-3 PUFAs (n-3 LC-PUFAs).

Low **dietary intakes** and blood levels of n-3 LC-PUFAs have been associated with a higher risk of developing depressive symptoms in observational studies, but their use in the treatment of various depressive disorders has a mixed record. Some studies have reported improved scores on depression rating scales with DHA supplementation as an **adjunct to therapeutic counseling**. In clinically diagnosed patients with major depressive disorder, treatment with n-3 LC-PUFAs had **significant antidepressive effects**. It has been suggested that n-3 LC-PUFAs might **enhance psychiatric treatments** because of their ability to augment medical therapies and the low occurrence of side effects associated with them.

Kuan-Pin Su and colleagues at the China Medical University, Taiwan, conducted a randomized, double-blind controlled trial to examine the potential preventive effects of n-3 LC-PUFAs on the development of depressive symptoms in hepatitis C patients being treated with interferon-alpha. Previous work by these investigators had reported significantly lower levels of DHA, but not EPA in red blood cell membranes between depressed and nondepressed patients undergoing interferon-alpha treatment (3.2 ± 0.09 vs. $3.4 \pm 0.34\%$, respectively). With those observations in mind,

they designed their study to provide short-term EPA, DHA or placebo supplementation prior to the onset of interferon-alpha treatment in patients with hepatitis C.

Participants were recruited by hepatologists from the Liver Center of China Medical University Hospital, Taiwan, who determined the eligibility of patients for combination therapy with weekly peginterferon α -2b and daily ribavirin, an antiviral medication. Participants



with a previous major depressive episode, a lifetime history of psychotic disorders or alcohol or drug dependence within a year prior to the study, or who had any unstable chronic medical condition were ineligible. Of the 162 patients who were randomized and treated for 2 weeks, 152 completed the 24-week combined drug therapy and were included in the analysis. Participants were 51 years of age, on average, and 48% were male. At randomization, participants were given capsules containing 2.5 g/day of EPA or 1.75 g/day of DHA or 800 mg of high oleic acid oil as a control to consume daily for 2 weeks. After the fatty acid treatment, interferon-alpha treatment began and patients were evaluated at weeks 2, 4, 6, 8, 12, 16, 20 and 24 for the occurrence of any major depressive episode using the [Mini-International Neuropsychiatric Interview](#). The investigators analyzed red blood cell membrane fatty acids at randomization, at the end of n-3 LC-PUFA treatment and at week 24. The primary outcome was the incidence of a major depressive episode at any time during the 24-week post-fatty acid treatment period.

The severity of depressive and neurovegetative symptoms (functional impairments such as sleep disorder, loss of interest, hopelessness, inability to concentrate) was assessed using the Hamilton Depression and Neurotoxicity Rating Scales, given at enrollment, the beginning of combination drug treatment and the same times as the clinical patient evaluations.

EPA and DHA measurements in the red blood cells after the 2-week intervention showed significant increases in EPA and DHA in the respective treatment groups compared with the values at enrollment. There were no changes in the control group. After 24 weeks, the incidence of a major depressive episode was significantly lower in the patients who consumed the EPA supplement compared with the DHA group and the control (Table). Among all participants, 22% developed

an interferon-alpha-induced depressive episode at some point during the 24-week treatment. Those who developed a depressive episode had lower EPA levels at enrollment compared with participants who did not have such an episode ($2.0\% \pm 0.6$ vs. $2.5\% \pm 0.9$, $P = 0.03$).

Table. Incidence and time to occurrence of a major depressive episode in 152 hepatitis C patients treated with EPA or DHA for 2 weeks prior to 24 weeks of combined therapy with interferon-alpha and ribavirin

Outcome at 24 wk	Control	EPA	DHA	P
Interferon-induced major depressive episode, % (n)	30 (15)	10 (5)	28 (14)	0.04
Time to depressive episode, wk	5.3 \pm 3.2	12.0 \pm 5.8	11.7 \pm 6.4	0.002

Pretreatment with either EPA or DHA resulted in a significant delay in the onset of a major depressive episode compared with the control (12 vs. 5 wk). Results from the secondary measures of depressive symptom severity showed increased scores over time for the Hamilton Depression Scale evaluations that peaked at 20 weeks. There were no significant differences among the EPA, DHA and control groups, although those in the EPA group had significantly lower scores at weeks 4 and 8 compared

with the DHA and control groups. The Neurotoxicity Scale scores increased markedly over the first 8 weeks and diminished thereafter, remaining significantly higher at 24 weeks than fatty acid or drug treatment baseline values.

Pretreatment with EPA for 2 weeks prior to interferon-alpha therapy in patients with hepatitis C significantly reduced the incidence of interferon-induced depressive episodes. DHA had no effect on depressive episodes, but both EPA and DHA significantly delayed the onset of symptoms.

The key finding of this trial was the significant reduction in the incidence of a major depressive

episode in hepatitis C patients who were pretreated with EPA for 2 weeks prior to drug therapy with interferon-alpha and ribavirin. Both EPA and DHA led to a significant delay in the onset of symptoms, but only

EPA was associated with a lower incidence of depressive episodes. Others have also reported that EPA is more effective than DHA in the treatment of depressive symptoms. A meta-analysis of 15 controlled trials of EPA or EPA + DHA in the treatment of depressive symptoms concluded that supplements containing 60% or more of EPA in treatments using EPA + DHA were more effective than those having a lower proportion of EPA. Randomized controlled trials of n-3 LC-PUFAs in diverse settings seldom yield as clear-cut responses as reported in this study. With confirmation by other investigators, EPA might be on its way to reducing the depressive episodes that occur in a considerable proportion of hepatitis C patients facing challenging drug therapy.

Su KP, Lai HC, Yang HT, Su WP, Peng CY, Chang JP, Chang HC, Pariante CM. Omega-3 fatty acids in the prevention of interferon-alpha-induced depression: results from a randomized controlled trial. Biol Psychiatry 2014; Jan. 24. doi: 10.1016/j.biopsych.2014.01.008. [PubMed]

Fish Intake, not Red Blood Cell Omega-3s Linked to Poorer Cognitive Performance in Older Adults

More frequent consumption of fish, especially fatty fish, and higher blood levels of long-chain omega-3 PUFAs (n-3 LC-PUFAs) have been associated with a lower risk

of developing dementia and cognitive decline in several prospective cohort studies of older adults. As is often the case, not all studies agree. Fewer investigators have asked the basic question, does fish or n-3 LC-PUFA consumption affect normal cognitive perfor-

mance in older individuals? Studies in Norway and the U.K. reported a direct association between higher fish consumption and cognitive performance in adults 70 to 74 and 70 to 79 years of age, respectively. However, a randomized trial in the same individuals in the U.K. who were supplemented with 700 mg per day of EPA + DHA or a placebo for 2 years reported no significant decline in cognitive performance in either group. A study in the Netherlands of 807 individuals aged 50 to 70 years reported no association between plasma n-3 PUFAs and cognitive performance on 5 domains. A study of

middle-age adults reported that n-3 LC-PUFA intakes were inversely related to the risk of impaired cognitive function. There are few cross-sectional studies that have examined this question, especially using biomarkers, in cognitively healthy older individuals.

Vanessa Danthiir and colleagues at the University of Adelaide, Australia, wondered whether long-term fish consumption or more recent n-3 LC-PUFA intakes were associated with cognitive performance in older adults with normal cognitive function. They also considered the influence of the APOE-ε4 allele, which is associated with a higher risk of Alzheimer's disease and the spectrum of dementia. The researchers recruited participants 65 to 90 years of age from metropolitan Adelaide and excluded those who were taking any n-3 PUFA supplements, had experienced head injury, brain trauma, stroke or transient ischemic attacks, coronary artery bypass surgery, degenerative neurological disease, had a history of alcohol or drug abuse, were taking drugs known to interfere with cognition, had various other medical or mental conditions or scored below 22/27 on a telephone-administered version of the Mini Mental State Examination (MMSE). This score was equivalent to a score of <24/30 on the standard MMSE test.

To assess historical fish consumption, the investigators used the Lifetime Diet Questionnaire which asked how frequently participants consumed several species of fatty fish and other fish during childhood, early adulthood, adulthood and middle-age, given the choices of daily, 2 to 3 times/week, 2 to 3 times/month, rarely or never. Current fish consumption was also obtained using a food frequency questionnaire. The investigators measured n-3 LC-PUFAs in red blood cell membranes using gas chromatography.



Cognitive function was evaluated using a test battery of 2 or more tests for the following 10 functions: perceptual speed, simple/choice reaction time, speed of memory scanning, reasoning speed, inhibition, psychomotor speed, reasoning, working memory, short-term memory and retrieval fluency.

A total of 390 participants were enrolled and 388 provided data for all variables. Historical fish consumption data came from 352 participants obtained 3 months

Few observational studies have examined whether fish consumption or tissue omega-3 PUFAs are associated with cognitive function in older adults. A cross-sectional study measured dietary fish consumption and red blood cell omega-3 PUFAs to answer this question.

Table. Standardized regression coefficients (P value) for the relationship between current fish consumption and cognitive performance in 388 cognitively normal adults aged 65 to 90 years

Cognitive test	Total fish intake	Fatty fish	White fish
Perceptual speed	-0.09 (0.05)	-0.07 (0.17)	-0.07 (0.14)
Inhibition	-0.11 (0.028)	-0.11 (0.03)	-0.05 (0.33)
Simple/choice reaction time	-0.13 (0.008)	-0.02 (0.69)	-0.15 (0.002)
Reasoning speed	-0.10 (0.045)	-0.04 (0.48)	-0.10 (0.043)
Memory scanning speed	-0.10 (0.047)	-0.07 (0.13)	-0.06 (0.22)

after the baseline assessments. Data for all variables were available for 339 of these participants.

At baseline, the participants reported eating fish an average of twice a week, with 60% of intake attributable to white fish. Red blood cell membrane concentrations of EPA, DHA and total n-3 LC-PUFAs, but not DPA, were associated with weekly consumption of all fish and fatty fish, as expected. When the findings on the cognitive performance tests were analyzed in terms of their association with current fish consumption, using the whole sample (388 participants) and a multivariate model, total fish consumption was significantly associated with slower cognitive speed for inhibition, simple/choice reaction time, reasoning speed and memory scanning (Table). More frequent consumption of fatty fish was significantly associated with poorer inhibitory processes and eating white fish more often was associated with slower simple/choice reaction time. Sex or APOE-ε4 status interactions did not occur in any analytical model. Controlling for historical fish consumption did not appreciably alter these findings.

Analysis of the potential effect of historical or habitual fish consumption at different stages of life—childhood through middle age—revealed modest, but significant inverse associations between total fish intake in childhood and perceptual speed and simple/choice reaction times in both the entire sample and just those for whom historical data were available. Cognitive scores were unrelated to fish consumption at all other life stages.

The relationships between the cognitive scores and the total and individual n-3 LC-PUFAs in the red blood cell membranes of all the participants showed no significant associations with any fatty acid in any of the statistical models. The researchers did find a significant interaction between red blood cell membrane EPA concentration and sex for reasoning and perceptual speeds. In males, increasing EPA was associated with faster reasoning and perceptual speeds, but the opposite was true for females. In these participants, EPA constituted

less than 20% of the DHA present and about 10% of the total n-3 LC-PUFAs in the red blood cell membranes.

Current total fish consumption was weakly but significantly associated with slower perceptual and reading speeds, memory scanning speed, inhibition and simple/choice reaction time cognition tests. Red blood cell omega-3s were not associated with any test of cognitive function.

These findings do not support the hypothesis that greater fish consumption or higher n-3 LC-PUFA status, as reflected in red blood cell n-3 LC-PUFA concentrations, are associated with better cognitive performance in cognitively normal older adults. Lack

of association between fish consumption and cognitive performance has been [previously reported](#), as has the [absence of effect](#) on mental well-being of two doses of n-3 LC-PUFA supplementation for 26 weeks in older Dutch adults. A negative association between higher fish consumption and memory or language measures was reported in the [highest quartile](#) of fish intake in elderly men in the Netherlands. In a prospective study of aging Canadians, [higher plasma concentrations](#) of EPA and DHA were reported in cognitively impaired and demented individuals, respectively, compared with controls.

Biomarkers of n-3 LC-PUFA status are generally considered more accurate estimates of fish or n-3 LC-PUFA intake than estimates of dietary intake, which are subject to greater measurement error. In this study, which assessed both dietary and red blood cell n-3 LC-PUFAs, the red blood cell data were not associated with any evaluation of cognitive function. Whether this discrepancy casts doubt on the validity of the dietary intake data as a reflection of n-3 LC-PUFA status cannot be answered. That the associations between current and childhood dietary

fish intakes and various cognitive tests were relatively weak (β -coefficients for the regression analyses associated with statistically significant results were generally around -0.1) suggests that these observations might not be of clinical significance.

The investigators also observed an inverse association between higher red blood cell EPA concentrations in women and measures of perceptual and reasoning speed. However, when the analysis controlled for fish

The study also reported an inverse association between higher red blood cell EPA concentration and perceptual and reasoning speeds in women. Controlling for fish intake abolished this relationship. In men, EPA was positively associated with these tests, but the relationships were not significant.

intake, the relationship remained negative, but was no longer significant. In men, the association with EPA was in a positive direction, but was not statistically significant.

This study warrants attention for several reasons. Chief among them is the association between esti-

mated fish consumption and modestly poorer speeds in several tests of cognitive function. The investigators performed an extensive battery of cognitive tests to assess diverse mental functions. They also reported biomarker and dietary data, although the results for each diverged. Their analysis accounted for many potential confounding variables. In a novel aspect of dietary assessment, the researchers evaluated historical fish consumption for different stages of life. They reported that fish consumption during childhood, but not at other life stages up to middle age, was modestly and inversely associated with perceptual speed and simple/choice reaction time. These findings are a reminder that research yields surprising results, sometimes where least expected.

Danthiir V, Hosking D, Burns NR, Wilson C, Nettelbeck T, Calvaresi E, Clifton P, Wittert GA. Cognitive performance in older adults is inversely associated with fish consumption but not erythrocyte membrane n-3 fatty acids. J Nutr 2014;144:311-320. [PubMed]

Worth Noting

Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: A review of the interactions between inflammation and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry 2014;53C:23-34. [PubMed]

Bellino S, Bozzatello P, Rocca G and Bogetto F. Efficacy of omega-3 fatty acids in the treatment of borderline personality disorder: a study of the association with valproic acid. J Psychopharmacol 2014;28:125-132. [PubMed]

Persons JE, Robinson JG, Ammann EM, Coryell WH, Espeland MA, Harris WS, Manson JE, Fiedorowicz JG. Omega-3 fatty acid biomarkers and subsequent depressive symptoms. Int J Geriatr Psychiatry 2013; Dec. 11. doi: 10.1002/gps.4058 [PubMed]

Giles GE, Mahoney CR, Kanarek RB. Omega-3 fatty acids influence mood in healthy and depressed individuals [Review]. Nutr Rev 2013;71:727-741. [PubMed]

■ VISUAL FUNCTION

Topical Long-Chain Omega-3s Reduce Inflammation and Improve Eye Surface in Model of Dry Eye Syndrome

Dry eye syndrome (dry eye) is one of the leading reasons for patient visits to ophthalmologists in part because its

Dry eye, a relatively common condition prevalent among women and older adults, undermines one's quality of life. Treatment with artificial tears eases symptoms but may not reduce inflammation.

symptoms are unpleasant. They include pain, dryness, grittiness, itchiness, burning, light sensitivity, difficulty reading, driving and doing computer work, all of which undermine one's quality of life. Dry eye is a condition of insufficient tears to lubricate the eye and is common throughout the world. In the U.S., the condition affects about 3.3 and 1.7 million women and men

over the age of 50 years, respectively, and 10 to 20% of adults worldwide. It is even more prevalent in Asian populations, including Japanese and Chinese. Dry eye afflicts women more often than men and is associated with certain medications such as estrogen therapy, antidepressants, antihistamines and antihypertensives. It also develops with increasing age, certain medical conditions, environmental exposures to smoke, wind and dry climate, extensive computer usage, the use of contact lenses and with eye surgeries, such as LASIK.

Dry eye syndrome is usually accompanied by the increased production of inflammatory cytokines in the ocular surface cells and in tears. These contribute to the symptoms of dry eye and the severity of the condition. Patients commonly use artificial tears to relieve

Long-chain omega-3 PUFAs have been associated with a lower risk of some eye diseases and have recently been studied in dry eye syndrome. Until now, these fatty acids have been provided only by dietary intake. This article describes their use in topical eye drops.

symptoms. Depending on their formulation and period of use, artificial tears may not improve the ocular surface, reduce inflammation or affect the underlying meibomian gland dysfunction. However, eye drops containing anti-inflammatory agents have been associated with improved symptoms and reduced cyto-

kines, although products with topical steroids may have serious side effects. Eye drops containing alpha-linolenic acid were reported to improve the symptoms of dry eye and significantly reduce the inflammatory mediators associated with the condition. One report described the effectiveness of topical treatment with resolvin E1, a derivative of EPA, in reducing corneal staining by 80% and maintaining goblet cell density in a mouse model of dry eye syndrome. With these exceptions, the only studies on the effects of long-chain omega-3 PUFAs (n-3 LC-PUFAs) in dry eye disease provided these PUFAs via dietary intake. This study in an animal model reports the effect of n-3 LC-PUFAs furnished as a constituent of therapeutic eye drops.

Dr. Zhengri Li and colleagues at Chonnam National University, Gwangju, Korea, investigated the effectiveness of lubricant eyedrops containing one of two concentrations of n-3 LC-PUFAs with or without the addition of 0.1% hyaluronic acid (HLA) in dry eye syndrome. The study was performed using a desiccating stress model of dry eye syndrome in mice treated systemically with scopolamine. Eye drops with HLA have been associated with improved lissamine green staining and symptom frequency scores in patients with eye disease, but HLA not a component of all artificial tear products. The research objective was to assess eye disease severity using corneal irregularity scores assessed as previously described and fluorescein staining. To assess inflammatory responses, the investigators measured the concentration of inflammatory cytokines and markers of oxidative stress in the conjunctiva in response to the condition alone and the varying treatments.

The experimental design included (1) untreated animals not exposed to dry eye conditions or topical treatment; (2) dry eye exposed animals without eye drops; (3) dry eye animals treated with 0.1% HLA drops; (4) dry eye animals treated with 0.02% n-3 LC-PUFAs alone;

(5) dry eye animals treated with 0.2% n-3 LC-PUFAs; (6) dry eye animals treated with 0.02% n-3 LC-PUFAs and 0.1% HLA drops; (7) dry eye animals treated with 0.2% n-3 LC-PUFAs and 0.1% HLA drops. Eye drops (3µL) were given 4 times per day initiated at the induction of dry eye syndrome.

Investigators compared the effectiveness of eye drops containing omega-3 PUFAs at two levels, with or without hyaluronic acid, in mice with dry eye syndrome.

Mice were evaluated for corneal irregularity scores ranked on a 6-point scale and fluorescein staining on a 4-point scale at 5 and 10 days after treatment. The animals were sacrificed 10 days after treatment began.

The investigators measured the concentrations of 3 inflammatory markers

and 2 indicators of lipid peroxidation in conjunctiva tissue. These markers were interleukins (IL)-1 β , IL-17 and interferon gamma-induced protein 10 (IP-10), hexanoyl-lys (HEL), a marker of acute lipid peroxidation, and 4-hydroxynonenal (4-HNE), a marker of chronic lipid peroxidation. The conjunctiva lines the eyelid and covers the white part of the eye. It contains epithelial and goblet cells.

Five days after dry eye conditions were induced, corneal irregularity scores increased significantly in the dry eye animals compared with the untreated controls (3.86 ± 0.54 vs. 0.42 ± 0.49 , $P < 0.05$). The effects of the different treatments compared with the various groups are shown in the Table. Compared with the dry eye control animals, those treated with drops containing only HLA or 0.02% n-3 LC-PUFAs did not differ from the controls, even though their scores were lower. The animals treated with 0.2% n-3 LC-PUFAs alone had significantly lower corneal irregularity scores compared with the dry eye controls and treatments with only 0.1% HLA or the low-dose n-3 PUFAs. Overall, the eyedrops containing 0.2% n-3 LC-PUFAs plus HLA provided most effective treatment to improve corneal surface irregularities. Findings after 10 days of treatment were similar to those at 5 days.

Results from the corneal fluorescein staining, a test used to detect corneal damage and foreign bodies, followed a pattern similar to the corneal surface irregularities scores. The most effective treatment was the combination of 0.1% HLA and 0.2% n-3 LC-PUFAs, which significantly exceeded the improvements observed with HLA alone, 0.02% n-3 LC-PUFAs alone, 0.2% n-3 LC-PUFAs alone, or the combination of 0.02% n-3 LC-PUFAs with 0.1% HLA.

Table. Summary of the changes in corneal surface scores (mean \pm SD) after 5 days' treatment with different eye drop solutions and the significance levels of comparisons among the groups

Treatment							
	Untreated control	Dry eye only	0.1% HLA* alone	0.02% n-3s* alone	0.2% n-3s alone	0.02% n-3s + HLA	0.2% n-3s + HLA
Corneal surface irregularities scores							
Treatment comparisons	0.42 \pm 0.49	3.86 \pm 0.54	3.14 \pm 0.57	3.13 \pm 0.69	2.15 \pm 0.59	3.12 \pm 0.72	1.20 \pm 0.50
vs untreated		<0.05					
vs dry eye only			NS	NS	<0.05	NS	<0.05
vs HLA alone				NS	<0.05	NS	<0.05
vs 0.02% n-3s					<0.05	NS	<0.05
vs 0.2% n-3s						<0.05	<0.05
vs 0.02% n-3s + HLA							<0.05

*Abbreviations: HLA, hyaluronic acid; n-3s include EPA and DHA

Mice with dry eye syndrome treated with eyedrops containing 0.2% omega-3 LC-PUFAs and 0.1% hyaluronic acid experienced significant and substantial reductions in corneal integrity scores, corneal damage, inflammatory cytokines and oxidative stress markers.

Conjunctival tissue cytokines increased significantly with the induction of dry eye syndrome, but were reduced the most with eyedrops containing 0.2% n-3 LC-PUFAs and HLA. IL-1 β concentrations were significantly lower with drops having only HLA, 0.2% n-3 PUFAs, or 0.02% n-3 LC-PUFAs plus HLA, but the greatest suppression

occurred with the 0.2% n-3 LC-PUFAs plus HLA treatment. In these animals, inflammatory markers were equivalent to the untreated animals without dry eye. Tissue levels of IL-17 and IP-10 were significantly lower with 0.2% n-3 LC-PUFAs alone, but the lowest concentrations were observed with the 0.2% n-3 LC-PUFAs plus HLA.

The two markers of oxidative stress increased significantly with the induction of dry eye, but the concentrations of HEL were unaffected by any of the eye drop preparations. In contrast, the concentrations of 4-HNE were significantly reduced with drops containing 0.2% n-3 LC-PUFAs alone and further reduced when the drops contained both 0.2% n-3 LC-PUFAs and HLA.

This study is the first demonstration of the effectiveness of topical n-3 LC-PUFAs in the alleviation of dry eye syndrome in an animal model. The investigators provided HLA and two concentrations of n-3 LC-PUFAs in artificial tear drops and observed the greatest reductions in corneal integrity and damage scores with drops containing 0.2% n-3 LC-PUFAs plus 0.1% HLA. In addition, 0.2% n-3 LC-PUFAs alone significantly lowered the concentrations of 3 inflammatory markers and a marker for chronic lipid peroxidation. These reductions were further improved by the addition of 0.1% HLA. This study, the report on resolvin E1 and the involvement of n-3 LC-PUFAs and their derivatives in corneal nerve regeneration as shown in animal studies, along with human studies on the dietary consumption of n-3 LC-PUFAs, all indicate that n-3 LC-PUFAs are potentially important in the treatment dry eye syndrome.

Li Z, Choi JH, Oh HJ, Park SH, Lee JB, Yoon KC. Effects of eye drops containing a mixture of omega-3 essential fatty acids and hyaluronic acid on the ocular surface in desiccating stress-induced murine dry eye. Curr Eye Res 2014; Feb. 21. [PubMed]

Long-Chain Omega-3 Consumption Associated with Improved Symptoms in Patients with Dry Eye Syndrome

The rationale for exploring the potential benefits of long-chain omega-3 PUFAs (n-3 LC-PUFAs) in dry eye syndrome (dry eye) stems from their well known anti-inflammatory properties and from the reduced incidence of certain eye diseases among individuals who consume fish or these PUFAs. In 2005, an epidemiological study reported that

Observational reports and a limited number of intervention studies have suggested that higher intakes of long-chain omega-3 PUFAs may be linked to lower incidence and severity of the condition. The anti-inflammatory properties of omega-3 PUFAs or their derivatives have also been reported in dry eye syndrome.

the consumption of tuna or higher amounts of n-3 LC-PUFAs was associated with a significantly lower risk of developing dry eye. Since then, various investigators have explored dietary supplementation with n-3 LC-PUFAs or mixtures containing these PUFAs plus n-6 PUFA, antioxidants and other substances in patients with dry eye.

In this report, investigators from Ghaziabad, Uttar Pradesh, India, present the results of a randomized control trial using n-3 LC-PUFA supplements in 518 patients with dry eye syndrome. The investigators noted that this region in northern India has a dry,

Researchers in northern India examined the effects on symptoms and objective measures of eye function in 518 patients with dry eye syndrome who consumed long-chain omega-3 PUFAs or placebo for 3 months.

windy climate with high exposure to ultraviolet radiation, and diets devoid of n-3 LC-PUFAs, all of which favor the development of dry eye. Eligible patients who reported symptoms of dry eye were recruited from 2 eye centers and enrolled unless they had pre-existing ocular

disease, a past history of herpetic eye disease, liver disease, diabetes, LASIK surgery or were taking tetracycline or corticosteroids. Other exclusions included pregnancy, HIV or hepatitis B and C, post-menopausal status or psychiatric disease. Participants discontinued topical medications and use of contact lenses prior to treatment. On average, participants were 39 years of age.

Participants (268 females, 250 males) were randomly assigned to consume 1 g per day of EPA and DHA (ratio ~2:1) or a corn oil placebo for 3 months. Participants were evaluated and scored at baseline and after 3 months for frequency of symptoms and adequacy of tear production (Schirmer's I test), tear breakup time (TBUT), ocular surface integrity (Rose Bengal score) and conjunctival impression cytology for cellular changes and goblet cell density. One eye from each patient was selected at random for evaluation. At each monthly visit, participants received a detailed ocular examination by an independent ophthalmologist for measurement of best corrected visual acuity, slit lamp examination and meibomian gland function.

After 3 months' treatment, symptom scores improved in both the n-3 LC-PUFA and placebo groups, but the magnitude of the improvements was significantly greater in the n-3 LC-PUFA group (change in score, 2.02 ± 1.0 vs. 0.48 ± 0.2 , active treatment and placebo, respectively). Likewise, the improvement in the Schirmer's I test scores was significantly greater in the n-3 LC-PUFA group compared with the placebo group. The greatest difference in scores between the two groups was in the tear breakup time, which increased by 2.54 ± 2.34 in the n-3 LC-PUFA participants and by 0.13 ± 0.16 in the placebo group. These improvements reflected a reduction in the

A small study in Japanese patients reported significant improvements in eye pain and objective measurements of the eyes and tears with EPA-rich supplementation for 12 weeks. As described in the [December 2013 PUFA Newsletter](#), investigators in Spain reported that patients with clinically diagnosed meibomian gland dysfunction who consumed 1.3 g of DHA-rich n-3 LC-PUFAs daily for 3 months experienced significant reductions in their subjective and objectively measured symptoms and tear levels of inflammatory mediators. These investigators have since reported that patients with dry eye who consumed a commercial supplement containing DHA-rich n-3 LC-PUFAs, antioxidant vitamins and other nutrients, providing 1.5 g/day of n-3 LC-PUFAs (1.05 g DHA), for 12 weeks experienced a significant reduction in symptoms, improved tolerance of contact lenses and decreased use of artificial tears.

A multicenter study in France reported that patients with dry eye who consumed a supplement with n-3 and n-6 PUFAs, vitamins and zinc for 3 months had significantly reduced numbers of human leukocyte antigen-DR positive cells, indicating a reduction in a conjunctival inflammatory marker, but symptoms did not improve. The anti-inflammatory pro-resolving derivatives of EPA and DHA, [resolvins E1 and D1](#), reduced leukotriene D4 in cultured rat goblet cells and halted the secretion of this inflammatory mediator. As reviewed by Cortina and Bazan, DHA and the derivatives of EPA and DHA reduce the symptoms of dry eye in animal models, increase the regeneration of damaged corneal nerves and have strong anti-inflammatory effects in conditions associated with dry eye. Overall, only a handful of studies have examined the effects of n-3 LC-PUFAs in dry eye syndrome, many with small numbers of participants and varying treatments and doses. To date, all have reported significant symptom improvements.

number of participants with the shortest breakup times (<5 sec) from 62 to 40 and an increase in those with

Key findings from the study were significant improvements in symptom scores and tear breakup times in participants who consumed long-chain omega-3 PUFAs for 3 months compared with the control group.

the longest breakup times (>10.1 sec) from 28 to 89 participants. Scores for conjunctival impression cytology improved only in the n-3 LC-PUFA group.

In summary, this study observed significant improvements in symptom scores and tear breakup times in patients treated with

n-3 LC-PUFA supplements for 3 months compared with those who took the placebo capsules. That these results were observed in relatively young adults compared with other studies of dry eye in older adults, suggests that the effects of n-3 LC-PUFA consumption are independent of age. Most important, n-3 LC-PUFA consumption eases the discomfort of dry eye disease and might protect the eye from further injury and inflammation.

Bhargava R, Kumar P, Kumar M, Mehra N, Misbra A. A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. In J Ophthalmol 2013;6:811-816. [PubMed] Open Access.

Worth Noting

Merle BM, Benilan P, Puche N, Bassols A, Declourt C, Souied E. Circulating omega-3 fatty acids and neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci 2014;55:2010-2019. [PubMed]

CLINICAL CONDITIONS

Higher Serum Long-Chain Omega-3s Linked to 33% Lower Risk of Type 2 Diabetes

Whether the consumption of fish or long-chain omega-3 PUFAs (n-3 LC-PUFAs) found mainly in fish and shellfish are associated with the risk of developing type 2 diabetes is uncertain. In spite of many studies having searched for a relationship, the evidence for any risk reduction is meager. A review of 16 prospective studies on fish or seafood consumption or n-3 LC-PUFA intakes and incident type 2 diabetes concluded that there were neither harms nor benefits in terms of diabetes risk, but that alpha-linolenic acid may be associated with a modestly lower risk. Another meta-analysis of 11 randomized trials of n-3 LC-PUFAs and insulin sensitivity found no association between n-3

Most studies have reported no association between the consumption of omega-3 PUFAs and the risk of type 2 diabetes. There are few data on diabetes risk based on blood levels of these fatty acids. A new study reports diabetes risk based on dietary intakes and blood levels of omega-3 PUFAs.

LC-PUFA intervention and insulin sensitivity. Other studies have agreed and reported differences in outcomes between geographical regions.

A major problem with the meta-analyses of these data is the heterogeneity among the studies. Obtaining precise estimates of n-3 LC-PUFA consump-

tion from dietary intake data, especially when fish consumption is low, is also fraught with wide variability. A few studies have used biomarkers of n-3 LC-PUFA status instead of dietary consumption, but results to date have not shown any association between n-3 LC-PUFAs and risk of type 2 diabetes. However, higher levels of linoleic acids were inversely associated with risk in the biomarker study.

Reasons for the lack of association between higher intakes of fish or n-3 LC-PUFAs are unclear, although some have suggested that methylmercury found in varying amounts in fish might be a contributing factor. This contaminant has also been associated with a higher risk of cardiovascular disease in Finnish men. Thus, it seemed reasonable to examine the risk of type 2 diabetes in the men who had participated in previous studies on heart disease risk factors. There were 2,212 participants from Eastern Finland who were between 42 to 60 years of age at enrollment and free of impaired fasting glucose and type 2 diabetes at baseline. The average duration of follow-up was 19 years. Food intakes, glucose tolerance tests, fasting blood sample collections and medical assessments were conducted at 4, 11 and 20 years after enrollment.

During the follow-up period, 422 men developed type 2 diabetes. In multivariate-adjusted analysis, men in the highest quartile of serum total n-3 LC-PUFAs (EPA + DPA + DHA) had a 33% lower risk of developing type 2 diabetes compared with men in the lowest quartile (Table). DHA and DPA were also inversely related to risk, but EPA and ALA were not. Those in the highest quartile of total n-3 LC-PUFAs had a slightly, but significantly, higher BMI, more leisure physical activity, higher income and educational attainment, lower serum linoleic acid, higher hair mercury, serum LDL-cholesterol, lower triglycerides and serum insulin levels, lower

Table. Risk* of incident type 2 diabetes by quartile of serum n-3 PUFAs in Finnish men aged 42 to 60 years monitored for an average of 19 years

Fatty acid	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P
Total n-3 LC-PUFAs	1	0.71 (0.54 – 0.93)	0.78 (0.6 – 1.02)	0.67 (0.51 - 0.87)	0.01
EPA	1	1.07 (0.81 – 1.40)	0.96 (0.73 – 1.27)	0.85 (0.64 - 1.13)	0.15
DPA	1	0.77 (0.59 – 1.01)	0.73 (0.56 – 0.96)	0.72 (0.55 – 0.94)	0.02
DHA	1	0.81 (0.62 – 1.06)	0.71 (0.54 – 0.94)	0.66 (0.51 – 0.87)	0.003
ALA	1	0.87 (0.67 – 1.14)	0.88 (0.67 – 1.17)	0.82 (0.62 – 1.10)	0.22

*Expressed as hazard ratios (95% CI). Analysis adjusted for age, examination year, BMI, family history of type 2 diabetes, smoking history, current smoking ≥ 20 cigarettes per day, education, leisure time activity, alcohol intake and serum linoleic acid.

insulin resistance and greater alcohol intake compared with men in the lowest total n-3 LC-PUFA quartile.

This study in middle-age and older men reported a 33% lower risk of type 2 diabetes in those with the highest serum concentrations of total n-3 LC-PUFAs, DHA and DPA. Serum levels of EPA, ALA and fish consumption were not associated with diabetes risk.

In secondary analyses, the investigators analyzed the associations between incident type 2 diabetes and quartiles of hair mercury content, the consumption of fish, EPA + DHA and alpha-linolenic acid and none was associated with the risk of type 2 diabetes. The lack of an association with dietary EPA + DHA and fish consumption suggests that these estimates are not sufficiently precise

compared with serum fatty acid concentrations, which were significantly associated with risk. The authors raised this point in their discussion of these findings. A notable feature of this study is the availability of both dietary intake data and biomarker measurements for n-3 LC-PUFAs. The findings comparing these two assessments argue in favor of the biomarker assessment because it has substantially less measurement error

associated with it. Lacking from the study, however, are data for women. The Kuopio Ischemic Heart Disease study has provided a wealth of evidence on the factors in men associated with heart disease and closely related conditions. These observations challenge the data from many previous results.

Virtanen JK, Mursu J, Voutilainen S, Uusitupa M, Tuomainen TP. Serum omega-3 polyunsaturated Fatty acids and risk of incident type 2 diabetes in men: the Kuopio Ischemic Heart Disease Risk Factor Study. Diabetes Care 2014;37:189-196. [PubMed]

Worth Noting

Mahendran Y, Ågren J, Uusitupa M, Cederberg H, Vangipurapu J, Stančáková A, Schwab U, Kuusisto J, Laakso M. Association of erythrocyte membrane fatty acids with changes in glycemia and risk of type 2 diabetes. Am J Clin Nutr 2014;99:79-85. [PubMed] Open Access

Sari Niinistö, Hanna-Mari Takkinen, Liisa Uusitalo, Jenna Rautanen, Jaakko Nevalainen, Michael G. Kenward, Mirka Lumia, Olli Simell, Riitta Veijola, Jorma Ilonen, Mikael Knip, Suvi M. Virtanen. Maternal dietary fatty acid intake during pregnancy and the risk of preclinical and clinical type 1 diabetes in the offspring. Br J Nutr 2014;111:895-903. [PubMed]

