PUFA NEWSLETTER

Volume 7 Issue 1

March 2003 Contents

Editorial

Relaunch of the *PUFA Newsletter*

The March 2003 issue of the *PUFA Newsletter* marks the relaunch of this quarterly newsletter solely in electronic form. It aims to keep scientists and physicians, health professionals and educators up-to-date on the leading scientific advances in polyunsaturated fatty acids (PUFAs). Articles bring perspective to the broad horizon of PUFA research from infant development, cardiovascular disease, and mental health, to recommendations for fatty acid consumption from different countries and health authorities. We focus particular attention on the long-chain omega-3 PUFAs (n-3 LC-PUFAs) that are associated with reduced cardiac mortality and other health benefits. These fatty acids, once nearly exclusively found in seafoods, especially fatty fish, are now available in some countries in supplemented foods such as eggs, margarine, bread, and infant formula.

We dedicate this inaugural electronic issue to the memory and achievements of James Magee, the newsletter's first editor. From the establishment of the newsletter in 1996, until his untimely death in August 2002, James applied his masterful prose to alert scientists and health professionals worldwide to the key research on LC-PUFAs. The *PUFA Newsletter* chronicled the rapidly expanding science on LC-PUFAs that established the n-3 LC-PUFAs in fatty fish as among the most potent cardioprotective substances known.

We invite you to subscribe. The *PUFA Newsletter* is complimentary and will be published quarterly, solely in electronic form. Future issues will feature interviews with PUFA scientists, policy statements and recommendations, readers' comments, and controversial issues. We welcome your comments and suggestions.

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Cardiovascular Health

Consumption of n-3 LC-PUFAs Reduces Risk of Death in Heart Disease Patients

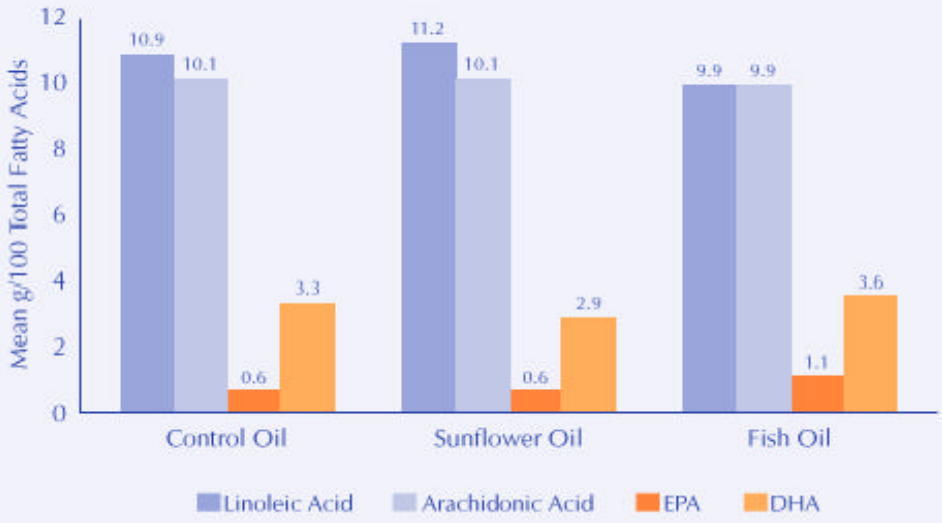
Any substance that reduces the risk of cardiac patients dying by a stunning 45% or more makes a scientist ask, "How does that happen?" A consumer might ask, "Where can I get it?" For the past 30 years, scientists have been seeking to understand how fish oils confer their remarkable heart-protecting properties. Several possible answers include the ability of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) to:

- lower blood triglycerides significantly;
- reduce the tendency for platelets to aggregate and initiate clot formation;
- inhibit the development of electrical abnormalities or arrhythmias in the heart;
- stimulate the production of substances that reduce inflammation in the arteries.

Another protective strategy focuses on the behavior of arterial plaques. Plaques are fatty and cellular deposits in the lining of arteries that develop and enlarge over decades, accumulate various lipids and cells, become inflamed, and eventually burst, causing heart attacks, strokes, and sometimes death. Their steady growth diminishes blood flow to the heart and can completely choke an artery. When they rupture, they can disperse small blood clots to the heart, lungs, kidney, and other organs, causing serious damage. Although we know a great deal about the build up of materials in these plaques, we don't know what makes them break or how to prevent them from doing so. At present, there are only two ways of reducing plaques: 1) remove them by means of balloon angioplasty or surgical excision, or 2) reduce their size through strict dietary change and control. What if plaques could be made less fragile and dangerous?

Thinking along these lines, researchers in the laboratory of Philip Calder, University of Southampton, UK, investigated the effect of different dietary fats on the fatty acid composition of arterial plaques in people who consumed diets rich in fish oil (1.4 g n-3 LC-PUFAs/day), sunflower oil (3.6 g linoleic acid/day), or a blended control fat for 7-190 days prior to carotid artery surgery to remove plaque. The consumption of n-3 LC-PUFAs from fish oil, in contrast to sunflower oil or a blended control oil, was accompanied by a significant increase in the n-3 LC-PUFA content of carotid plaques and plasma low density lipoprotein lipid fractions (see figure). Moreover, the proportion of eicosapentaenoic acid, a major n-3 LC-PUFA in fish oil, in the plaques increased with the length of time the diets were consumed. These findings indicate that the fatty acid composition of plaques can be manipulated by dietary fatty acid intake.

Effects of Dietary Oil Consumption on Selected Fatty Acids in Carotid Plaque Phospholipids



Abbreviations: EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid

Absolute difference between fish oil and sunflower oil:
Linoleic acid p=0.0015 EPA p<0.001 DHA p<0.01

In turn, properties of the plaque and its interaction with constituents in the blood may be affected by changes in its fatty acid composition.

To see whether the incorporation of n-3 LC-PUFAs in the arterial plaque was associated with functional changes, the researchers examined the distribution of the types of plaque lesion among the three dietary groups. Lesions in patients consuming fish oil longer than 46 days had lesions significantly different from those in the control and sunflower oil groups. Lesions were grouped according to modified American Heart Association classification. Omega-3 LC-PUFA content was highest for type IV plaques and lowest for type VI plaques. This means that those consuming fish oil had more plaques with a well formed fibrous cap, rather than a thin inflamed cap. The implication is that such lesions are less likely to rupture.

A third aspect of plaque welfare investigated by this team was inflammation. Macrophages, cells involved in inflammatory responses, are incorporated into arterial plaques as they grow. Their presence enhances plaque inflammation and instability. Using staining techniques specific to a macrophage marker, the intensity of macrophage staining was compared in plaque sections from patients in the three dietary groups. Plaques from the fish oil-fed patients had significantly less staining than those from the control and sunflower oil groups. For patients consuming fish oil longer than 46 days, staining was significantly less than in those who consumed fish oil for a shorter time. Further, plaques with the highest intensity of staining (highest macrophage level), had significantly lower levels of n-3 LC-PUFAs than plaques with more moderate staining and macrophage content.

These findings indicate that carotid lipids exhibit substantial lipid turnover, and their composition and characteristics can be favorably altered by modest dietary intake of n-3 PUFAs. For people with carotid plaques that warrant surgery, this study suggests that increasing consumption of n-3 LC-PUFAs will beneficially modify the characteristics of these lesions and their plasma lipids. Such a dietary modification is also likely to significantly reduce macrophage infiltration into plaque and reduce the likelihood that existing lesions will rupture with harmful consequences. This is the first study to show that the consumption of n-3 LC PUFAs can increase plaque stability and lower plaque inflammation, which has the potential to lower the chance of serious cardiac events, including fatal ones. “Increased plaque stability” may now be added to the list of the cardiovascular benefits of consuming n-3 LC-PUFAs.

Thies F, Garry JMC, Yaqoob P, Rerkasem K, Williams J, Shearman CP, Gallagher PJ, Calder PC, Grimble RF. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomized controlled trial. *Lancet* 2003;361:477-485.

Do Dietary n-3 LC-PUFAs Reduce the Risk of Stroke?

The benefits of the consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in coronary disease have been well established, but it is less certain if these fatty acids protect against stroke. One reason for the ambivalence is that data may combine all types of stroke, and the underlying mechanisms differ in various stroke subtypes. One might expect fish or n-3 LC-PUFA consumption to reduce the risk of ischemic or thrombotic strokes, which are related to clot formation. However, hemorrhagic strokes may be less affected, or even increased in cases of very large intakes, as observed in native Greenlanders and Inuit. Data support these expectations, but they are not consistent.

Much of the information on dietary fat intake and stroke comes from epidemiological studies. This literature has shown that high intake of saturated fat increases risk of ischemic stroke and high intake of linoleic acid (LA) lowers it. However, there are studies that contradict this view, showing a protective effect with saturated fat intake and increased risk associated with LA. For example, in a nested case-control study of 197 cases from a sample of 7,450 Japanese women aged 40-85 years, intake of LA was associated with 37% fewer strokes. Intake of saturated fatty acids was higher among cases, but consumption of n-3 LC-PUFA did not

differ between cases and controls.

In the U.S. Nurses' Health Study, which studied the diet and health patterns of more than 79,000 women for 14 years, fish consumption twice or more per week was associated with a 51% lower chance of thrombotic stroke compared with women who ate fish less than once a month. Those who ate fish five or more times a week had a 33% lower chance of thrombotic stroke, indicating that large fish intakes did not give additional protection.

In a recent Spanish case-control study, people eating the most fish (average of 46 grams/day) in a population with high fish consumption and stroke incidence were twice as likely to incur a stroke or cerebral infarction as those with the lowest fish intake, 11 grams/day. When the data were analyzed in terms of n-3 LC-PUFA intake, risk was increased nearly as much, but did not quite reach statistical significance. In this study, low fish consumption protected against ischemic stroke.

Dr. He and associates at the Harvard School of Public Health, Boston, Mass., report that men aged 40 to 75 years who consume fish at least once a month have a 44% lower risk of ischemic stroke compared with men who eat fish less than once a month. Their study included 43,671 male participants in the U.S. Health Professionals Study who had no heart disease, diabetes, or stroke. Subjects' diets and health patterns were tracked for 12 years and their fish consumption was grouped according to how often they ate fish, from less than once a month, to five or more times/week. The majority (62%) of strokes observed were ischemic.

Those who ate even small amounts of fish – one to three times/month – had significantly lower ischemic stroke compared with those who ate fish less than once/month. Fish consumption had no effect on hemorrhagic stroke. Notably, the chance of having ischemic stroke did not increase beyond the level of one serving of fish/week. Thus, this study confirms earlier observations that even relatively low fish consumption has important cardiovascular protective effects.

The nature of epidemiological studies indicates that one cannot simply extrapolate from the protective mechanisms that appear to operate in the heart and coronary arteries to those at work in the head. However, it is encouraging that most studies so far show that modest fish consumption has protective effects in ischemic stroke. When it comes to eating fish, we are serving both the heart and the head.

He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, Ascherio A. Fish consumption and risk of stroke in men. *JAMA* 2002;288:3130-3136.

Never Too Late to Benefit from Fish

Some of the most convincing data available on the protective effects of consuming fish oil or long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) have come from large-scale epidemiological studies. While such observations do not prove cause and effect, they give useful insight into relevant dietary factors involved in cardiovascular disease (CVD). From these studies have come persuasive demonstrations that fatty fish consumption reduces risk of CVD, the chance of sudden death owing to cardiac arrhythmias, and cardiac mortality among those who already have established disease.

Perhaps the most convincing epidemiological study showing the protective effects of n-3 LC-PUFAs on cardiac mortality, especially from sudden death, is the GISSI-Prevenzione study of more than 11,000 patients who already had a myocardial infarction. In that study, those who consumed about one gram/day of n-3 LC-PUFAs had a 45% lower chance of sudden death and a 30% reduced risk of CVD death. Subsequent analysis indicated that benefits on reducing mortality from all causes were observed as early as three months after the consumption of n-3 LC-PUFA supplements. Total mortality was reduced by 41%. The reduced chance of sudden death was statistically significant at four months and risk was lowered by 53%. However, subjects in the GISSI study were predominantly 50 to70 years of age.

The study by Lemaitre and colleagues, University of Washington, Seattle, Wash., published early

this year fills in the gap for older adults. These investigators studied a cohort of men and women from four U.S. communities, aged 65 years or more, whose average age was 78. Subjects, who were free of heart disease and stroke at baseline, were followed for four years. Cases included those who experienced either fatal ischemic heart disease or nonfatal myocardial infarction; controls were matched closely to cases. The researchers compared the incidence of events and fatty acid composition of plasma phospholipids.

Two thirds of heart disease-related deaths could be attributed to arrhythmias. Those who died had significantly lower baseline plasma phospholipid concentrations of n-3 LC-PUFAs, specifically eicosapentanenoic acid (EPA) and docosahexaenoic acid (DHA), as well as higher concentrations of linoleic acid . Taking into consideration potentially confounding factors, the level of EPA and DHA in plasma phospholipids was associated with a 70% lower risk of fatal ischemic heart disease. The level of alpha-linolenic acid, the vegetable source precursor of EPA, was associated with half the risk of fatal cardiac death. In contrast, a high level of linoleic acid increased the chance of fatal cardiac death by 2.4 times. Increased risk associated with linoleic acid adds to the controversy about the healthfulness of consuming an abundance of unsaturated vegetable oils characteristic of Western diets.

The results of this study indicate that fatty fish or n-3 LC-PUFAs consumption by older people reduces the risk of fatal heart disease. The remaining gap in the epidemiological repertoire concerning the cardioprotective effects of consuming n-3 LC-PUFAs is the demonstration that providing modest amounts of these fatty acids to healthy young adults, who have no signs of heart disease, will similarly reduce the toll of cardiac mortality. With the extensive knowledge already in hand, it is worth asking whether progressive health policy must await such a primary prevention trial.

Lemaitre RN, King IB, Mozaffarian D, Kuller LH, Tracy RP, Siscovick DS. Omega-3 polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the cardiovascular health study. *Am J Clin Nutr* 2003;77:319-325.

Be Choosy About Your Fish

It may seem like common sense, but the species of fish you eat and the way it is prepared makes a difference to its health-protecting properties. It is widely accepted that the benefits associated with fish consumption relate largely to their long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), which are found in few other foods. Few studies, however, have described or analyzed their findings according the type of fish consumed. In particular, U.S. studies, where the consumption of fish is low, seldom have the ability to make this type of distinction. But Mozaffarian and coworkers have shown that fish is not simply "fish."

They compared the effects on cardiac mortality of eating tuna, fried fish or fish sandwiches, and other broiled or baked fish species in adults aged 65 or older, who were free of CVD at baseline. Subjects were monitored for 9.3 years. Because they were related, the consumption of tuna and "other fish" was combined. Eating tuna or other broiled/baked fish reduced the risk of cardiac death, particularly those from cardiac arrhythmia, by nearly half compared with the fried fish eaters. Those who ate tuna or other fish three times/week or more had a 49% lower chance of fatal heart disease and a 58% reduced likelihood of fatal cardiac arrhythmia compared with those eating tuna or other fish less than once/month. Eating fried fish or fish sandwiches not only failed to guard against cardiac fatality, it tended to make it more likely.

Several differences between the two types of fish consumption can be suggested. Fried fish and commercially prepared fish sandwiches are high in fat because of the fat added during preparation and cooking. Fish sandwiches are usually made with lean white fish, the kind with the least n-3 LC-PUFAs. In addition, the kind of fat added is likely to contain saturated and partially hydrogenated fat with trans fatty acids. Neither of these help the heart. So, choose fatty fish species for the most n-3 LC-PUFAs and prepare them with as little additional fat of any kind.

Mozaffarian D, Lemaitre RN, Kuller LH, Burke GL, Tracy RP, Siscovick DS. Cardiac benefits of fish consumption may depend on the type of fish meal consumed: the cardiovascular health study. *Circulation* 2003;107:1372-1377.

Cardiovascular Disease: Vascular and Endothelial Function

Exercise and EPA Improve Blood Flow

One of the ways in which long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) contribute to heart health is by improving vascular function. This has several aspects, one of which is greater relaxation of the muscles lining the arteries. As these relax, blood flows with greater ease and blood pressure may be reduced. The mechanisms involved in arterial muscle relaxation are affected by n-3 LC-PUFAs. They also respond to exercise. The question is, do n-3 LC-PUFAs enhance the effect of exercise on vascular function? If so, both interventions might boost arterial function, especially in subjects whose vascular function is impaired by atherosclerosis.

To answer this question, Tagawa et al. measured blood flow in the forearms of subjects with stable coronary artery disease, before and after they consumed eicosapentaenoic acid (EPA), an n-3 LC-PUFA, for 3 months. Exercise consisted of a rhythmic handgrip exercise. When forearm blood flow was measured following exercise, blood flow increased. The same experiment conducted after EPA supplementation resulted in arterial endothelial cell function. When the participants were given a drug that blocks nitric oxide (NO) synthesis, the blood flow response to EPA consumption was significantly reduced. This finding indicated that EPA was exerting its effect on blood flow through mechanisms that involve NO.

The study is important for several reasons. First, it showed that EPA consumption improves vasodilation and blood flow, and will further improve the benefit of exercise on blood flow. Second, the results using NO blocking agents indicate the type of mechanism through which n-3 LC-PUFAs are most likely acting to improve blood flow. For subjects with impaired circulatory responses, vascular function can be improved with exercise or n-3 LC-PUFAs, and even more so with both. This is good news for people with cardiovascular disease.

Tagawa T, Hirooka Y, Shimokawa H, Hironaga K, Sakai K, Oyama J, Takeshita A. Long-term treatment with eicosapentaenoic acid improves exercise-induced vasodilation in patients with coronary artery disease. *Hypertens Res* 2002;25:823-829.

EPA or DHA Improve Arterial Elasticity

The elasticity of the vascular system is an important indication of its functional capability. When elasticity is reduced, such as often happens in heart disease and type 2 diabetes, the result is increased blood and pulse pressure, greater workload on the heart, and diminished coronary blood flow. These complications increase coronary risk. Vascular function is improved with fish oil, dicisahexaenoic acid (DHA), or alpha-linolenic acid, but the individual effects of DHA and eicosapentaenoic acid (EPA), the main long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) in fatty fish, have not been reported until now.

The subjects in this study were overweight men and women with dyslipidemia. They were randomly assigned to consume three grams/day of EPA, DHA, or placebo oil for seven weeks. Systemic arterial compliance, a measure of arterial elasticity, was determined before and after supplementation. After seven weeks, arterial compliance was significantly increased by EPA (36%) and DHA (27%) compared with the placebo. Differences between EPA and DHA were not significant. Blood pressure was unaffected. Thus, fish oil or purified n-3 LC-PUFAs improve arterial compliance without necessarily affecting blood pressure. This study and the preceding one extend the evidence that n-3 LC-PUFAs improve vascular function.

Nestel P, Shige H, Pomeroy S, Cehun M, Abbey M, Raederstorff D. The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *Am J Clin Nutr* 2002;76:326-330.

ECG Unaffected by Daily Fish Oil

Substantial evidence indicates that long-chain omega-3 fatty acids (n-3 LC-PUFAs) reduce and prevent cardiac arrhythmias that lead to sudden death. So, it seemed reasonable to look for changes in the electrocardiogram that might reflect the consumption of n-3 LC-PUFAs. Geelen et al. fed 3.5 grams/day of fish oil or high oleic acid sunflower oil to 84 older men and women for 12 weeks. They recorded12-lead electrocardiograms (ECGs) in subjects at the start and end of the intervention period. Daily fish oil intake had no effect on ECG patterns compared with sunflower oil. The authors speculate that results may be different in more susceptible populations. Things are seldom straightforward.

Geelen A, Brouwer IA, Zokd PL, Kors JA, Swenne CA, Katan MB, Schouten EG. (n-3) Fatty acids do not affect electrocardiographic characteristics of healthy men and women. *J Nutr* 2002;132:3051-3054.

Maternal and Infant Health

DHA-Enriched Egg Extends Gestation Time

One of the greatest risks to infant mortality and poor development is low birthweight – less than 2,500 grams or 5 lb., 8 oz. In the United States, one in 13 babies weighs less than 2,500 grams at birth. A majority of these tiny infants are born before term; that is, less than 37 weeks after gestation. They face serious health problems in early life and risk lifelong impairments. How can a woman ensure a full-term pregnancy and a healthy newborn?

Many factors reduce the chance of preterm birth and low birthweight. These include good medical care during pregnancy, avoiding smoking and alcohol consumption, and good nutrition. The latter includes vitamins, minerals, a healthy balanced diet that permits adequate weight gain, and foods rich in long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). These fatty acids are essential for optimum brain growth and development of the fetus. They may also increase the likelihood of a full-term pregnancy.

Pregnant women in the Faroe Islands have high fish consumption, longer gestation, and higher infant birthweight than women in the United States. This difference has been attributed to the high intake of n-3 LC-PUFA. Thus, Carlson's research team at the University of Kansas Medical Center, USA, thought it appropriate to see whether increasing the intake of docosahexaenoic acid (DHA), an n-3 LC-PUFA found mainly in fatty fish, could affect gestation time and birthweight in U.S. women. Because American women have one of the world's lowest intakes of n-3 LC-PUFA, they are good candidates for such a study.

For this study, DHA was provided by DHA-enriched eggs, which contained an average 133 mg of DHA/egg compared with 33 mg/egg in ordinary eggs. Women were given 12 DHA-enriched eggs a week and encouraged to eat as many as possible. The number of eggs consumed was determined by interview. Gestational age in the DHA-supplemented group was significantly increased by six days. When birthweights in the two groups were compared, those from the DHA-supplemented mothers weighed on average 83 grams more, but this increase was not statistically significant. The DHA level in infant red blood cell phospholipids in the supplemented group was significantly higher than the unsupplemented group. DHA in maternal red cell lipids did not differ. All other variables in the two groups were similar. Thus, a modest increase in DHA consumption significantly increased gestation in healthy U.S. women, a finding with potentially important clinical implications. It would be interesting to learn whether a larger intake of n-3 LC-PUFA would affect birthweight.

Smuts CM, Huang M, Mundy D, Plasse T, Major S, Carlson SE. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet Gynecol* 2003;101:469-479.

DHA-Enriched Egg Yolk Provides Infants n-3 LC-PUFAs

For about the first six months of life, the infant obtains LC-PUFAs from breast milk or LC-PUFA-supplemented infant formula. After weaning, food sources of LC-PUFA may be limited. Because brain growth and development continue at least through

the first 18 months, provision of these essential fatty acids may be uncertain. Biosynthesis from short chain precursor fatty acids likely provides small amounts.

Standard egg yolk is a potential source of long chain essential fatty acids after weaning, but is not rich in n-3 LC-PUFAs. It is a good source of iron, but its use may be restricted by concerns about cholesterol. Eggs produced by n-3 LC-PUFA-fed hens are enriched primarily in DHA, but this comes at the expense of reduced arachidonic acid content. Makrides and associates at the Women's & Children's Hospital in Adelaide, Australia, decided to evaluate the effectiveness of DHA-enriched egg yolk in the diets of healthy 6-month old infants.

They fed these infants, who had been breast or formula-fed from birth, four DHA-enriched or standard egg yolks a week for six months. Enriched eggs provided nearly five times as much n-3 LC-PUFAs as standard eggs. Before and after the dietary treatment they measured red blood cell fatty acid content, plasma cholesterol, and other nutritional parameters.

As the table below shows, formula-fed infants had half the DHA content in their red cells as breast-fed infants at the 6-month baseline (2.7% vs 5.6%). This was not surprising because breast milk, but not infant formula, provides n-3 LC-PUFAs. At 12 months, feeding DHA-enriched eggs significantly increased the DHA level in red cells by 30% in both breast and formula-fed infants compared with regular eggs (table). Red cell DHA in infants consuming DHA-enriched eggs did not differ from the breast-fed control infants (4.1 vs 4.8), but was lower than breast-fed infants receiving the DHA-rich eggs at 12 months (4.1 vs 6.7). These observations mean that providing DHA from DHA-enriched egg yolk to 6-month-old formula-fed infants can make up for their not receiving DHA in breast milk in the first six months of life.

With regard to arachidonic acid (AA), the DHA-enriched eggs had less than half the amount of regular eggs. Feeding infants DHA-enriched eggs decreased red cell AA by about 10% in both breast and formula-fed infants compared with regular eggs or control (table). The addition of egg yolk to the diet had no effect on plasma cholesterol concentration in either group of infants.

Red Blood Cell Fatty Acids in Infants Fed Regular or DHA-Enriched Egg Yolk for Six Months

	BREAST-FED COHORT			FORMULA-FED COHORT		
Fatty Acid	Control	Regular egg yolk	n-3 egg	Control	Regular egg yolk	n-3 egg
DHA[1] 6 mo	$5.2^{a,2}$	$5.4^{a,2}$	$5.6^{a,2}$	$2.7^{b,2}$	$2.6^{b,2}$	$2.7^{b,2}$
12 mo	$4.8^{a,d,3}$	$4.1^{a,2}$	$6.7^{b,3}$	$3.0^{c,2}$	$3.3^{c,3}$	$4.1^{d,3}$
AA[1] 6 mo	$15.8^{a,2}$	$15.6^{a,2}$	$15.8^{a,2}$	$14.1^{b,2}$	$14.2^{b,2}$	$14.4^{b,2}$
12 mo	$15.3^{a,2}$	$15.3^{a,2}$	$13.9^{c,2}$	$15.0^{a,2}$	$15.1^{a,2}$	$14.5^{b,2}$

1 Percent of total phospholipid fatty acids; DHA = docosahexaenoic acid; AA= arachidonic acid

a-c Values with different superscript letters indicate significant differences between dietary groups. See original paper for details.

2,3 Values with different superscript numbers indicate significant differences by time within dietary group, $p < 0.05$

This study is valuable for demonstrating that DHA-enriched egg yolks are an effective dietary source of LC n-3PUFA in the second six months of life. When consumed regularly for six months, they overcome the lower red cell DHA that results from feeding infant formula. DHA-enriched eggs offer an additional means of providing n-3 LC-PUFAs to infants, especially those fed formula that is not enriched with n-3 LC-PUFAs.

Cognitive Performance in 7-Year Olds Unrelated to LC-PUFA Status at Birth

There is intense interest in knowing whether low maternal and infant long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) intakes in pregnancy and infancy have long-lasting effects. Availability of adequate amounts of the LC-PUFAs, DHA and AA, affects neurodevelopment and function. When supplements of LC-PUFAs are provided, measures of development and neurofunction improve, especially in preterm infants, but the results of long term studies are scarce and inconsistent.

In this study of 306 children born at term, Bakker and co-workers at the University of Maastricht, The Netherlands, evaluated cognitive performance at seven years of age using the Kaufman Assessment Battery for Children. Assessment of LC-PUFA status at birth was determined from fatty acid analysis of phospholipids in venous cord blood obtained at birth. After controlling for maternal intelligence and parenting skills, the results showed no significant association between cognitive performance and the concentration of either DHA or AA at birth.

LC-PUFA status at seven years was unrelated to measures of cognitive performance. This study does not support the view that the LC-PUFA status of term infants at birth is related to impaired cognitive performance in childhood. These findings provide some reassurance to mothers whose dietary intake of LC-PUFAs, especially of the omega-3 family, may have been less than what is recommended today. However, they are unlikely to be the final word.

Bakker EC, Ghys AJA, Kester ADM, Vles JSH, Dubas JS, Blanco CE, Hornstra G. Long-chain polyunsaturated fatty acids at birth and cognitive function at seven years of age. *Eur J Clin Nutr* 2003;57:89-95.

Newborn Sleep Patterns Related to Maternal DHA Status

Nervous system function is challenging to measure in young infants. Investigators have found that sleep patterns in infancy are related to central nervous system (CNS) function and are associated with neurologic changes during development. Sleep behavior has also been linked to essential fatty acid status. Thus, they provide a useful way to evaluate the effects of long-chain polyunsaturated fatty acids (LC-PUFA) status on infant development.

Cheruku and colleagues at the University of Connecticut, USA, studied infant sleep patterns and their relationship to maternal omega-3 LC-PUFA status. They collected sleep recordings from 17 healthy term infants for the first two days post partum and determined the percent time spent in each of four sleep categories. Sleep categories included quiet sleep, active sleep, sleep-wake transition, and wakefulness. Maternal plasma phospholipid fatty acids at delivery were measured and docosahexaenoic acid (DHA) content was used to divide mothers into two groups: high and low DHA (>3.0% and <3.0% by weight), respectively.

DHA ranged from 1.91% to 4.5% by weight. Maternal age, race, education, parity, and gestation time and infant characteristics were equivalent in the two groups. Maternal DHA was positively associated with wakefulness and inversely related to active sleep and sleep-wake transition times. These findings suggest that higher DHA concentrations are associated with more mature sleep and wakefulness patterns. The authors pointed out that less time spent in active sleep and more in quiet sleep, as observed in the infants exposed to greater levels of DHA, suggest greater CNS maturity.

Cheruku SR, Montgomery-Downs HE, Farkas SL, Thoman EB, Lammi-Keefe CJ. Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. *Am J Clin Nutr* 2002;76:608-613.

Flaxseed Oil Does Not Increase DHA in Human Milk

Pregnant and nursing women have few food choices rich in long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), especially docosahexaenoic acid (DHA), from which to obtain adequate amounts for themselves and their infants. The challenge is even greater for strict vegetarians who must rely on DHA mainly from the conversion of alpha-linolenic acid (ALA), which is found in some plants.

Humans perform this conversion rather poorly. The question arises, will foods rich in ALA, such as flaxseed oil, provide enough DHA in pregnancy and lactation? The fetus' and infant's need for DHA in early brain development makes this question highly important.

This study examined the effectiveness of consuming flaxseed oil, rich in ALA, to provide DHA in breast milk. Seven lactating women consumed 10.7 grams/day ALA from flaxseed oil for four weeks. At the end of four weeks, DHA in breast milk, plasma, and red blood cells remained the same. ALA and two other n-3 LC-PUFAs in breast milk, plasma, and red blood cells increased significantly after the supplementation and returned to baseline by four weeks after supplementation. Flaxseed oil did not increase DHA in breast milk.

Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr* 2003;77:226-233.

Pregnant Canadian Women Short on LC-PUFAs

Because trends in long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) intake by pregnant Canadian women are not known, investigators estimated the dietary intake of LC-PUFAs and their precursors in 55 pregnant women in British Columbia, Canada. Fatty acids in plasma phospholipids were measured to determine the relationship with dietary intake. Average daily intake of the LC-PUFAs eicosapentaenoic acid, docosahexaenoic acid (DHA), and arachidonic acid were 78 ± 2, 160 ± 20, and 121 ± 8 mg/day, respectively. The authors concluded that intake of DHA may be below possible needs for fetal and maternal tissue DHA accretion in some Canadian women.

Innis SM, Elias SL. Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. *Am J Clin Nutr* 2003;77:473-478.

Cuban Mothers and Infants Well Nourished in LC-PUFAs

In 1998, samples of blood and breast milk were obtained from a cross-sectional sample of 56 mothers and term infants in Havana, Cuba, two months post partum. Values for breast milk long-chain polyunsaturated fatty acids (LC-PUFAs), docosahexaenoic acid (DHA) and arachidonic acid (AA), were higher than those found in the United States, Australia, and many developed countries. Essential fatty acid deficiency was exceedingly rare, if present at all. Similarly, red blood cell DHA levels exceeded published values for samples of U.S. women. It is of interest that linoleic acid, AA and total n-6FA levels were higher than in U.S. women, but the ratio of n-6FA:n-3FA was lower than for U.S. women (3.51 vs. 4.3). Cuban women received a weekly ration of one pound of high-fat fish and thus had a rich source of LC n-3PUFAs. Measures of visual acuity in Cuban infants were within normal values.

Krasevec JM, Jones J, Cabrera-Hernandez A, Mayer DL, Connor WE. Maternal and infant essential fatty acid status in Havana, Cuba. *Am J Clin Nutr* 2002;76:834-844.

Exposure to PCBs Linked to Lower Maternal Arachidonic Acid

Women in the Faroe Islands have high fish consumption and exposure to polychlorinated biphenyls (PCBs). This study examined the relationship between PCB exposure and long-chain polyunsaturated fatty acids (LC-PUFA) status in pregnant women at the time of birth. Maternal and cord serum phospholipid levels of the n-3 LC-PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were similar but arachidonic acid (AA) concentration in cord blood greatly exceed that in maternal blood (16.5% vs 9.14%). Maternal PCB levels were positively associated with EPA and DHA concentrations, but negatively related to AA level. At least one PCB congener (a particular form of PCB) is known to decrease AA concentration in rat liver and to inhibit enzymes necessary for the conversion of linoleic acid to AA. It is therefore possible that PCB accumulation in maternal tissue may hinder the synthesis of AA during pregnancy.

Grandjean P, Weihe P. Arachidonic acid status during pregnancy is associated with polychlorinated biphenyl exposure. *Am J Clin Nutr* 2003;77:715-719.

DHA and AA Status at Birth Unrelated to Cognitive Performance at Four

The availability at birth of the essential fatty acids, docosahexaenoic acid (DHA) and arachidonic acid (AA) – fatty acids essential for optimal brain growth and development – has been related to cognitive development. Short term studies have shown an advantage to higher levels of DHA, but long term data are limited and inconsistent. These investigators studied the relationship of AA and DHA status at birth in 128 healthy term infants with cognitive development assessed at four years. Researchers found no significant correlations between cognitive scores and plasma or red blood cell phospholipid AA and DHA. These findings provide no evidence to support a relationship between AA and DHA status at birth and cognitive performance four years later.

Ghys A, Bakker E, Hornstra G, van den Hout M. Red blood cell and plasma phospholipid arachidonic and docosahexaenoic acid levels at birth and cognitive development at 4 years of age. *Early Human Development* 2002;69:83-90.

Clinical Conditions: Type 2 Diabetes Mellitus

Seal Meat Reduces Type 2 Diabetes Risk in Greenland Inuit

The relentless worldwide increase in the number of people with type 2 diabetes has created a pressing international health concern. In the United States, nine of every 100 people over the age of 20 have the disease or its early warning signs, which accounts for some 17 million people. Prevalence, the number of people are already afflicted, has increased 2.3-3.3 times in 25 years in the United States, 2.0-2.8-fold over 10 years in England, and 3.9 times over 18 years in Egypt. There are no signs that its development is abating. Ways of halting this health epidemic are urgently needed.

Epidemiological evidence has documented a lower prevalence of Type 2 diabetes and impaired glucose tolerance, a harbinger of the disease, in populations consuming large amounts of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), such as Greenland and Alaskan Inuit. However, incidence – the number of new cases – in these groups is increasing rapidly. This change has been linked to changes in diet and decreased consumption of traditional foods, especially marine animals.

In this cross-sectional study, Jørgensen and colleagues from the Steno Diabetes Center in Denmark, measured 2-hour glucose tolerance in 917 adult Inuit in three areas of Greenland to assess the prevalence of diabetes. Seventy percent of subjects with diabetes had not previously been diagnosed. Prevalence of type 2 diabetes was high at 9.7%; prevalence of impaired glucose tolerance, an early sign of type 2 diabetes, was 12.2%. Risk of diabetes was inversely related to frequent consumption of seal meat and fresh fruit and positively associated with family history, age, body mass index, and high alcohol consumption. Seal meat and fat are rich in n-3 LC-PUFAs. Interestingly, the study found no effect of dietary intake of other sea mammals or fish on diabetes or glucose tolerance. This latter finding contrasts with observations in Alaskan Inuit in which lower levels of some n-3 LC-PUFAs were found in subjects with abnormal glucose intolerance. Long term prospective studies are needed to clarify this issue.

Jørgensen ME, Bjeregaard P, Borch-Johnsen K, Backer V, Becker U, Jørgensen T, Mulvad G. Diabetes and impaired glucose tolerance among the Inuit population of Greenland. *Diabetes Care* 2002;25:1766-1771.

EPA or DHA Improves Lipids and Impairs Glucose in Some Type 2 Diabetics

Long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) improve blood lipids in subjects with type 2 diabetes. There has been some concern, however, about the possible adverse effects of fish oil consumption on glucose control in diabetes. Two recently published meta-analyses of 18 or more studies on the consumption of n-3 LC-PUFAs

in diabetic subjects concluded that there were no significant effects on glucose control. Doubts continue to linger.

It is not known whether individual n-3 LC-PUFAs would have the same effect on lipid, glucose and insulin responses in type 2 diabetics as the combination of several n-3 LC-PUFAs present in fish oil. To find out, Woodman and colleagues at the University of Western Australia tested the effects of purified eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on glycemic control, blood pressure, and serum lipids in 51 moderately obese, type 2 diabetic subjects, who were also being treated for high blood pressure. Subjects were randomly assigned to consume four grams a day of EPA, DHA or olive oil for six weeks.

Serum Lipids and Glucose in Moderately Obese Type 2 Diabetics Before and After 4 Grams/Day or DHA Supplementation for 6 Weeks

SERUM LIPIDS OR GLUCOSE	OLIVE OIL	EPA	DHA	p
Tryglycerides (mmol/L)				
Baseline	1.74	1.34	1.62	
After intervention	1.68	1.11	1.35	<0.05
Total Cholesterol (mmol/L)				
Baseline	4.57	4.48	4.49	
After intervention	4.61	4.42	4.55	NS
LDL Cholesterol (mmol/L)				
Baseline	2.71	2.66	2.74	
After intervention	2.77	2.68	2.88	NS
HDL Cholesterol (mmol/L)				
Baseline	1.06	1.21	0.99	
After intervention	1.07	1.22	1.03	NS
HDL_2 Cholesterol (mmol/L)				
Baseline	0.38	0.49	0.32	
After intervention	0.38	0.56	0.38	<0.05
HDL_3 Cholesterol (mmol/L)				
Baseline	0.68	0.73	0.67	
After intervention	0.69	0.66	0.65	<0.05
Fasting Glucose (mmol/L)				
Baseline	7.96	7.46	8.25	
After intervention	7.55	8.49	8.80	<0.002
Fasting Insulin (mmol/L)				
Baseline	14.57	14.16	16.54	
After intervention	13.69	13.84	15.98	NS

As expected, both EPA and DHA reduced plasma triglycerides but had no significant effect on total HDL or LDL levels (table). DHA or EPA consumption increased HDL_2 significantly and EPA intake decreased HDL_3 significantly. Fasting glucose was significantly increased by DHA and EPA consumption, but there were no changes in insulin sensitivity or secretion after the treatment. Neither fatty acid affected blood pressure, heart rate, or total cholesterol levels.

These results showed that purified EPA and DHA, when consumed at the level of four grams a day for six weeks, are associated with mildly impaired glucose tolerance. It should be noted, however, that this dose is substantially greater than what would be consumed with regular fatty fish meals, perhaps as much as four times greater. These findings also indicate that purified fatty acids have no advantage over fish oils or fatty fish as sources of n-3 LC-PUFAs. This might be an important consideration in patient management of type 2 diabetes.

Woodman RJ, Mori TA, Burke V, Puddey IB, Watts GF, Beilin LJ. Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *Am J Clin Nutr* 2002;76:1007-1015.

Fish Oil Partly Corrects Lipid Profiles in Type 2 Diabetics

Diets in the United States and other western countries are characterized by high intakes of omega-6 fatty acids and low consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), particularly the long chain omega-3 type. The relative imbalance created by these large differences in types of dietary polyunsaturated fatty acids has been associated with the development of atherosclerosis and may affect some types of lipid disorders, including the dyslipidemia characteristic of type 2 diabetes.

Subjects with type 2 diabetes have elevated triglyceride (TG) levels, lower high density lipoprotein (HDL) and smaller low density lipoprotein particles compared with nondiabetic subjects. These alterations in lipid profiles are linked to the greatly increased risk of cardiovascular disease seen in diabetic subjects.

The consumption of fish oil or purified long n-3 LC-PUFAs consistently reduces plasma triglyceride levels and may increase HDL in healthy and diabetic subjects. The effect of consuming n-3 LC-PUFAs on lipoprotein size has been inconsistent.

In this research, Petersen and colleagues from the Royal Veterinary and Agricultural University in Denmark compared the effect of dietary supplementation with four grams a day of fish oil or corn oil in 42 type 2 diabetic subjects. Participants consumed the supplements for eight weeks and plasma lipoproteins were measured before and after supplementation. As expected, fish oil reduced plasma TG concentrations. Two of three types of HDL subfractions, HDL_{2a} and HDL_{2b}, were significantly increased with fish oil supplementation. There was no change in any lipoproteins in corn oil subjects. LDL cholesterol and the concentration of small dense LDL particles were not significantly changed in comparison with the corn oil supplemented subjects.

The consumption of fish oil partly corrected the disordered lipid profile in these diabetic subjects by reducing TG levels and increasing HDL subfractions. Fish oil had no effect on the atherogenic LDL level and particle size. The consumption of n-3 LC-PUFAs found in fatty fish has potential benefit in type 2 diabetes where cardiovascular disease is the leading cause of mortality.

Petersen M, Pedersen H, Major-Pedersen A, Jensen T, Marckmann P. Effect of fish oil versus corn oil supplementation on LDL and HDL subclasses in type 2 diabetic patients. *Diabetes Care* 2002;25:1704-1708.

Clinical Conditions: Cancer

No Omega-3 Link with Breast Cancer in Post-Menopausal Dutch Women

Cross-cultural population studies have suggested that type of dietary fat is related to incidence of breast cancer. The Inuit, Japanese, and some Asian populations have much lower rates of breast cancer than those observed in the United States and several European countries. In contrast, data from within one country have generally not supported such an association. Studies conducted in single countries or among countries with similar dietary habits seldom have the wide range or diversity of fat intakes such as one sees in comparisons among different countries.

This prospective cohort study, begun in 1986, studied 62, 573 women aged 55 to 69 yr for 6.3 years. Food intake was assessed using a 150-item food frequency questionnaire. Fat and fatty acid intakes were grouped according to quintiles of intake and relative risks were calculated for the consumption of various types of fat, including specific fatty acids.

A significant protective trend for the consumption of alpha-linolenic acid, a short-chain omega-3 fatty acid, was observed in multivariate analysis, but eicosapentaenoic, docosahexaenoic, linoleic or arachidonic acids were not associated with risk.

Voorrips LE, Brants HAM, Kardinaal AFM, Hiddink GJ, van den Brandt PA, Goldbohm RA. Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. *Am J Clin Nutr* 2002;76:873-882.

Fish Fatty Acids Lower Risk of Advanced Prostate Cancer

Several lines of evidence have linked long-chain omega-3 fatty acids (n-3 LC-PUFAs) with reduced likelihood of prostate cancer. For example, studies in cultured cells and laboratory animals have demonstrated a protective effect of eicosapentaenoic acid and docosahexaenoic acid on prostate cancer. Men with benign prostate hyperplasia, a forerunner of prostate cancer, have lower levels of n-3 LC-PUFAs in their prostate tissue, serum, and red blood cell membranes. Prostate tumors also have a high level of enzyme activity that increases the production of prostaglandins and thromboxanes, substances that promote inflammation and other responses. The activity of this enzyme is reduced by n-3 LC-PUFAs. As in breast cancer, results from epidemiological studies have been inconsistent.

This study reports the findings on fish consumption and prostate cancer in a cohort of 47,882 men in the Health Professionals Follow-up Study, initiated in the United States in 1986. Fish intake was assessed by a food frequency questionnaire upon entry in 1986, and repeated in 1990 and 1994. During 12 years of followup, 2482 cases of prostate cancer were diagnosed, including 617 with advanced disease and 278 with metastatic cancer, the most advanced stage of the disease.

Risk of prostate cancer was significantly reduced in subjects who consumed fish more than three times a week compared with men who ate fish less than twice a month (multivariate relative risk, 0.56, a 44% lower risk). The association was strongest for men with metastatic disease. Each daily half-gram increase of "marine fatty acid from food" was associated with a 24% lower risk of metastatic disease. The authors noted that marine fatty acids might account for part of the effect, but that other factors in fish may be involved. We have not heard the last word on prostate cancer and n-3 LC-PUFAs.

Augustsson K, Michaud DS, Rimm EB, Leitzmann MF, Stampfer MJ, Willett WC, Giovannucci E. A prospective study of intake of fish and marine fatty acids and prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2003;12:64-67.

Epidemiological Studies Seldom Link Fish Consumption with Hormone-Related Cancers

The evidence relating lower risk of breast or prostate cancer with diets rich in long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) is, at best, inconsistent. Studies in cultured cancer cells have been more encouraging. Omega-3 LC-PUFAs usually inhibit the proliferation of cultured cancer cells. Other metabolic activities associated with the development or progress of cancer cells have also been inhibited by these fatty acids. Why are epidemiological and laboratory observations so often conflicting?

This review collected and evaluated the epidemiologic evidence relating fish consumption and hormone-dependent cancers from cohort and case-control studies conducted within populations. The authors discussed the limitations of these types of studies that may contribute to the inconsistency of results. These include dietary variable—fish, type of fish, seafood, n-3 LC-PUFAs, range of consumption etc.—as well as large differences in study design, sample size, and duration of followup.

Inverse associations between fish or marine fatty acid consumption and breast or prostate cancer risk have mostly been observed in populations where fish consumption is much greater than in the United States. However, as this review showed, most epidemiologic studies do not report an inverse association between fish or marine fatty acid consumption and risk of hormone-related cancers.

Terry PD, Rohan TE, Wolk A. Fish consumption seldom linked to hormone-related cancers in epidemiological studies. *Am J Clin Nutr* 2003;77:532-543.

Clinical Conditions: Mental Health

No Link Between Dietary Fat and Dementia

How much and which types of fat we consume have been linked to many diseases, notably cardiovascular disease. Studies in animals and human subjects have associated the intake of different types of fat to various mental illnesses. In particular, consumption of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) has been associated with improvements in depression, bipolar disorder, and other conditions. Are they involved in the development of dementia in the elderly? The Rotterdam Study indicated no.

Designed as a prospective cohort study, the Rotterdam Study examined risk factors for several diseases in the elderly. It began in the early 1990s and included 5,395 noninstitutionalized subjects aged 55 or older. Engelhart and colleagues at the Erasmus Medical Center, The Netherlands, studied the relationship between types and amounts of dietary fat consumed, including LC-PUFAs, and the risk of dementia and its subtypes. Subjects were continuously monitored for incident dementia and dietary assessments were performed every three years. Such careful monitoring is necessary to detect cases of illness as soon as possible.

After an average followup of six years there were 197 cases of dementia, 146 of which were Alzheimer's Disease. High intake of total fat, saturated fat, trans fat and cholesterol was unrelated to the development of any type of dementia. Neither low intake of monounsaturated, polyunsaturated, omega-6 LC-PUFAs, nor omega-3 LC-PUFAs were related to increased risk of dementia, Alzheimer's Disease, or vascular dementia.

In discussing their findings, the authors mentioned various factors that may have introduced bias into the study. They contrasted the present results with previously published observations from the same population, which had documented increased risk of dementia with high total fat and low fish consumption after two years' followup. At that time, the number of cases was considerably smaller, which, the authors say, would have affected the statistics and contributed to greater bias. Because a few other studies, including a randomized controlled trial, reported improved memory with PUFA supplementation, the authors believe it is too soon to take dietary fat off the "watch" list.

Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JCM, Breteler MMB. Diet and risk of dementia: Does fat matter? The Rotterdam Study. *Neurology* 2002;59:1915-1921.

Low Dose EPA Benefits Depressed Patients

Depression is an illness for which current treatments have only limited efficacy. The number of people affected, is difficult to estimate because many with the affliction do not seek treatment. In western countries, the incidence has increased steadily since World War II, but in Asia its prevalence is low. For example, the prevalence in the United States is estimated at 6%; France, 16%; and Taiwan, less than 2%. Diets in these countries differ substantially, as do other factors. Dietary fat and long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs), are potential candidates in the development and treatment of the condition. Recently, n-3 LC-PUFAs were associated with significant clinical improvements in depressed subjects. It was also reported that depressed patients have lower eicosapentaenoic acid (EPA) levels than those without depression.

Scientists from the United Kingdom tested the efficacy of adding different amounts of EPA to the existing treatment of 70 depressed patients. Subjects were randomly assigned to receive one, two, or four grams a day of purified EPA or placebo for 12 weeks. Depression was assessed before and after supplementation using three different rating scales.

Significant improvement in depression was observed in all three rating scales in the subjects consuming one gram a day of EPA. Contrary to what might have been expected, significant benefits were not found in any other treatment groups. No biochemical or other clinical data were reported that might

provide clues to this observation. The authors concluded that a modest dose of one gram of EPA a day was effective in the treatment of depressed patients. Larger clinical trials are needed to confirm these results. Comparison with docosapentaenoic acid singly and combined with EPA would also be worthwhile.

Peet M, Horrobin D. A dose-ranging study of the effects of ethyl-eicosapentaenoate in patients with ongoing depression despite apparently adequate treatment with standard drugs. *Arch Gen Psychiatry* 2002;59:913-919.

LC-PUFA Intake and Status

Biomarkers for LC-PUFAs: Adipose Tissue and Skeletal Muscle

To study the effect of habitual or usual consumption of different foods or nutrients one needs reliable measures of long term consumption. Subjects' memories tend to be unreliable, interviews biased and too general, and commonly available tissues such as blood and urine may reflect only recent intake. These tissues can also be affected by other events, such as exercise, smoking, and disease.

The possibility of examining tissue membranes, where fatty acids can be deposited, is another way to assess customary fatty acid intake. The membranes of red blood cells, for example, have served in the study of fatty acid availability in pregnancy. Adipose tissue, an abundant source of fatty acids, is appealing because of its slow turnover and relative resistance to acute disease. It may be particularly suitable for fatty acids that come mainly from the diet and are not made from carbohydrates. For these reasons, adipose tissue content of long-chain polyunsaturated fatty acids (LC-PUFAs) and certain other kinds of fatty acids may be a good biomarker or mirror of long-term dietary intake.

Baylin and colleagues from the Harvard School of Public Health, USA, and the Universidad de Costa Rica, used a validated food frequency questionnaire to determine dietary intake in a sample of 503 healthy middle-aged subjects. They measured fatty acids in samples of adipose tissue taken from the upper buttock.

Adipose tissue content of linoleic and alpha-linolenic acids were highly correlated with the dietary consumption of these fatty acids. Correlation coefficients, a measure of the strength of the association, were 0.58 and 0.34, respectively. In contrast, adipose tissue level of arachidonic acid was not highly correlated with dietary intake. Previously published studies have indicated that arachidonic acid is not stored to a large extent in adipose tissue and tissue levels are not responsive to increases in dietary consumption.

Fish intake was moderately related to adipose tissue omega-3 (n-3) LC-PUFAs. Docosahexaenoic acid (DHA) was directly related to fish intake up to about 100 grams of fish/ week. The correlation coefficient for DHA in adipose tissue was 0.18. The authors concluded that adipose tissue DHA content could be a suitable marker for fish consumption. It was noted, however, that subjects in this study had relatively low fish consumption, an average of 14.5 grams a day. The suitability of adipose tissue as a measure of n-3 LC-PUFAs remains to be investigated in people who eat substantial quantities of fish.

Skeletal muscle phospholipids may also be suitable markers of dietary LC-PUFA intake. There is evidence that skeletal membrane fatty acid composition responds to dietary change in human subjects and animals. Some evidence in infants suggests that skeletal muscle phospholipid is more responsive to dietary LC-PUFAs than red blood cells. In a recent study of this question by Andersson and colleagues at Uppsala University, Sweden, healthy men and women were fed diets high in saturated or monounsaturated fatty acids. Subgroups from each dietary group were given fish oil capsules or placebo for three months and samples of quadriceps muscle taken at the end of the diet period.

These investigators found that consuming 3.6 grams of n-3 LC-PUFAs for three months increased the proportion of n-3 LC-PUFAs in skeletal muscle phospholipids by two and a half times in both

dietary groups. Muscle DHA content was doubled and eicosapentaenoic acid (EPA) content increased five-fold in response to n-3 LC-PUFA supplementation. In skeletal muscle triglycerides, DHA content doubled, but EPA was not detected. Thus, phospholipids are a better place to measure n-3 LC-PUFAs in skeletal muscle. The fish oil supplement significantly reduced the level of arachidonic acid in muscle phospholipids. When the ratio of omega-6 to omega-3 PUFAs was compared after fish oil supplementation, the ratio was reduced to a third of the value in the unsupplemented groups.

This study clearly demonstrated the responsiveness of skeletal muscle lipids to differences in dietary fat composition. Skeletal muscle readily incorporates n-3 LC-PUFAs into phospholipid. Although the study did not measure the relation between dietary fatty acid intake and muscle fatty acid composition, it showed clearly that muscle is an important reservoir for n-3 LC-PUFAs and its membrane fatty acid composition responds to diet similarly to the membranes in other tissues such as red blood cells.

Baylin A, Kabagambe EK, Siles X, Campos H. Adipose tissue biomarkers of fatty acid intake. *Am J Clin Nutr* 2002;76:750-757.

Norwegian Teens Shun Fish Consumption

Like teenagers the world over, Norwegian adolescents living in coastal regions are abandoning the dietary traditions of their parents in favor of more sugar and fat-rich foods, sedentary lifestyles, and smoking. As this study documents, these 13- and 14-year-olds, with high blood cholesterol levels, had little intake of marine fish or omega-3 fatty acids. Inland teens consumed fish rarely if at all. Ten to 20% of students reported smoking daily, 8-13% rarely or never exercised, and 30% had no risk factors protective of heart disease. Can we motivate the next generation to change such habits?

Brox J, Bjørnstad E, Olaussen K, Østerud B, Almdahl S, Løchen ML. Blood lipids, fatty acids, diet and lifestyle parameters in adolescents from a region in northern Norway with a high mortality from coronary heart disease. *Eur J Clin Nutr* 2002; 56:694-700.

Fatty Acid Profiles Related to Age in Japanese Women

In Japan, another country with plenty of coastline, women of all ages consume seafood, as reflected in their plasma fatty acid levels. Kuriki and colleagues determined dietary intakes and plasma fatty acids in 79 female dietitians 32 to 66 years old. Total, saturated, monounsaturated and long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) intakes were highest in those aged 43-50 years and lowest in the eldest group. There were no differences in age groups for n-3 LC-PUFA intakes, suggesting that seafood is consumed by women of all ages. Plasma fatty acid concentrations for all fatty acids except arachidonic acid were correlated with age. Eicosapentaenoic acid, but not docosahexaenoic acid, was correlated with dietary intake in all age groups. It would be interesting to know whether these observations would be confirmed in a larger sample.

Kuriki K, Nagaya T, Imaeda N, Tokudome Y, Fujiwara N, Sato J, Ikeda M, Maki S, Tokudome S. Discrepancies in dietary intakes and plasma concentrations of fatty acids according to age among Japanese female dietitians. *Eur J Clin Nutr* 2002;56:524-531.

Recommendations for LC-PUFA Consumption

American Heart Association Recommends Eating Fatty Fish Twice Weekly

In an update of its science advisory statement on fish consumption and heart disease, first issued in 1996, the American Heart Association (AHA) unequivocally said that data support its dietary recommendation to "include at least two servings of fish per week (particularly fatty fish)." The data also support the inclusion of vegetable oils such as soybean, canola, walnut, and flaxseed, and foods high in alpha-linolenic acid (ALA), such as walnuts and flaxseeds.

The AHA science advisory statement reviewed epidemiological and clinical studies linking fish consumption or the intake of long-chain omega-

3 polyunsaturated fatty acids (n-3 LC-PUFAs) to the risk of heart disease. Although most studies have reported lower cardiac mortality and reduced chance of sudden death, particularly among survivors of a myocardial infarction, a few have failed to find a benefit associated with fish consumption. Some have noted a cardioprotective effect with fatty fish, but not with lean or fried fish.

Data relating fish consumption to the risk of stroke are less abundant. Nevertheless, current evidence indicates that small intakes of fish are associated with a lower risk of stroke, particularly ischemic stroke. Very high intakes of n-3 LC-PUFAs, such as those of native Arctic populations, appear to increase the chance of hemorrhagic stroke. The majority of strokes in countries with Western dietary habits are ischemic.

The AHA authors also examined the data linking ALA with reduced cardiac mortality. Although there are fewer of these studies, the authors concluded that the evidence favors a beneficial effect of ALA on coronary heart disease and noted that this topic warrants further clarification. The benefits from ALA do not appear to be as wide-ranging as for n-3 LC-PUFAs.

In addition to summarizing the various mechanisms through which n-3 LC-PUFAs appear to exert their beneficial effects, the AHA statement addressed the safety and efficacy of fish and n-3 LC-PUFA consumption. Current intake of n-3 LC-PUFAs in the United States from both fish and plant foods is among the world's lowest, approximately 1.6 grams/day. ALA accounts for nearly all of it. Americans consume four times as much beef and three times as much chicken as fish and shellfish. Although all fish contain some n-3 LC-PUFAs, fatty fish such as salmon, rainbow trout, mackerel, and sardines are the richest food sources. Thus, the AHA dietary recommendations suggest eating "at least two servings of fish a week" and emphasized fatty fish.

The AHA statement discussed the contribution of fish oil supplements to boosting n-3 LC-PUFA consumption. It suggested that heart disease patients increase their intake of n-3 LC-PUFAs to about one gram/day. This amount is close to the level used in the GISSI-Prevention Study, the largest secondary prevention trial to show a reduction in cardiac mortality in people with heart disease. For some people, consuming one gram of n-3 LC-PUFAs/day may be difficult to achieve solely from foods; they may need to rely on fish oil supplements. Fish oil capsules vary in concentration, so one must read the label carefully. The AHA statement noted that the U.S. Food and Drug Administration (FDA) considers the consumption of up to three grams of n-3 LC-PUFAs a day to be safe for most people.

A potential drawback to increased fish consumption is methylmercury contamination in some species. The AHA statement noted that some fish species posed a greater risk than others and that the risk was greatest for pregnant women. The paper cited the FDA guidelines, noting the species with higher methylmercury content. Currently, the FDA advises pregnant women to avoid eating swordfish, shark, king mackerel, and tilefish, but says it is safe for others to consume up to 7 ounces (about 200 grams) a week of these species. For middle-aged and older men and postmenopausal women, Kris-Etherton and co-authors wrote, "the benefits of fish consumption far outweigh the risks within the guidelines established by the FDA and Environmental Protection Agency." Consuming fish known to have the lowest mercury levels and eating a variety of species is the best approach to increase fish consumption safely. How about salmon for supper tonight?

Kris-Etherton P, Harris WS, Appel LJ, AHA Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids and cardiovascular disease. *Circulation* 2002;106:2747-2757.