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PUFA Newsletter Staff
Editor.........Joyce A. Nettleton, DSc, RD
Publicist/web manager ...... Angela Dansby

Letters and editorial comments should be submitted to Dr. Nettleton at joyce@fatsoflife.com
PUFA Newsletter at http://www.fatsoflife.com

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Yasushi Saito, M.D., Chiba University, Japan
Norman Salem, Jr., Ph.D., National Institutes of Health, USA
Editorial

In Sickness and in Health

This quarter’s newsletter covers the gamut of long-chain polyunsaturated fatty acid (LC-PUFA) activity, from preterm infancy through adulthood, in healthy and seriously ill subjects. It also breaks from custom by including two reports based on animal studies. The first, “Men, Mice and Membranes,” describes how cell culture and animal studies can provide a basis for estimating human requirements for docosahexaenoic acid, a major omega-3 LC-PUFA many consider at least conditionally essential to human health. The second on retinal composition and function in baboons, reports findings from studies we simply can’t do in human subjects. This work is probably as close as it gets to the human condition.

The newsletter also includes several reports on LC-PUFAs in preterm infants, who particularly need these essential nutrients. A large study comparing the growth of preterm infants fed mainly human milk, fortified formula, or combinations of the two is noteworthy for readers particularly interested in infant nutrition.

Also summarized are three reports on fish or omega-3 LC-PUFAs in patients with cancer. These show the difficulty in generalizing the activity of LC-PUFAs in various types of cancers.

Two other reports clarify the effects of omega-3 LC-PUFAs on oxidative stress. It seems clear that using the most advanced techniques to measure the effects of LC-PUFAs on increased production of harmful “reactive oxygen species” produces the most reliable assessments. Should we throw out previous studies based on cruder assessments?

Prompted by September’s article on the conversion of alpha-linolenic acid to its long-chain derivative fatty acids, Norman Salem Jr. and colleagues offer another perspective. Keep both articles handy – we haven’t heard the last on this topic.

The PUFA Newsletter appreciated hearing from reader Sharon Monerrubio in Wollongong, Australia, whose comments are included in a letter to the editor inside.

Holiday greetings and wishes for peace.

Joyce A. Nettleton, DSc, RD
Editor, PUFA Newsletter
joyce@fatsoflife.com

PUFA Newsletter Staff
Editor ........... Joyce A. Nettleton, DSc, RD
Publicist/web manager ...... Angela Dansby
Sponsor ............ DSM Nutritional Products (formerly Roche Vitamins Ltd.), Kaiseraugst, Switzerland, http://www.rochevitamins.com
Letters and editorial comments should be submitted to Dr. Nettleton at joyce@fatsoflife.com
PUFA Newsletter at http://www.fatsoflife.com

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Alpha-Linolenic Acid Conversion Revisited

A recent article\(^1\) in the *PUFA Newsletter* indicated that in adult men and women the “average estimated conversion of . . . alpha-linolenic acid to n-3 LC-PUFA metabolites and docosahexaenoic acid was 17.3 ± 12.8 and 3.6 ± 3.8 percent, respectively (mean + SD).” This is likely to be an overestimate of the actual overall conversion rates for several reasons.

The first reason is the small amount of ingested alpha-linolenic acid available for liver elongation and desaturation. It is estimated that as much as 60% of dietary alpha-linolenic acid is oxidized during the first week. Moreover, much of the dietary alpha-linolenic acid is used for the biosynthesis of cholesterol and non-essential fatty acids. Most of the remaining alpha-linolenic acid goes to other organs including skin, adipose tissue, and muscle. In our animal studies, the maximal amounts of alpha-linolenic acid that appeared in plasma and liver represented only 0.6% and 3% of the oral dose, respectively. Thus, only a small percentage, about 5%, of dietary alpha-linolenic acid may be available for liver synthesis of n-3 LC-PUFAs. The amount of metabolites released by the liver into the plasma as measured in human studies is an imperfect index of metabolism.

Another concern is the calculation of the conversion rate, the amount of stable isotope-labeled alpha-linolenic acid that is converted to n-3 LC-PUFAs. These studies generate plots of the amount of isotope in a particular plasma fatty acid over time. The area under the curve is then calculated (Figure 1) and a ratio of the product to the precursor can be determined. However, the area under the curve calculation does not render a value for the total amount of the labeled fatty acid in the plasma as the units are expressed as quantity x time. The occurrence of time in the units reflects the persistence of the label in plasma. These time-course curves represent an appearance phase, when the labeled product enters the plasma, and a longer disappearance phase as the product is removed from the plasma. Fatty acids characteristically have an extended period of disappearance as they may be acylated, for example, to complex lipids. The period of disappearance of the initial substance or precursor will contribute to the area under the curve, but may not be associated with product formation.

Some have calculated a “percent conversion” for a particular fatty acid by taking the sum of the area under the curve for all fatty acids and expressing the area for a specific fatty acid as a percent of the sum. The difficulty with such measurements is that they do not correspond to any important metabolic parameter, nor do they give a net conversion rate or a percent of label converted to a product. To overcome these difficulties Pawlosky and colleagues used a compartmental modeling procedure for alpha-linolenic acid metabolism\(^2\). This procedure is based on the precursor-product relationship and the dynamic flux of
isotope between the relevant pools of metabolites. In this study, eight adults subsisting on a controlled beef-based diet were given one gram of deuterated-alpha-linolenic acid and the rate of isotope appearance in plasma was measured. Deuterated-alpha-linolenic acid in plasma represented only about 0.3% of the oral dose, or 3 mg.

From the isotope measurements in alpha-linolenic acid-derived LC-PUFAs, fractional synthetic rates, i.e., the fraction of the labeled substrate that contributed to product formation, were derived. Using the fractional synthetic rates they estimated that only 0.2% of the plasma alpha-linolenic acid was destined for synthesis of eicosapentaenoic acid (EPA). Sixty-three percent of EPA was converted to docosapentaenoic acid, 37% of which was then converted to docosahexaenoic acid (DHA). Based on the time course curves they estimated maximum amounts of EPA, docosapentaenoic acid, and DHA in plasma of 100, 25 and 8 micrograms, respectively. These amounts correspond to a conversion rate of one gram alpha-linolenic acid in the order of < 0.02% for total n-3 LC-PUFAs or 0.002% for conversion to DHA. These estimates of alpha-linolenic acid conversion do not include that occurring in other non-accessible compartments, including metabolites retained by the liver and conversion in other organs. Support for this conclusion comes from studies by Hoffman and coworkers3 and Vermunt and associates4 who reported similar concentration-time-course curves. That is, the relative magnitude of the time course curves representing the labeled isotopes LNA, EPA, DPA and DHA exhibit a similar quantitative relationship to those of Pawlosky et al. These studies demonstrate a much lower conversion of alpha-linolenic acid to n-3 LC-PUFAs in adults than was suggested by Emken1.

If the conversion rates used to estimate the amounts of n-3 LC-PUFAs formed from alpha-linolenic acid in Western diets are too high, then the estimates of n-3 LC-PUFAs supplied from metabolism will also be too high. Mean intakes of EPA and DHA from Western diets are estimated at about 50-100 mg/day. Daily synthesis of n-3 LC-PUFAs from a Western diet containing 1.1 to 1.6 g alpha-linolenic acid per day using the higher conversion rates were estimated by Emken to be 277 and 398 mg for men and women, respectively. These estimates represent a 3-8 fold greater supply of n-3 LC-PUFAs from synthesis compared with dietary intake. However, in volunteers subsisting on a controlled beef-based diet, a diet not unlike that of many Americans, we calculate that the total amount of n-3 LC-PUFAs synthesized was only about 27 mg/d2.

A conversion rate of 3.6% of alpha-linolenic acid should lead to increased plasma DHA with alpha-linolenic acid supplementation. However, many human studies have shown that alpha-linolenic acid supplementation produces only modest increases in EPA and docosapentaenoic acid, and no increase in DHA. A recent study reported that DHA in breast milk did not increase with alpha-linolenic acid supplementation. In contrast, it is well known that DHA supplementation increases plasma and breast milk DHA. Similarly, estimates of infant biosynthesis of 65 mg/d of n-3 LC-PUFAs are inconsistent with the decreases in infant brain and bloodstream DHA levels that occur after birth when infant diets contain alpha-linolenic acid but not DHA.

In conclusion, we believe the estimates and interpretations currently put forward as best estimates can be substantially improved. The best estimates of alpha-linolenic acid conversion to n-3 LC-PUFA are much smaller than those claimed. More rigorous determinations of n-3 fatty acid metabolism must serve as the foundation for more accurate nutritional conclusions and dietary recommendations.

1 Emken EA. Alpha-linolenic acid conversion to n-3 LC-PUFAs. PUFA Newsletter September 2003.
Cardiovascular Health

**Tuna Oil Improves Vascular Function in Healthy Middle-Aged People**

Improvements in vascular function have been reported in subjects with cardiovascular disease who consumed fish oil or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). Responses include reduced production of proinflammatory markers, which result from endothelial cell activation, and improved vasodilation and forearm blood flow. These effects contribute to reduced risk of cardiovascular events. It is not known, however, whether the consumption of fatty fish or fish oil results in similar vascular responses in healthy adults.

To find out, Khan and associates at the University of Dundee, UK, enrolled 173 healthy volunteers, 118 men and 55 postmenopausal women, aged 40-65 years, in a randomized, double blind, placebo-controlled trial to test the vascular responses to supplements of six different oils: placebo (25% soy, 75% fractionated coconut oil), oleic acid-rich sunflower oil, placebo plus evening primrose oil as a source of gamma-linolenic acid, placebo plus soybean oil as a source of alpha-linolenic acid, placebo plus tuna oil for the n-3 LC-PUFA docosahexaenoic acid (DHA), and a 50/50 mixture of tuna and evening primrose oils. Supplements were provided for 8 months at a dose of 10 g oil/day for sunflower oil and of 5 g/day (plus 5 g/day placebo) for evening primrose, soy, and tuna oils. The amount of n-3 LC-PUFAs consumed in the two tuna oil groups was 1.1 to 1.2 g/day. Tuna oil contains predominantly DHA and in this study, the DHA to eicosapentaenoic acid (EPA) ratio was 5.5 to 1.

Vascular responses in forearm skin, as reflected by forearm skin perfusion, were measured using Doppler imaging technology following the local application of acetylcholine and sodium nitroprusside. These substances elicit endothelium-dependent and independent responses, respectively. This imaging technology is a non-invasive way of examining the flow of moving red blood cells in microvessels.

Vascular responses to acetylcholine were significantly improved in subjects who consumed tuna oil, but not in those consuming any other oil supplements.

Table 1. Peak vasodilator responses to acetylcholine and plasma phospholipid DHA concentrations in healthy adults before and after consuming selected oil supplements for eight months. The authors assume that the microvasculature in skin is representative of microvessels elsewhere in the body, and is a suitable surrogate for cardiovascular and endothelial function in general. Several published studies suggest that such blood flow measurements in skin are significantly correlated with systemic endothelial function in subjects with peripheral vascular disease.

<table>
<thead>
<tr>
<th>Supplemental oil</th>
<th>Before/after supplementation</th>
<th>Peak acetylcholine response(^1) (interquartile range)</th>
<th>(P)</th>
<th>Plasma phospholipid DHA(^2)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Before</td>
<td>5.64 (3.67-6.80)</td>
<td>NS</td>
<td>2.47 ± 1.99</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>4.67 (3.18-5.72)</td>
<td></td>
<td>2.13 ± 1.72</td>
<td></td>
</tr>
<tr>
<td>Evening primrose oil</td>
<td>Before</td>
<td>5.81 (5.07-7.14)</td>
<td>NS</td>
<td>2.39 ± 2.38</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>6.64 (4.99-8.69)</td>
<td></td>
<td>2.03 ± 2.12</td>
<td></td>
</tr>
<tr>
<td>Tuna oil</td>
<td>Before</td>
<td>5.00 (3.38-5.87)</td>
<td>0.02</td>
<td>2.67 ± 2.52</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>5.85 (4.77-7.37)</td>
<td></td>
<td>4.60 ± 2.12</td>
<td></td>
</tr>
<tr>
<td>Primrose/tuna mix</td>
<td>Before</td>
<td>5.26 (4.48-7.12)</td>
<td>NS</td>
<td>2.41 ± 1.46</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>6.18 (4.79-7.22)</td>
<td></td>
<td>4.62 ± 2.79</td>
<td></td>
</tr>
</tbody>
</table>

\(1\) Ratio of peak to baseline response in forearm skin.
\(2\) Percent of total fatty acids.
Men, Mice, and Membranes: Linking Cell and Animal Studies for Human DHA Requirements

Brain and eye tissues in living human beings are exceedingly difficult to access for research studies, so researchers usually turn to experimental animals or cultures of cells from particular nervous tissues. How relevant the findings are to people clouds this experimental approach. This issue is particularly relevant to studies of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), which accumulate in nervous tissues during human development. In this study, a group of French scientists attempted to determine the precise relationship between cell models and developing animals in order to measure the amount and rate of docosahexaenoic acid (DHA) incorporation in nervous tissue membranes. DHA is the predominant n-3 LC-PUFA in the membranes of brain and nervous tissue.

Using five different rat neural tissues (retina, cerebellum, frontal cortex, hippocampus, striatum) Alessandri et al. of the Institut National de la Recherche Agronomique, France explored the effect of feeding graded levels of DHA on the incorporation of DHA into these tissues. They used rats weaned at 3 weeks of age, born to mothers depleted of n-3 LC-PUFAs, and fed them experimental diets for 5 weeks. Dietary DHA ranged from zero to 400 mg/100 g diet. DHA concentrations in phospholipids of brain tissue and cultured cells were measured at baseline and after the dietary period or 3 days of culture, respectively. The incorporation of DHA into cultured human cells (three lines of cultured cells from human nervous tissue, one retinoblastoma and two different neuroblastoma lines) was studied using cells grown in a medium containing serum albumin, which binds DHA. Different amounts of DHA were added to the medium and cells were grown for 3 days, after which lipids were extracted and analyzed for DHA. Concentrations of DHA incorporated into the cultured cells and rat brain tissues were then plotted and compared mathematically.

After exposure to DHA, the DHA-depleted brain tissue incorporated DHA to a maximum concentration of 21.8% to 28.8% of total fatty acids. The retina avidly took up DHA to a maximum of 45.9% total fatty acids in phosphatidylethanolamine phospholipids. In cultured cells, the maximum DHA concentration ranged from 31.8% to 39.2% of total fatty acids. The pattern of the incorporation or the dose-response curve was similar in nervous tissue and cultured cells.

From the dose-response curves, the authors calculated the amount of dietary DHA required to reach half the maximum concentration of DHA, known as DHA50. For the retina, this value was 4 mg DHA/100 g diet, but for brain tissues, the value ranged from 11.9 to 18.0 mg DHA/100 g diet. These observations confirm the avidity or preferential uptake of DHA by the retina (Table 1).

Because the pattern of DHA incorporation and mathematical ratios calculated for rat brain tissues and cultured human cells were equivalent, the authors suggest that data from human cell culture and animal feeding studies can be linked and used to estimate dietary needs. Of particular interest, the authors used their observations to suggest how infant nutritional requirements for brain DHA levels could be approximated. Their estimates, based on the concentration of DHA in brain phospholipids, suggest that human infants, who are normally not deficient in DHA at birth, should consume about 180 mg DHA/100 g diet. This level is equivalent to breast milk DHA of 0.8% total fatty acids. To put these numbers in perspective, the World Health Organization reported that breast milk DHA ranges from 0.07% to more than 1.0% total fatty acids, with an average of 0.34%. The level of DHA added to infant formula in the United States is 0.35% total fatty acids.


**Postpartum Depression and Maternal DHA Status: How Strong is the Link?**

Postpartum depression is estimated to affect 10% to 15% of women after they give birth, although mild mood alterations may be much more common. Prevalence of postpartum depression has been linked to low maternal docosahexaenoic acid (DHA) status after pregnancy.

---

**Table 1. Dose-response effects of dietary DHA on DHA concentration in ethanolamine phospholipids of rat brain regions and retina, and 3 human cell lines.**

<table>
<thead>
<tr>
<th>Rat nervous tissue or human cell</th>
<th>Minimum DHA (depletion)(^1)</th>
<th>Maximum DHA(^2)</th>
<th>DHA dose for 50% maximum DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% by wt total fatty acids</td>
<td>% by wt total fatty acids</td>
<td>mg/100g diet or μmol/L of culture medium</td>
</tr>
<tr>
<td>Cortex</td>
<td>6.6 ± 0.5</td>
<td>28.8</td>
<td>18.0</td>
</tr>
<tr>
<td>Striatum</td>
<td>6.0 ± 0.6</td>
<td>21.8</td>
<td>11.9</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>6.6 ± 0.7</td>
<td>26.2</td>
<td>16.6</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>6.6 ± 0.6</td>
<td>24.7</td>
<td>13.1</td>
</tr>
<tr>
<td>Retina</td>
<td>11.6 ± 2.4</td>
<td>45.9</td>
<td>4.0</td>
</tr>
<tr>
<td>Human neuroblastoma-91</td>
<td>3.9 ± 0.5</td>
<td>31.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Human neuroblastoma-5Y</td>
<td>5.9 ± 0.5</td>
<td>32.7</td>
<td>5.7</td>
</tr>
<tr>
<td>Human retinoblastoma</td>
<td>7.4 ± 0.6</td>
<td>39.2</td>
<td>6.6</td>
</tr>
</tbody>
</table>

\(^1\) Concentration with no dietary or cell-medium DHA

\(^2\) DHA value extrapolated from double reciprocal dose-response relationships
De Vriese et al. reported earlier this year that among 48 women with healthy single births, the 10 women who developed postpartum depression had significantly lower concentration of DHA in their plasma phospholipids and cholesteryl esters. Depression unrelated to pregnancy has also been associated with low levels of long-chain omega-3 fatty acid (n-3 LC-PUFA) in serum and red blood cell phospholipids. The n-3 LC-PUFA DHA is the major polyunsaturated fatty acid in nervous tissue membranes. It is well known that plasma phospholipid DHA levels diminish during the last trimester of pregnancy and lactation, while DHA accumulates in the fetus and breast-fed or DHA-supplemented infant.

Two recent studies investigated the association between DHA and postpartum depression. Otto et al. from Maastricht University in the Netherlands examined the risk of maternal postpartum depression in 112 Caucasian women in relation to DHA status at delivery and 32 weeks later. Llorente and colleagues from the Baylor College of Medicine in Texas, the U.S. Department of Agriculture, and the Mayo Clinic in Minnesota looked at the effect of providing 200 mg DHA/day or placebo on mothers’ self-assessed rating of depression and the concentration of DHA in plasma phospholipids 4 months after delivery.

Otto and colleagues measured DHA levels in the plasma phospholipids of 112 women at delivery and 32 weeks later. Women completed the Edinburgh Postnatal Depression Scale questionnaire at 32 weeks postpartum for retrospective determination of likely postpartum depression. Women with scores below 10 were considered non-depressed and those scoring above 10, “possibly depressed.” DHA functional status was expressed as the ratio of DHA to Osbond acid, the docosapentaenoic acid of the n-6 family. When DHA is limited, the production of Osbond acid increases, so the change in the ratio between these two fatty acids was used as an index of the functional status of DHA.

The postpartum increase of this functional DHA status index was significantly lower in women in the “possibly depressed” group (2.34 ± 5.56) compared with non-depressed women (4.86 ± 5.41) 32 weeks after delivery (p = 0.03), Table 1. However, DHA status at the time of delivery and at 32 weeks postpartum was not correlated with depressive symptoms.

In this study, the prevalence of postpartum depressive symptoms, 21% or 24 women, was somewhat greater than the 10% to 15% commonly reported, but similar to previously published values for the Netherlands (20%). The authors suggested that women whose DHA status recovered more slowly after giving birth were more likely to develop postpartum depression. Interestingly, these investigators did not observe an increase in depression among lactating women whose DHA status is usually reduced.

In the second study by Llorente et al., mothers planning to breastfeed exclusively for at least 4 months were randomly assigned to consume 200 mg DHA/day or placebo for 4 months after delivery. Of the 138 who were initially enrolled, 89 completed the study. Mothers used a self-rating scale, the Beck Depression Inventory, to assess mood at baseline.

Table 1. DHA status at delivery and 32 weeks postpartum in nondepressed and possibly depressed women.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Delivery</th>
<th>Change at 32-wk postpartum</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nondepressed</td>
<td>Possibly depressed</td>
<td>Nondepressed</td>
</tr>
<tr>
<td>% wt/wt</td>
<td>(88)</td>
<td>(24)</td>
<td>(88)</td>
</tr>
<tr>
<td>DHA</td>
<td>3.93 ± 0.97</td>
<td>3.82 ± 0.82</td>
<td>-0.68 ± 0.98</td>
</tr>
<tr>
<td>DHA/DPA²</td>
<td>9.52 ± 5.35</td>
<td>10.97 ± 6.97</td>
<td>4.86 ± 5.41</td>
</tr>
</tbody>
</table>

¹ Nondepressed women had depression rating scores below 10; possibly depressed had scores of 10 or higher.
² Ratio of 22:6n-3 to 22:5n-6
(delivery), 3 weeks, 2 and 4 months. Diagnosis of depression was confirmed by structured clinical interview and the Edinburgh Postnatal Depression Scale in a study subsample. DHA in plasma phospholipids was measured at baseline and after 4 months.

Both placebo and DHA-supplemented women, 45 and 44 women respectively, had similar scores on the depression rating scales at baseline and all times thereafter (Table 2). Nine women in the placebo group (21%) and 11 in the DHA-supplemented group (24%) had scores indicating possible depression. Scores declined similarly in both groups over the 4-month period suggesting improved mood. There was no association between any depression rating and phospholipid DHA at baseline or at 4 months. As expected, DHA concentration in plasma phospholipids was maintained in women receiving the DHA supplement, 3.15 ± 0.78 mg/dl and 3.40 ± 0.97 mg/dl at baseline and 4 months, respectively, and declined in the placebo group from 3.31 ± 0.70 to 2.27 ± 0.87 mg/dl (Table 2). After 4 months, DHA concentration was significantly higher (about 50% greater) in the supplemented women compared with the placebo group, p<0.05.

Both studies documented the decrease in maternal plasma phospholipid DHA following birth. The study by Otto et al. reported a statistically significant association between risk of postpartum depression at 32 weeks and low functional DHA status, using an index based on two LC-PUFAs. Postpartum depression was not significantly related to phospholipid DHA levels alone, but rather to changes in both n-6 docosapentaenoic acid and DHA. By contrast, the Llorente study found no significant relation between depression at delivery or 16 weeks postpartum and phospholipid DHA status or supplementation. Prevalence of postpartum depression was similar in the two studies, but possibly the smaller sample size, too small a dose, and large variability in Llorente’s study diminished the power of the statistical analysis. Provision of DHA during pregnancy rather than after delivery might also have affected the results. The Llorente study confirmed that DHA supplementation after delivery prevents the decline in maternal plasma phospholipid DHA that usually occurs after birth and is a physiological phenomenon. These studies indicate that postpartum depression involves more than plasma phospholipid DHA levels, but this does not diminish the value of providing adequate DHA for pregnant and nursing women.

De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: Further evidence that lowered n-PUFAs are related to major depression. Life Sciences 2003; 73:3181-3187.


Table 2. DHA in maternal plasma phospholipids and self-assessed depression rating at baseline and 4 months postpartum in 89 women consuming placebo or 200 mg DHA/day1.

<table>
<thead>
<tr>
<th>Fatty acid or score</th>
<th>Baseline</th>
<th>4 Months</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>DHA suppl</td>
<td>Placebo</td>
</tr>
<tr>
<td>DHA (mg/dl)</td>
<td>3.31 ± 0.70</td>
<td>3.15 ± 0.78</td>
<td>2.27 ± 0.87</td>
</tr>
<tr>
<td>Depression score²</td>
<td>0.5 ± 4.2</td>
<td>7.1 ± 4.7</td>
<td>4.8 ± 5.9</td>
</tr>
</tbody>
</table>

145 and 44 women in placebo and DHA-supplemented groups, respectively
²Beck Depression Inventory self-assessed rating scale

PUFA Newsletter December 2003
Direct Evidence that DHA Affects Retinal Function in Preterm and Term Baboons

The retina in humans and higher primates concentrates docosahexaenoic acid (DHA) one of two main long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) found in fatty fish. Retinal function is correlated with retinal DHA concentration in rats and rhesus monkeys, but such correlations in humans must be inferred from indirect measurements of retinal fatty acid composition, usually of plasma or red blood cells. Commonly used measures of retinal function are electroretinograms and preferential looking tests. Both are difficult to obtain in very young or premature infants. For these reasons, direct measurements in baboons serve as surrogates for tests in human infants.

Diau and colleagues at Cornell University, NY determined the effect of dietary LC-PUFA supplementation on retinal fatty acid composition and function in term and preterm infant baboons. Term baboons were fed either breast milk, which provided n-3 LC-PUFAs, or human infant formula without LC-PUFAs but containing ample amounts of their dietary precursors linoleic and alpha-linolenic acids. Preterm infants were fed the same formula with or without LC-PUFAs. The supplementary LC-PUFAs were DHA at 0.3% energy, and arachidonic acid, an omega-6 LC-PUFA, provided at 0.6% energy. Each group consisted of 4 animals.

Electroretinograms were performed on the preterm infant baboons at 28 days after birth, which corresponds to the age of normal term birth. Electroretinograms were performed on all groups at 4 weeks corrected age, 3 to 5 days before death. Retinas were analyzed for fatty acid composition. Body weight was measured at birth and death. Although the preterm infants weighed less at birth than the term infants, at the time of death there were no significant differences in body weight among the groups. The study may have not have had sufficient statistical power to detect significant differences in growth.

Analysis of the baboon breast milk and supplemented formula indicated similar concentrations of DHA, 0.68% and 0.61% total fatty acids, respectively, but breast milk had half the concentration of arachidonic acid as the formula, 0.62% and 1.21%, respectively.

Retinal fatty acid composition is shown in Table 1. Preterm and term infants consuming formulae without LC-PUFAs had significantly lower DHA concentrations than the breast-fed infants. Unsupplemented infants also had approximately twice the concentration of the n-6 LC-PUFA, docosapentaenoic acid, thought to be compensatory for the lack of DHA. Provision of LC-PUFAs to the preterm infants raised DHA concentration to that of breast-fed infants. Neither prematurity nor LC-PUFA supplementation affected the concentration

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Preterm infants</th>
<th>Term infants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No LC-PUFAs</td>
<td>+ LC-PUFAs</td>
</tr>
<tr>
<td>Docosahexaenoic acid, 22:6(n-3)</td>
<td>14.4 ± 0.55a</td>
<td>18.44 ± 1.20b</td>
</tr>
<tr>
<td>Docosapentaenoic acid, 22:5(n-6)</td>
<td>2.32 ± 0.53a</td>
<td>1.11 ± 0.27bc</td>
</tr>
<tr>
<td>Arachidonic acid, 20:4(n-6)</td>
<td>10.24 ± 0.41</td>
<td>10.07 ± 0.33</td>
</tr>
<tr>
<td>22:5(n-6)/22:6(n-3)</td>
<td>0.16 ± 0.04</td>
<td>0.06 ± 0.02</td>
</tr>
</tbody>
</table>

* Means in rows with different superscripts are significantly different from one another, p<0.05

Table 1. Selected fatty acid concentrations in retinas of preterm and term infant baboons fed diets with or without LC-PUFAs.

PUFA Newsletter December 2003
of arachidonic acid in the retina.

Comparisons of five electroretinogram parameters for each infant group and correlation studies showed that retinal function was directly related to the level of DHA in the retina. Both preterm groups had similar electroretinographic responses on the first measurements, corresponding to term birth, but only the LC-PUFA-supplemented infants showed improved retinal function at the second measurement. This observation is consistent with functional data from human studies. These data are among the first direct evidence that retinal DHA concentration is linked to retinal function in primates. More detailed discussion of the specific electroretinographic responses is given in the paper.

The electroretinographic data showed that retinal function was related to DHA concentration, which was affected by diet, prematurity and breast-feeding. None of the treatments achieved the level of retinal function observed in the breast-fed group. Breast-feeding involves differences in environment and milk composition that may contribute to retinal function. This study, in a primate model close to humans, underscores the importance of providing preterm and term infants DHA directly. Premature infants and late-term primates have the ability to convert the 18-carbon precursor of DHA, alpha-linolenic acid, to DHA, but this biosynthetic ability appears insufficient to support optimal retinal function in the neonatal period. Thus, the study adds support for supplementing preterm and term infant formula with DHA for the development of optimal retinal composition and function.


Maternal Smoking in Pregnancy Associated with Lower Breast Milk- DHA

Infants born to mothers who smoke during pregnancy are more likely to have impaired intrauterine growth, preterm birth, lower birthweight, and lower measures of neurodevelopment compared with infants born to nonsmoking women. They also have a pattern of long-chain polyunsaturated fatty acids (LC-PUFAs) in their blood that suggests lower utilization of docosahexaenoic acid (DHA) a primary omega-3 (n-3) LC-PUFA essential to infant development. The effect of smoking on the composition of breast milk has not been reported until now.

In this report from the San Paolo Hospital, Milan, Italy Agostoni and colleagues studied women enrolled in a study on human milk composition through extended lactation. Socioeconomic information was obtained from mothers at study enrollment and dietary habits assessed for 3 months prior to delivery, at delivery, and 3 and 6 months thereafter. Breast milk was obtained at the end of feeding (hindmilk) for a 24-hour period after the first production of milk following delivery, and at 1, 3, and 6 months after delivery. Of the 92 participants, 61 were nonsmokers who had never smoked, and 31 smoked at least 5 cigarettes/day prior to pregnancy. Twenty-one of the latter stopped smoking by the fourth month of pregnancy and 8 reduced their smoking during pregnancy.

For the first 6 months, the number of breastfeeding mothers declined from 92 to 63 at 1 month, 50 at 3 months, and 30 at 6 months. Birth weight and gestational age of the infants born to smoking and nonsmoking mothers did not differ significantly at birth, and throughout the 6-month follow-up period the growth characteristics were not different either. Dietary patterns, including lipids and fish consumption, were similar at all times of assessment for both groups of mothers. Energy and protein intake were greater for nonsmoking women at 3 and 6 months, respectively, while monounsaturated fatty acid intake was significantly greater for smoking women at 6 months.

Profiles of PUFAs in breast milk were comparable in the two groups throughout lactation. However, DHA concentrations as a percent of total fatty acids were significantly lower in smoking women than nonsmoking mothers, but by 6 months, these percentages were comparable in the two groups and comparable to values observed at one month.
In the first month of lactation, total milk lipid content increased in both groups, but to a significantly higher concentration in nonsmoking women (4.54 + 2.13 vs 3.64 + 1.63 g/dl, p<0.05). Because the DHA concentration was almost similar in both groups, the difference in total fat meant that DHA content in milk from nonsmoking women was significantly greater (11.23 + 6.47 vs 7.90 + 4.48 mg/dl, p<0.05). At the end of 3 months, this difference in DHA content remained significant (9.36 + 3.14 vs 7.00 + 3.50 mg/dl, p<0.05). At 6 months, the difference persisted, but was no longer statistically significant, partly because the sample size was greatly reduced. These comparisons are shown in Figure 1. Note that time is not drawn to scale in the figure.

The lower fat and DHA content of milk from smoking mothers translated into reduced DHA intake by the infant. From estimates of maternal milk production and infant weight the amount of DHA consumed by the infant was determined. These calculations showed that infants of smoking mothers consumed 33% less DHA at one and three months of lactation than infants of nonsmoking mothers. A trend for lower DHA and arachidonic acid consumption was apparent for the entire 6-month period.

This study showed that maternal smoking adversely affects the DHA content of breast milk, particularly in early lactation. Considered along with the known adverse effects of smoking on the smoker’s health and fetal development, this study adds another reason to urge pregnant women to avoid smoking altogether.


**Outcomes in Preterm Infants Fed Human Milk, Fortified Formula, or Both**

A large multi-center study from 17 neonatal units in 3 countries assessed the developmental outcomes of 463 preterm infants born at less than 33 weeks gestation, weighing from 750 to 1805 grams and fed one of four diets. After enteral feeding was established, diets were: 1) energy and protein-fortified human milk predominantly; 2) a combination of fortified human milk and formula providing 50% or more total energy from human milk; 3) fortified formula and human milk with less than 50% of total energy from human milk; 4) predominantly nutrient-enriched preterm formula with or without long-chain polyunsaturated fatty acids (LC-PUFAs). The study assessed growth plus visual, cognitive, motor, and language development at study day 1, term, and intervals from 2 to 12 months corrected age.

Results showed that preterm infants fed predominantly human milk from first enteral feeding until term corrected age and thereafter until 6 months corrected age were smaller than similar infants fed predominantly fortified formula over the same time. After 6 months corrected age, differences were not statistically significant. By 12 months corrected age, infants...
fed predominantly human milk, fortified formula, or combinations of the two had similar growth measurements. Infants fed predominantly human milk appeared to have the advantage over their counterparts fed mainly fortified formula in visual acuity scores and reduced incidence of serious illnesses and hospital readmission. Differences attributable to LC-PUFA supplementation were not reported.


**Compendium: Mechanisms of Action of LC-PUFA Effects on Infant Growth and Neurodevelopment**

The October issue of the *Journal of Pediatrics* includes a supplement with 12 articles addressing, “Mechanisms of Action of LC-PUFA Effects on Infant Growth and Neurodevelopment.” Topics include: perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids (LC-PUFAs), plausible mechanisms for the effects of LC-PUFAs on growth, results of randomized controlled trials on neurodevelopment in term infants supplemented with LC-PUFAs, building blocks of cognition, learning language, sleep-wake states, visual development and G-protein-coupled signaling.

**Clinical Conditions: Cancer**

**Omega-3 LC-PUFAs May Promote Weight Gain in Advanced Disease**

Advanced cancer is often accompanied by profound metabolic disorder, weight and tissue loss, and lack of appetite. This condition is called cachexia. To overcome cachexia and boost nutrient intake, patients are given oral supplements high in energy, protein, and other enrichments, sometimes including long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). It is particularly challenging to combat the weight loss characteristic of advanced cancer. The provision of n-3 LC-PUFAs has been associated with reduced weight loss and improved quality of life in these patients. In the study described here, the effects of oral supplements rich in energy and protein with or without the n-3 LC-PUFA eicosapentaenoic acid (EPA), a major n-3 LC-PUFA and antioxidants, were compared in patients with advanced pancreatic cancer and cachexia.

Two hundred patients with advanced pancreatic cancer were enrolled in the study from 12 medical centers in 6 countries. In addition to receiving medical care, 105 patients were randomized to consume 2 cans/day of an oral supplement (control group). Each can provided 310 kilocalories, 16 g protein, and 6 g fat. Ninety-five patients in the experimental group consumed the same supplement with the addition of 1.1 g EPA and mixed antioxidants per can. Average EPA consumption was 1.5 g/day. Patients were assessed for body weight, lean body mass, dietary intake, plasma fatty acids, and quality of life at baseline, 4 and 8 weeks.

After 4 weeks, 148 patients remained in the study and at 8 weeks 110 remained. Forty-five patients were lost to followup from each of the control and experimental groups. Survival at 4 and 8 weeks was 75% and 55% of patients, respectively. Patients consumed an average of 1.4 cans of supplement per day with no significant difference between the groups for supplement or total dietary intake. However, patients in the n-3 LC-PUFA supplemented group who completed 8 weeks had a significant
increase in total dietary intake from baseline amounting to an additional 15 g protein and 224 kcal per day. Mean survival from enrollment for all patients was 130 days with no significant difference between dietary groups.

At baseline, patients were losing 3.3 kg body weight per month. Over the 8-week period, weight loss was reduced significantly to 0.25 kg and 0.37 kg/month in the experimental and control groups, respectively. Differences between groups were not significant. Similarly, lean body mass changed from a net loss of about 2 kg/month prior to the study to a net gain of 0.27 kg and 0.12 kg/month in the experimental and control groups, respectively. Differences were statistically significant (p<0.001) compared with baseline within each group but not between groups. These changes in weight stabilization and gain in lean body mass suggest a benefit of the oral supplements.

Because there was no difference between the control and EPA-supplemented groups in weight gain or lean body mass, there appeared to be no specific benefit associated with the provision of EPA. However, examination of the dose-response curves in each group for the amount of supplement consumed suggested a benefit of the EPA-supplement on weight gain and increased lean body mass, but not for the control supplement. Increases in plasma phospholipid EPA in the experimental group were also strongly correlated with increased lean body mass.

Interpretation of the findings in this study was complicated by differences between the groups in the number of patients with more advanced disease, low mean levels of compliance, and undeclared use of n-3 LC-PUFA supplements at baseline. The suggestive findings from this study indicate that the potential benefit of n-3 LC-PUFAs in patients with confirmed cancer needs to be tested in patients with less severe disease, over a range of n-3 LC-PUFA intakes, under reasonably stringent conditions. However, this is easier said than done.

In this cohort, the proportion of current and former smokers was much greater among male than female smokers, 66.9% vs 4.5%, and smoking status was

<table>
<thead>
<tr>
<th>Fish consumption</th>
<th>Men (2798)</th>
<th>Women (3087)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 time/wk</td>
<td>6.2 %</td>
<td>6.3 %</td>
</tr>
<tr>
<td>1 or 2 times/wk</td>
<td>45.2 %</td>
<td>46.5 %</td>
</tr>
<tr>
<td>&gt;3 times/wk</td>
<td>48.6 %</td>
<td>47.1 %</td>
</tr>
</tbody>
</table>

In this prospective cohort study, Takezaki and colleagues from the Aichi Cancer Center Research Institute, Japan, examined the relationship between various environmental factors and common cancers among inhabitants of the rural Aichi Prefecture, Japan. Data on medical history, dietary habits, fish consumption and cooking methods, smoking, and exercise habits were obtained from 1985 to 1999. Valid responses were available from 5,885 men and women whose ages ranged from 40 to 79 years. Relative risk was calculated based on person-years calculated from enrollment to death, date of migration, or end of followup. Fish consumption was divided into 3 groups: less than once/week, one or twice/week, 3 or more times/week.

Fish Consumption Linked to Reduced Risk of Lung Cancer in Japanese

Although smoking is much more prevalent among Japanese men than among American or British men, the incidence and mortality rates from lung cancer are less than two-thirds. Why? Several epidemiological studies have suggested a protective effect of fish consumption against lung (and other) cancers, but data are inconsistent. Studies using animal models bearing human tumors have been more consistently positive. High fish consumption was also associated with less impaired pulmonary function among Japanese-American men who smoked.


Fish Consumption Linked to Reduced Risk of Lung Cancer in Japanese.
unrelated to fish consumption. The latter was similar between men and women and data confirmed the high prevalence of seafood in the Japanese diet (Table 1).

Although the observed number of lung cancer cases was small (51), relative risk of incident lung cancer was significantly and negatively associated with the frequency of individual fish and shellfish consumption, with those consuming fish 3 or more times/week having 68% lower risk (Table 2). When the risk ratios were calculated on the basis of household consumption, which is a surrogate index for individual consumption, and adjusted for age, sex, occupation, and smoking, a clear dose response relationship was evident. Households in which fish was consumed 3 or more times/wk had a 77% lower risk of lung cancer compared with a 57% reduced risk among households consuming fish once or twice a week. Controlling for more variables produced even lower relative risk values. Of interest is the observation that lung cancer risk was increased among individuals who consumed salty or dried fish more than once a week, but the increase was not statistically significant (RR =1.64 and 1.14 for middle and high fish consumers, respectively).

In addition to confirming the association between fish consumption and reduced risk of lung cancer, this report reviews in detail all previous epidemiological findings relating fish or fish oil consumption to lung cancer. Some reasons for the inconsistency among studies may be attributable to selection bias, variation in the types and preparations of fish consumed, and type of lung cancer. For example, the brining and drying of fish may reduce n-3 LC-PUFA content or introduce undesirable oxidation products that may affect susceptibility to lung cancer, particularly among smokers. The study also compared risk ratios by household cooking method. All preparations including deep-frying were associated with reduced risk, although the reductions observed for raw and deep-fried fish consumption were not statistically significant. Given the high prevalence of smoking among Japanese, these findings are remarkable.

<table>
<thead>
<tr>
<th>Frequency of fish consumption per week</th>
<th>Individual consumption(^1) (95% CI)</th>
<th>Adjusted household consumption(^1) (95% CI)</th>
<th>Adjusted household consumption(^2) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than once</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Once or twice</td>
<td>0.99 (0.48-2.03)</td>
<td>0.43 (0.20-0.95)</td>
<td>0.37 (0.16-0.83)</td>
</tr>
<tr>
<td>Three or more</td>
<td>0.32 (0.13-0.76)</td>
<td>0.23 (0.10-0.54)</td>
<td>0.19 (0.08-0.46)</td>
</tr>
<tr>
<td>(P) for trend</td>
<td>0.003</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\)Adjusted for age, sex, occupation, smoking status
\(^2\)Adjusted for age, sex, occupation, smoking status, drinking, exercise, consumption of meat, green-yellow vegetables, and salty/dried fish


**Omega-3 LC-PUFAs May Promote Weight Gain in Advanced Disease**

Advanced cancer is often accompanied by profound metabolic disorder, weight and tissue loss, and lack of appetite. This condition is called cachexia. To overcome cachexia and boost nutrient intake, patients are given oral supplements high in energy, protein, and other enrichments, sometimes including long-chain omega-3 polynsaturated fatty acids (n-3 LC-PUFAs). It is particularly challenging to combat the weight loss characteristic of advanced cancer. The provision of n-3 LC-PUFAs has been associated with reduced weight loss and improved quality of life in these patients. In the study described here, the effects of oral supplements rich in energy
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Fish Consumption Positively Associated with Breast Cancer in Postmenopausal Danish Women

Data linking the consumption of fish or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) with risk of breast cancer have generally found a protective effect, but findings are neither as consistent nor as strong as those for cardiovascular mortality. Studies in laboratory animals have more consistently found beneficial effects of n-3 LC-PUFAs than studies in people with breast cancer. Now, a report from the Institute of Cancer Epidemiology in Copenhagen, Denmark, has upset the applecart.
In this epidemiological study, Stripp and colleagues followed 23,693 postmenopausal women for a median of 4.8 years. Women aged 50 to 64 years, who consumed fish and had no history of cancer, were enrolled in a prospective study of diet, cancer, and health. During the study, 424 cases of primary breast cancer were diagnosed, and of these, tumor estrogen receptor status was obtained for 394 cases, or 93%. The majority of breast cancer tumors are estrogen receptor-positive, meaning that they respond to or grow in the presence of the hormone estrogen. Those that do not respond to estrogen are called estrogen receptor-negative. In their study, Stripp and colleagues determined the relationship between the incidence of breast cancer and tumor estrogen receptor status to fish consumption and the method of fish preparation.

As expected, the majority of the breast cancers diagnosed were estrogen-receptor positive, 77% versus 23% for estrogen receptor-negative. For 30 tumors, estrogen receptor status was unable to be determined. Fish consumption ranged from 11 to 86 g/day with the median intake being 36 g/day. Among breast cancer cases, median fish intake was 39 g/day. Median consumption of lean and fatty fish was 23 and 11 g/day, respectively, with fatty fish considered those with more than 8 g of fat per 100 g fish. Most common preparation methods were frying, processing (pickling, salting, and smoking), and boiling. Of the median intake of 36 g/day, median consumption by these preparation methods was 20, 11, and 6 g/day, respectively. Preparation methods were similar between those who developed breast cancer and the total study group.

Risk ratios for the incidence of breast cancer in these postmenopausal women showed a significant 13% increase in breast cancer risk with fish consumption (Table 1). Adjustment for potentially confounding variables did not alter the risk. Moreover, risk of breast cancer was increased by 47% in women in the highest quartile of fish consumption compared with those in the lowest. Women who consumed fatty fish had a lower increase in unadjusted risk, 8% vs 15%, compared with women who consumed mostly lean fish, but this difference disappeared when the risks were adjusted for confounding variables. Method of fish preparation affected unadjusted risk, but these differences also disappeared with adjustment for other variables.

High fish consumption was associated with an increase in estrogen positive tumors, but had no effect on the risk of estrogen negative tumors (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unadjusted Relative Risk (RR ± 95% CI)</th>
<th>Adjusted Relative Risk (RR ± 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fish intake, mean 25 g/day</td>
<td>1.13 (1.04-1.22)</td>
<td>1.13 (1.03-1.23)</td>
</tr>
<tr>
<td>Fatty fish intake²</td>
<td>1.08 (0.89-1.30)</td>
<td>1.11 (0.91-1.34)</td>
</tr>
<tr>
<td>Lean fish intake²</td>
<td>1.15 (1.01-1.31)</td>
<td>1.13 (0.99-1.29)</td>
</tr>
<tr>
<td>Fried fish</td>
<td>1.04 (0.91-1.19)</td>
<td>1.09 (0.95-1.25)</td>
</tr>
<tr>
<td>Processed fish</td>
<td>1.12 (0.94-1.34)</td>
<td>1.12 (0.93-1.34)</td>
</tr>
<tr>
<td>Boiled fish</td>
<td>1.21 (0.96-1.52)</td>
<td>1.09 (0.85-1.42)</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; Quartile of fish intake&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.54 (1.18-2.02)</td>
<td>1.47 (1.10-1.98)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Adjusted for parity, benign breast tumor, years of school, use and duration of hormone replacement therapy, body mass index, and alcohol consumption  
<sup>2</sup>225 women with intake of only one type of fish excluded from analysis  
<sup>3</sup>Highest quartile, >58 g/day compared with lowest quartile of fish intake, 0-26 g/day, RR=1.0
These risks did not change with adjustment for confounding variables.

This study is noteworthy because Danish women have the third highest fish consumption among 10 European countries, the range of fish intake was considerably greater than in some western populations, and substantial detail about the kinds of fish and preparation methods was obtained. Because the fat content of the fish and its cooking method had no effect on risk, it would appear that n-3 LC-PUFAs are unlikely to be involved with the increased risk observed in this study. Other substances that come to mind, such as environmental contaminants, have not been associated with detrimental effects on breast cancer risk from pesticide residues. The study poses at least two challenges: to find out what in fish or fish-rich diets in Denmark is linked to increased risk of estrogen receptor positive breast cancer, and to communicate to postmenopausal Danish women that n-3 LC-PUFAs, strongly linked with fatty fish, are unlikely to harm their health.


Only recently have methods become available to assess lipid peroxidation in the body reliably. This is done by measuring the amount of degradation products, known as F2-isoprostanes, in urine. F2-isoprostanes are produced when arachidonic acid, an n-6 LC-PUFA, undergoes free radical peroxidation. Trevor Mori and colleagues at the University of Western Australia have shown that meals containing fish or purified n-3 LC-PUFAs consumed by diabetics or mildly hyperlipidemic men were associated with decreased excretion of F2-isoprostanes. In a follow-up study reported here, these investigators compared the effect of purified EPA or DHA supplementation on oxidative stress and markers of inflammation.

<table>
<thead>
<tr>
<th>Tumor estrogen-receptor status</th>
<th>Unadjusted Relative Risk</th>
<th>Adjusted Relative Risk&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1.14 (1.03-1.25)</td>
<td>1.14 (1.03-1.26)</td>
</tr>
<tr>
<td>Negative</td>
<td>1.02 (0.83-1.24)</td>
<td>1.00 (0.81-1.24)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Adjusted for parity, previous benign breast tumor surgery, education, use and duration of hormone replacement therapy, body mass index, and alcohol consumption

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**Clinical Conditions: Type 2 Diabetes**

**Purified n-3 LC-PUFAs Reduce Oxidative Stress**

One of the controversies about the safety of consuming fish oils or purified long-chain omega-3 fatty acids (n-3 LC-PUFAs) is their potential to increase oxidative stress. What does this mean? Because these fatty acids are highly unsaturated with five and six double bonds in the n-3 LC-PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively, they are particularly susceptible to oxidation.

Double bonds in a fatty acid’s structure make it vulnerable to oxidation, a process that can produce free radicals or highly reactive oxygen species, such as superoxide. These molecules are unstable, and in seeking stability, they interact with and damage other molecules, including DNA, lipids, proteins, and lipoproteins. They also deplete supplies of antioxidants, molecules designed to neutralize the harm from excess oxidation products. For this reason, it is important to ensure adequate intake of antioxidants such as vitamin E, when consuming purified fish oils. An abundance of free radicals creates oxidative stress. But does the consumption of highly unsaturated n-3 LC-PUFAs actually increase oxidative stress? This is an important question to settle.

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Only recently have methods become available to assess lipid peroxidation in the body reliably. This is done by measuring the amount of degradation products, known as F2-isoprostanes, in urine. F2-isoprostanes are produced when arachidonic acid, an n-6 LC-PUFA, undergoes free radical peroxidation. Trevor Mori and colleagues at the University of Western Australia have shown that meals containing fish or purified n-3 LC-PUFAs consumed by diabetics or mildly hyperlipidemic men were associated with decreased excretion of F2-isoprostanes. In a follow-up study reported here, these investigators compared the effect of purified EPA or DHA supplementation on oxidative stress and markers of inflammation.
in type 2 diabetic patients. Diabetics have two to four times greater risk of cardiovascular disease and have increased oxidative stress.

Study subjects were 51 nonsmoking, type 2 diabetic men and postmenopausal women being treated for hypertension. They averaged about 61 years of age. After a 3-week stabilization period, subjects were stratified by gender, age, and body mass index, and randomly assigned to consume either 4 g/day of EPA, DHA, or olive oil placebo capsules for 6 weeks. The n-3 LC-PUFAs were provided as ethyl esters and contained somewhat more antioxidant tocopherols (ca 2.6 mg/g) than the placebo (1.9 mg/g). Blood pressure and blood and urine samples were obtained at baseline and the end of the intervention.

Measurement of plasma and platelet phospholipid fatty acids confirmed compliance with the intervention. As expected, groups consuming either EPA or DHA significantly increased the phospholipid concentrations of these fatty acids, respectively, in both platelets and plasma, compared with the olive oil group. In both n-3 LC-PUFA groups, the concentration of arachidonic acid, the major n-6 LC-PUFA, and total n-6 fatty acids decreased significantly. DHA supplementation had no effect on EPA concentrations and vice versa in platelets or plasma phospholipids. However, the changes in total n-6 and total n-3 LC-PUFAs with DHA supplementation were less than and significantly different from those observed after EPA supplementation.

Excretion of F2-isoprostanes was significantly decreased in groups consuming EPA and DHA in contrast to a slight increase in excretion in the placebo group (Figure 1). Baseline excretion of these substances was also positively correlated with body mass index and indices of diabetic control, such as baseline fasting glucose, and glycated hemoglobin (hemoglobin with sugar attached to it).

Supplementation with n-3 LC-PUFAs had no significant effect on any of the inflammatory markers measured when compared with the placebo group. Changes in urinary F2-isoprostanes were not associated with changes in platelet or plasma LC-PUFA concentrations. However, changes in urinary F2-isoprostanes were positively associated with changes in the inflammatory marker, tumor necrosis factor-α, and in glycated hemoglobin, although changes in these markers were not statistically significant. The authors discuss a variety of ways in which n-3 LC-PUFAs may be linked to oxidative stress, inflammatory responses, and leukocyte and enzyme activities. This study provides critical evidence of the benefit of n-3 LC-PUFAs for reducing oxidative stress in type 2 diabetics, and the potential ways in which oxidative stress, inflammatory responses, and atherosclerosis may be connected.

Clinical Conditions: Ulcerative Colitis

Fish Oil n-3-LC-PUFAs Reduce Oxidative Stress

Patients with ulcerative colitis, a severe inflammatory disease of the large intestine or bowel, exhibit oxidative stress. The latter condition results from the excess production of highly reactive products of oxidation, known as free radicals and reactive oxygen species. These oxidation products do considerable damage to epithelial cells lining the intestine and blood vessels and to particular molecules such as DNA, lipids, and proteins. Free radicals result from the metabolism of polyunsaturated fatty acids. Provision of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFAs) has been shown to reduce oxidative stress in some patients, but results in ulcerative colitis have been inconsistent.

One treatment used in ulcerative colitis to reduce the production of free radicals is sulfasalazine, a drug thought to scavenge or capture free radicals. It has also been suggested that a combination of sulfasalazine and n-3 LC-PUFAs might be superior to either treatment separately. Barbosa and colleagues at Londrina State University in Brazil decided to find that out.

Nine patients with mild to moderate clinically confirmed ulcerative colitis were randomly assigned to receive 4.5 g/day of n-3 LC-PUFAs from fish oil or the same amount of soy oil for 2 months. After 2 months receiving only sulfasalazine, patients were switched to the other treatment for an additional 2 months. Nine healthy subjects matched by age and sex served as controls for the measurement of oxidative stress. All patients were treated with 2 g/day sulfasalazine and disease activity and oxidative stress were assessed at baseline and 2 and 6 months after treatment. Disease severity was assessed by sigmoidoscopy and histologic activity scores. Oxidative stress was assessed using six different assays in plasma and red blood cells.

Clinical evaluation of patients after 2 months of treatment showed no significant differences between treatments; however, red cell sedimentation rates, a general reflection of clinical status and underlying disease, were significantly reduced in both groups compared with baseline values. Reduced red blood cell sedimentation rate may reflect general improvement in clinical condition.

Three measures of potential antioxidant capacity were significantly impaired in ulcerative colitis patients at baseline compared with healthy control subjects. After two months, two of these measures were improved with fish oil plus sulfasalazine treatment, but not with sulfasalazine alone. By a third measure, patients treated with sulfasalazine and fish oil were significantly improved over baseline and healthy control subjects, whereas patients treated only with sulfasalazine showed no improvement over baseline values.

Interpretation of these findings is made difficult by the inconsistency among the different measures of oxidative stress; the small number of patients; use of soy oil as a control, which may have increased oxidative stress; and the lack of clinical improvement in disease status. It may be that two months is insufficient time for substantial improvements in inflammation or antioxidant capacity. Consumption of n-3 LC-PUFAs from fish oil improved some measures of oxidative stress in patients with ulcerative colitis and was not associated with deterioration of clinical or biochemical status. Improvements in ulcerative colitis with n-3 LC-PUFAs remain equivocal.

Clinical Conditions: Pulmonary Function

Elite Athletes Breathe Easier with Fish Oil

Exercise-induced narrowing of the air passages, which is usually accompanied by breathing difficulties, is prevalent in elite athletes who exercise intensely and often. It occurs in some asthmatic subjects, too. Some evidence suggests that the condition in athletes differs from that observed in asthmatics. For example, elite athletes respond poorly to treatment with pharmacologic agents. Because long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) improve the respiratory function in some subjects with asthma and are known to reduce the production of proinflammatory mediators, Timothy Mickleborough at Indiana University, USA, and colleagues at the University of Wales, UK, tested the effect of fish oil supplementation on pulmonary function and production of proinflammatory mediators in 10 elite athletes with exercise-induced bronchoconstriction and 10 without. The study was a randomized, placebo-controlled, double blind, crossover study for 3-week dietary periods.

Ten male and female athletes, aged about 23 years on average and ranked at the collegiate or national level in their sport (triathletes, cross-country or track running), with clinically diagnosed exercise-induced bronchoconstriction but free of asthma, were matched with 10 elite athletes without this condition. All consumed a normal diet upon entry to the study and both groups had similar pulmonary function test results preceding exercise. Athletes with exercise-induced bronchoconstriction, however, had significantly reduced pulmonary function immediately following exercise, which they treated with inhaled bronchodilators.

Participants were randomized to consume either 6 g/day olive oil (placebo) or n-3 PUFAs providing 3.2 g/day eicosapentaenoic acid (EPA) plus 2.2 g/day docosahexaenoic acid for 3 weeks. After 2 weeks of consuming a normal diet, they were switched to the alternative treatment. Records were kept of diet and use of bronchodilators. Blood was collected at the beginning of the study and end of each treatment period. Pulmonary function tests were performed pre-exercise and at 6 intervals up to one hour after exercise. Urine samples were collected before and 3 times after exercise. All athletes completed the study.

Elite athletes with exercise-induced bronchoconstriction who consumed n-3 LC-PUFAs experienced a dramatic and significant improvement in their pulmonary function. Use of bronchodilators following exercise declined from an average of 58 ± 16 and 55 ± 17 puffs on the normal and placebo diets, respectively, to 39 ± 13 puffs during the last two weeks of consuming n-3 LC-PUFAs (p<0.05). Measures of pulmonary function in athletes with exercise-induced bronchoconstriction consuming n-3 LC-PUFAs were similar to those of athletes without breathing difficulties and contrasted markedly to the responses observed when they consumed a normal or placebo diet (Figure 1).
Usually, these athletes experience a marked decline in forced expiry volume, a measure of pulmonary function, in the first 15 minutes following exercise. This characteristic response was observed with the normal or placebo diet, but not with the n-3 LC-PUFA supplemented diet. Supplementation with n-3 LC-PUFAs did not improve physical performance. Moreover, it had no significant effect on pulmonary function in athletes without breathing difficulties.

In control athletes, inflammatory markers did not change as a result of diet or exercise. In athletes with exercise-induced bronchoconstriction, production of inflammatory markers increased following exercise when these athletes consumed a normal or placebo diet. Supplementation with n-3 LC-PUFAs resulted in a significant decrease in the production and excretion of several proinflammatory substances prior to and following exercise. Consumption of n-3 LC-PUFAs was accompanied by a significant increase in EPA in neutrophils, a type of white blood cell, in all study participants, but there was no change in neutrophil docosahexaenoic acid concentration. As expected, the omega-6 fatty acids, arachidonic and linoleic acids, decreased significantly with n-3 LC-PUFA consumption.

This study is the first demonstration that consumption of n-3 LC-PUFAs reduces the severity of exercise-induced bronchoconstriction in elite athletes. The increase in neutrophil EPA concentration and the reduced production and excretion of proinflammatory mediators are consistent with the well known anti-inflammatory effects of n-3 LC-PUFAs in other clinical states, such as asthma. These findings strongly suggest that exercise-induced bronchoconstriction is linked to over-responsive inflammatory pathways that are responsive to increased availability of n-3 LC-PUFAs. These findings contrast with those of a study using a similar dose of EPA in patients with asthma. The authors and editorialists suggest that the inflammatory processes in athletes with exercise-induced bronchoconstriction differ from those in asthma. For elite athletes with impaired pulmonary function, n-3 LC-PUFAs may offer effective treatment with the additional bonus of decreasing the amount of bronchodilators needed.


**Letter to the Editor**

**Omega-3 LC-PUFAs and Schizophrenia**

The study by Arvindakshan et al (2003) is of great value to researchers studying fatty acid supplementation in schizophrenia. A placebo group was not vital in this study because subjects acted as their own controls in the paired design. Other researchers have found a placebo group problematic, because subjects in the placebo group appeared to have changed their diet, perhaps as a result of learning about the beneficial effects of fatty acids.

The heartening aspect about Arvindakshan et al’s study was its relatively long duration, and follow-up assessment. This enabled a clearer picture of what can happen during the course of supplementation. As suggested by the late David Horrobin, a clear interaction between docosahexaenoic acid (DHA) and arachidonic acid occurred during supplementation, and the pattern was reversed during the washout period. The two series of fatty acids appear to obey their own homeostatic rules. There was some indication that DHA may have marginally increased even after washout, although a more explanatory picture might be evident from the data of each individual’s changes throughout the 32 weeks of observation. One wonders if the slight increase in DHA concentration and slight decrease in saturated fatty acids indicate an accretion of DHA in absolute terms? As suggested by Arvindakshan et al., DHA is the important structural fatty acid.

Sharon Monerrubio
University of Wollongong
Wollongong, NSW, Australia