PUFA NEWSLETTER

June 2005 Contents

Editorial

Pushing Back the Frontiers

Robust would be an understatement for research activity on polyunsaturated fatty acids (PUFAs). As this quarter's *PUFA Newsletter* describes, recently published articles confirm the positive effects of long-chain omega-3 PUFAs (n-3 LC-PUFAs) in reducing atrial fibrillation, improving heart rate variability, shifting lipoprotein profiles in children at high risk of heart disease, and improving neurodevelopment scores in premature infants.

As understanding grows, it is time to redraft our views on "essentiality," argues Stephen Cunnane of the University of Sherbrooke, Canada. He proposes scrapping the idea of essential and nonessential fatty acids in favor of dispensable and conditionally indispensable fatty acids, analogous to the framework now applied to amino acids. Write and tell us what you think.

Two reports inside add to the clinical relevance of n-3 LC-PUFAs. Data from the long-running Nurses' Study in the U.S. document an association between reduced risk of cataract with increased intake of n-3 LC-PUFAs in women over 45 years of age. However, a snapshot of data from the same study presents a fuzzier picture about this association. And alpha-linolenic acid found itself on the wrong side of the risk-benefit balance in the cataract study, but not in a careful study of fatty acids in different stages of prostate cancer, where its possible involvement has been highly controversial. What is it about this fatty acid? On the other hand, lower concentrations of n-3 LC-PUFAs were observed in advanced prostate cancer, but not organ-confined disease.

New studies from Tomohito Hamazaki's research group, linking n-3 LC-PUFAs to different behaviors in different population groups, support the view that these fatty acids affect behavior in widely varying circumstances, but measuring these effects can be tricky. At the clinical end of the spectrum, this newsletter briefly describes two papers relating to n-3 LC-PUFAs in psychiatry.

Other research advances in plant biotechnology and resolvins await coverage in the next issue. Fatty acids have come of age but no one would call the field mature just yet.

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Essential Fatty Acids: Time for a New Paradigm?

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Ambiguity in the use and meaning of the term essential fatty acid suggests that this term may now be obsolete. A new paradigm is proposed that recognizes the *conditional nature* of the dietary requirements for certain polyunsaturated fatty acids (PUFA) in humans.

Essential Fatty Acids: A Long Checkered History

Between 1930 and 1950, linoleate, alpha-linolenate (ALA), and arachidonate (AA) were named essential fatty acids because each was more or less able to meet the growth requirements of rats given fat-free diets. In the 1960s, it was found that rats could make AA from linoleate, so AA was no longer classified as an essential fatty acid. ALA was almost as good as linoleate or AA in supporting the growth of rats given fat-free diets but, paradoxically, diets deficient in? omega-3 (??n-3) PUFAs induced minimal symptoms of n-3 PUFA deficiency. Consequently, interest in n-3 PUFA declined and ALA gradually lost status as an essential fatty acid. Interest in the developmental importance of n-3 PUFA reemerged in the 1980s when docosahexaenoic acid (DHA), but not ALA, prevented the symptoms of n-3 PUFA deficiency in human infants.

Dietary "essential fatty acid deficiency" remains the principal approach to determining the type and amount of n-6 PUFA needed in the diet. Essential fatty acid deficiency occurs with the provision of either a fat-free diet or one containing saturated but no unsaturated fatty acids or PUFAs. The flaw in this model is that essential fatty acid deficiency is not nutrient specific: it is neither n-6 or n-3 PUFA deficiency, but a *combined* deficiency of both types of PUFAs. There are two reasons why essential fatty acid deficiency is an inappropriate model for establishing n-6 PUFA requirements: 1) by definition, an n-6 PUFA-deficient diet should be complete in all other nutrients known to be essential, including n-3 PUFA, 2) it takes much less linoleate to correct essential fatty acid deficiency when ALA is present in the diet than when it is not[1-3]. With ALA in the diet, the linoleate requirement in the rat is about 0.15 % of energy intake, or <10% of what is commonly believed. Thus, conceptually and practically, essential fatty acid deficiency is inadequate for modeling n-6 PUFA deficiency and requirements. Unfortunately, this also means that the true requirement for n-6 PUFA in all species including humans is still not known and has probably been overestimated.

Is Linoleic Acid Conditionally Dispensable?

It is perhaps not surprising that the flawed basis for n-6 PUFA requirements coincides with rising concern that excessive linoleate intake may be contributing to degenerative disease risk in individuals already consuming low amounts of n-3 PUFA[4-9]. If, as appears to be the case in rats, the linoleate requirement in healthy adults is under 0.5% energy, a dietary source of linoleate is probably unnecessary for weeks if not months. In this case, it seems reasonable to ask whether there are conditions in which linoleate could be "conditionally dispensable." This idea may seem radical, but it agrees with the old observation that healthy adults given fat-free linoleate-deficient diets had no reproducible symptoms of n-6 PUFA deficiency over several months. This is because healthy adults typically have linoleate stores averaging 1,000 g or more and are, therefore, not easily depleted of linoleate to the point of exhibiting symptoms of linoleate deficiency.

Is Docosahexaenoic Acid Conditionally Indispensable?

Without a dietary source of DHA it is unlikely (though not impossible) that visual and neurological development will be optimal during the first few years of life. Thus, despite the known ability of infants to convert ALA to DHA, the capacity of this pathway is commonly, if not always, insufficient to meet the needs for DHA. This results in low brain DHA and neurodevelopmental symptoms in infants not given dietary DHA. The need for DHA may or may not exist in healthy adults, but it is clearly "conditionally indispensable" in infants. A crucial point is that the ability to make some DHA does not prevent a need to provide DHA in the diet, because the pathway to make DHA has insufficient capacity to meet the developmental needs for DHA in humans.

A Precedent: Indispensable and Conditionally Indispensable Amino Acids

The two examples of linoleate and DHA suggest that several problems are connected to the term *essential fatty acid*: Are only the parent PUFAs (linoleate and ALA) needed in the diet or is there a *conditional* human requirement for

some long chain (LC)-PUFA, i.e. during infant development? Could linoleate or ALA actually be dispensable some of the time, either because one or more LC-PUFAs are more important or, in the case of linoleate, because healthy adults have abundant stores and minimal deficiency symptoms? Is there a dietary requirement for any of the PUFA intermediates, i.e., gamma-linolenate, dihomo-gamma-linolenate or eicosapentaenoate (EPA)?

A nutrient requirement has two components: 1) a key function that becomes reproducibly *dysfunctional* with dietary insufficiency of the nutrient, which can be avoided or corrected when the nutrient is consumed and 2) *insufficient capacity* to synthesize or store the nutrient under certain conditions, i.e. at one or more periods in the life cycle or perhaps in certain diseases. Since PUFAs exist as a series of precursor and product molecules, flexibility in defining their dietary essentiality according to different physiological or pathological conditions is both logical and necessary. This flexibility has a precedent with other organic nutrients, notably the indispensable and conditionally indispensable amino acids.

Over 20 years ago, essential and non-essential amino acids were officially reclassified as *indispensable* or *conditionally indispensable* amino acids, precisely to overcome the problem of excessively rigid terminology. The "essential/non-essential" terminology was unable to accommodate the clinical observations of a conditional need in infants for tyrosine and cysteine, both of which are "non-essential" (dispensable) in healthy adults. Tyrosine and cysteine are now recognized as dietary nutrients required during human infancy because infants have *insufficient capacity* to synthesize them from their respective precursor amino acids phenylalanine and methionine. Recognizing the conditional need for tyrosine and cysteine was a crucial step in better understanding the role of certain amino acids in human nutrition.

Conditionally Dispensable and Conditionally Indispensable Fatty Acids

"Conditional requirement" terminology is also applicable to PUFAs, but it has four caveats: 1) Because the possible nutritional importance of intermediate PUFA such as gamma-linolenic acid or dihomo-gamma-linolenic acid is presently inadequately known, this paradigm would be restricted to the five better-known PUFAs – linoleate, AA, ALA, EPA, and DHA; 2) The healthy human is either an "adult" (male or

non-pregnant and non-lactating female), or is in a "developmental state," i.e., pregnancy, lactation, or infancy through to adolescence; 3) Conditional dispensability applies to healthy humans and means that the requirement is low enough that *sporadic* intake as opposed to frequent or continuous intake is sufficient; 4) The paradigm could be modified under conditions that change PUFA requirements, e.g., development, aging, or specific disease states.

The following classification modifies a previous proposal[10]: a) There is inadequate evidence that any single PUFA is absolutely indispensable through the lifespan; hence, all PUFA are conditionally dispensable or conditionally indispensable; b) linoleate, AA, ALA and DHA are all conditionally indispensable in the developmental state; c) linoleate and AA are conditionally dispensable in adults; d) EPA is conditionally dispensable in the developmental state; (e) Any two of ALA, EPA, or DHA are conditionally dispensable in adults. The amounts of n-3 PUFAs that fulfill this proposal are consistent with ISSFAL recommendations (www.issfal.org.uk/PUFA%20intakes.htm). Distinguishing between conditional dispensability of linoleate and AA, but indispensability of ALA, EPA, or DHA in healthy adults is based on the low body reserves, greater role in health, and negligible adverse effect of n-3 PUFAs compared with n-6 PUFAs.

Like the developmental state, acute or chronic degenerative diseases are conditions for which PUFA requirements need to be evaluated separately from the healthy, adult state. For example, if a lower risk of heart attack in sedentary, middle-aged adults occurs with the consumption of n-3 PUFAs, then a higher intake of one or more n-3 PUFAs is conditionally indispensable for cardiovascular health in sedentary adults. The amount required to maintain cardiovascular health may well vary with genotype, age and other elements of lifestyle or environment.

Simply adding a "conditional" category to some essential fatty acids, i.e., DHA would be a "conditionally essential fatty acid" in infants or adults at heightened risk of heart attack is insufficient. Although this would incorporate conditionality into the dietary need for PUFAs, it would retain the implication that there are "primary" essential fatty acids that are more important and "secondary" (conditionally) essential fatty acids that are less so. Since there is no evidence that ALA can help prevent a heart attack, or reduce mortality in adults who have a robust intake of EPA or DHA (or

vice versa), there is no basis to consider ALA an essential fatty acid in healthy adults consuming sufficient EPA or DHA. Equally, healthy adults consuming adequate ALA presently have no demonstrable need for dietary EPA or DHA. Thus, "conditionally essential fatty acids" would not achieve anything in the long run. People working on the "essential amino acids" recognized this problem and avoided it by applying new terminology with no historical value implications.

The most important outcome of adopting this or an equivalent classification is provision of a framework for structuring research into specific roles of PUFAs in health and disease. This framework is lacking or nebulous if PUFAs are forced to be essential or non-essential when the physiological and metabolic importance of several long-chain-PUFAs resemble several common long-chain saturates or monounsaturates, even though, technically, all can be synthesized by humans. Developing valid dietary recommendations for specific PUFAs according to different conditions during the lifespan is also desirable, but is secondary to improving our understanding of the biology of PUFAs.
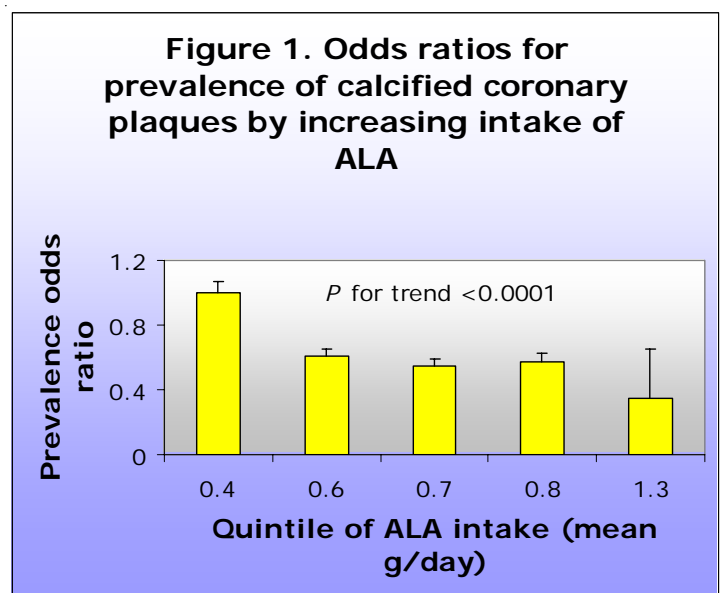
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Cardiovascular Health

Alpha-Linolenic Acid Inversely Linked to Arterial Plaque: Really?

The effectiveness of alpha-linolenic acid (ALA) in reducing the risk of heart disease and mortality continues to be controversial because the fatty acid is less potent than the long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) and its conversion to the LC forms is highly inefficient. Nevertheless, consumption of ALA has been linked to reduction of some inflammatory markers, lower serum triglycerides, and may have effects different from the n-3 LC-PUFAs. Weighing against the good news about the cardioprotective effects of ALA are some orange flags about its association with advanced prostate cancer. Others caution that the data are inconclusive.

In the study described here, Luc Djoussé and colleagues at the Boston University School of Medicine report an inverse association between ALA consumption and calcified coronary arterial plaque, a potential source of fatal heart attack. The researchers analyzed data from the Family Heart Study on families chosen at random and those known to have increased risk of coronary heart disease based on family history. The sample included 2004 white participants, 845 men and 1159 women, whose average age was 56 ± 13 years (range 32 to 93 years). ALA intake was estimated from food frequency questionnaires and calcified atherosclerotic plaque measurements from cardiac computed tomography scans.

Figure 1. Odds ratios for prevalence of calcified coronary plaques by increasing intake of ALA

P for trend <0.0001

Prevalence odds ratio — Quintile of ALA intake (mean g/day): 0.4, 0.6, 0.7, 0.8, 1.3

All images were read at a single site by trained analysts and expressed as the total score for calcified atherosclerotic plaque.

The most striking finding was the markedly reduced score (odds ratio) for the prevalence of calcified plaque with increasing intakes of ALA (Figure 1). At the highest consumption of ALA (mean 1.3 ± 0.3 g/day), the odds ratio for prevalent calcified plaque was 0.35 (CI 0.22 to 0.55), a 65% reduction compared with those having the lowest intake. The statistical model included the most adjustments for confounding variables. A significant trend for reduced plaque with increasing ALA intake was observed ($P<0.0001$).

One confounder is the sharply increased energy intake with increasing quintiles of ALA. At the lowest ALA level, participants consumed an average of 4700 ± 1200 kJ energy. At the highest ALA intake, energy consumption was more than double, $10,300 \pm 2200$ kJ. Body mass index across quintiles did not differ. How could this be? As William Harris pointed out in the accompanying editorial, when ALA consumption is corrected for energy intake, the range of intake becomes "flattened" (0.31% to 0.45% of energy compared with unadjusted intakes ranging from 0.35 to 1.25 g/day). Harris noted that ALA intakes, even at the highest level, were well below the Institute of Medicine's suggested adequate intake for ALA (0.6% to 1.2% energy). The Institute's values were based on current estimates of ALA consumption in the US population.

Harris also observed that the analysis did not control for saturated or *trans* fatty acid consumption. Both dietary constituents are strongly associated with increased risk of cardiovascular disease. Further, no account was taken of n-3 LC-PUFA consumption, which also increased across the quintiles of ALA intake. Not surprisingly, linoleic acid intake also increased with ALA consumption. Without adjustments for these potentially confounding factors, it is difficult to take the study findings at face value.

Djoussé L, Arnett DK, Carr JJ, Eckfeldt JH, Hopkins PN, Province MA, Ellison RC. Dietary linolenic acid is inversely associated with calcified atherosclerotic plaque in the coronary arteries. The National Heart, Lung, and Blood Institute Family Heart Study. Circulation 2005; 111:2921-2926.

Harris WS. Alpha-linolenic acid: a gift from the land? Editorial. Circulation 2005;111:2872-2874.

DHA Shifts Lipoprotein Subclasses in Children with High Cholesterol

The effects of dietary long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) on plasma lipoprotein profiles is complex and varies according to the health status of a person. Consumption of n-3 LC-PUFAs from fish or supplements usually leads to marked reductions in the lipoproteins that carry triglycerides but may also modestly increase in low-density lipoproteins. High-density lipoproteins, which are cardioprotective, may increase modestly or remain unchanged. Lipoprotein responses to dietary fatty acids are also related to genetic factors affecting risk of cardiovascular disease. For these reasons assessing the clinical importance of various dietary fatty acids calls for balancing benefits against risks.

Dr. Marguerite Engler and colleagues at the University of California, San Francisco, have been studying the effects of feeding docosahexaenoic acid (DHA) to children with genetically linked hyperlipidemia whose high cholesterol levels put them at greatly increased risk for cardiovascular disease. Engler's group recently reported that DHA supplementation improved endothelial function in these children by restoring flow-mediated dilation in the brachial (arm) artery, an effect that might delay the progression of arterial disease. Her team reports alterations in the distribution of lipoproteins by particle size in response to DHA supplementation that are thought to be less atherogenic.

Lipoprotein particles, the transport vehicles for cholesterol, fat, and other lipids in the blood, are distributed across many subclasses according to size and how much protein and lipid they contain. Larger more buoyant particles are associated with significantly reduced risk of cardiovascular disease and type 2 diabetes than smaller denser ones. It turns out that certain dietary fatty acids can alter the pattern of lipoprotein subclasses in a favorable direction.

Following a low-fat diet recommended by the American Heart Association 20 young people aged 9 to 19 years were randomized to consume 6 capsules/day containing a total of 1.2 g/day DHA or corn/soy oil placebo for 6 weeks. After a 6-week washout period, groups were switched to consume the alternative supplement for 6 weeks. Lipoprotein subclasses for low-density and high-density lipoproteins were measured by vertical-spin density-gradient ultracentrifugation at baseline and the end of each treatment period.
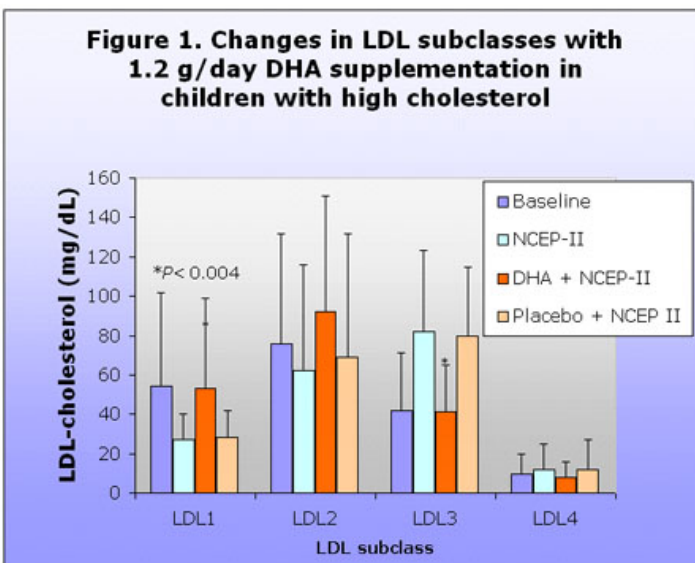
Results for the total triglycerides and lipoprotein cholesterol levels are presented in Table 1.

Table 1. Changes in triglycerides and total, VLDL, LDL, and HDL cholesterol (mean ± SD) in 20 subjects with elevated cholesterol levels after supplementation with 1.2 g/day DHA or placebo for 6 weeks
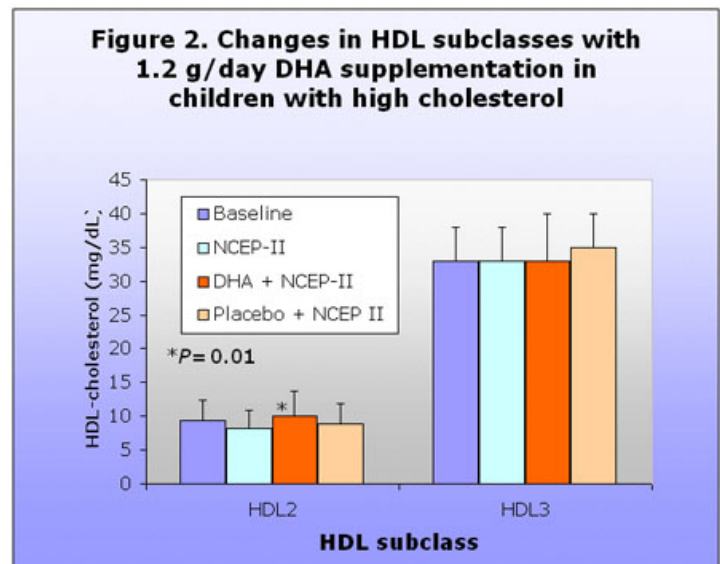
Lipid	Baseline	Dietary Treatment		
(mg/dL)		NCEP-II	DHA + NCEP-II	Placebo + NCEP-II
Triglycerides	133 ± 68	120 ± 50	114 ± 62	116 ± 67
Total cholesterol	275 ± 89	269 ± 88	285 ± 85	276 ± 90
VLDL cholesterol	19 ± 10	18 ± 7	17 ± 9	17 ± 10
LDL cholesterol	214 ± 89	210 ± 88	226 ± 86	216 ± 92
HDL cholesterol	42 ± 8	41 ± 7	43 ± 10	44 ± 8

Whether increased particle size is cardioprotective is controversial and the literature contradictory. Factors such as susceptibility to oxidation and ease of filtration of lipoprotein particles into the arterial wall affect the process of atherosclerosis and these, in turn, may be affected by the increased concentration of DHA. Some but not all studies suggest that enrichment of lipoproteins with DHA reduces susceptibility to peroxidation. Until our understanding of how lipoprotein characteristics affect atherosclerosis improves, clinical studies such as this will be disputed.

Compared with placebo, diet had no effect on any of these general measurements. However, when the individual subclasses of low and high-density lipoproteins were examined, DHA consumption had significant effects on the distribution of subclasses in both categories (Figures 1 and 2). Low-density lipoprotein subclass one increased significantly from 28 ± 14 to 53 ± 46 mg/dL whereas placebo had no effect. Subclass three fell significantly compared with placebo (41 ± 24 compared with 80 ± 35 mg/dL). These changes reflect a substantial shift from the smaller, denser, more atherogenic particles to the larger, less dense and less atherogenic subclass. In the high-density subclasses, DHA supplementation led to a significant increase in the HDL_2 subclass, which is the larger more buoyant category.

Although these findings are clear, their interpretation is not.

Figure 2. Changes in HDL subclasses with 1.2 g/day DHA supplementation in children with high cholesterol

Figure 1. Changes in LDL subclasses with 1.2 g/day DHA supplementation in children with high cholesterol

Engler MM, Engler MB, Malloy JM, Paul SM, Kulkarni KR, Mietus-Snyder ML. Effect of docosahexaenoic acid on lipoprotein subclasses in Hyperlipidemic children (the EARLY Study). Am J Cardiol 2005;95:869-871.

Fish Oil But Not Soy Oil Increases Heart Rate Variability in the Elderly

Long-chain omega-3 fatty acids (n-3 LC-PUFAs) are effective in reducing the risk of sudden death and fatal heart attack through their anti-arrhythmic properties, although results are not entirely consistent. A factor contributing to arrhythmia is reduced heart rate variability—the naturally occurring beat-to-beat changes in heart rate—that reflect changes in the autonomic nervous system. Low heart rate

variability is associated with increased risk of cardiovascular disease and mortality and is improved with the consumption of n-3 LC-PUFAs according to some studies. It is not known whether alpha-linolenic acid, an 18-carbon n-3 PUFA affects heart rate variability. There is one report that cell membrane content of alpha-linolenic acid was unrelated to heart rate variability.

Fernando Holguin and colleagues at the Instituto Nacional de Salud Publica in Mexico investigated the comparative effects of fish and soy oil on heart rate variability in 58 elderly nursing home residents aged 77 years on average (range 60 to 96 yr). Residents with cardiac pacemakers, arrhythmias, or taking anti-coagulants other than aspirin were excluded.

After a 2-month control period during which baseline heart rate variability was established, the participants were randomized to receive 2 g/day fish oil containing 1.7 g docosahexaenoic and eicosapentaenoic acids combined, or 2 g/day soy oil providing 136 mg/day alpha-linolenic acid. Supplements were consumed for 6 months. The investigators measured total heart rate variability and various frequency and time parameters of it every other day for 2 months prior to supplementation and throughout the study. Each treatment group was compared with its own pretreatment baseline data.

At the end of 6 months, those consuming fish or soy oil showed significant improvement in all six parameters of heart rate variability, with the exception of one parameter in the soy oil group. However, when the results were adjusted for age and mean heart rate, only the fish oil group showed a significant increase in total heart rate variability (beta from regression analysis = 0.23 (P=0.001) vs 0.08 (P=0.10) for the fish and soy groups, respectively. Four of the six parameters showed statistically significant improvements in the fish oil group. The soy oil group had a significant increase in the standard deviation of the normal resting heart rate variability. In addition, the response to fish oil appeared early, within the first 6 weeks, whereas it was not until 18 weeks after supplementation that a response to soy oil was observed.

These findings clearly demonstrate that fish oil, and by implication the n-3 LC-PUFAs it contains, can improve heart rate variability significantly in elderly people. Compared with a similar dose of soy oil, n-3 LC-PUFAs acted more quickly and effectively than the small amount of alpha-linolenic acid in the soy oil, whose effects in this study were minimal. However, the study did not compare equivalent doses of n-3 PUFAs. A 1.7 g/day dose of n-3 LC-PUFAs is nearly twice that recommended by the American Heart Association for people with cardiovascular disease, so the question remains whether a lower dose of fish oil or high level of alpha-linolenic acid would also improve heart rate variability in elderly and younger people.

Holguin F, Téllez-Rojo MM, Lazo M, Mannino D, Schwartz J, Hernández M, Romieu I. Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. Chest 2004;127:1102-1107.

Abuissa H, O'Keefe JH Jr, Harris W, Lavie CJ. Autonomic function, omega-3, and cardiovascular risk. Editorial. Chest 2005;127:1088-1091.

Table 1. Regression coefficients (95% CI) of the effect of fish or soy oil on parameters of heart rate variability in elderly people*

Parameter	Fish oil	P	Soy oil	P
Total heart rate variability, log10 (ms^2)/10^5	0.23 (0.10-0.35)	0.001	0.08 (-0.03-0.20)	0.1
High frequency component, log10 (ms^2)/10^5	0.24 (0.10-0.38)	0.001	0.13 (-0.0023-0.28)	0.057
Low frequency component, log10 (ms^2)/10^5	0.30 (0.16-0.43)	0.001	0.12 (-0.009-0.26)	0.06
SD of normal RR interval, log10 (ms^2)	20.17 (8.65-31.60)	0.001	13.17 (1.72-24.61)	0.02

*Adjusted for age, mean heart rate, and interaction between time and treatment

Short Takes

Pre-Surgical Long-Chain Omega-3s Reduce Post-Op Atrial Fibrillation

It is now accepted wisdom that long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) have antiarrhythmic effects and as a result, significantly reduce cardiovascular disease mortality. These fatty acids have been shown to reduce the risk of atrial fibrillation (disorganized heartbeats in the upper chambers of the heart) in many, but not all studies. Atrial fibrillation is the most common complication of coronary bypass surgery and significantly increases risk of stroke and mortality. Could something as simple as dietary PUFAs have an impact on the development of post-surgery atrial fibrillation and recovery?

This was the question posed by Leonardo Caló and colleagues at the San Filippo Neri Hospital in Rome, Italy. To get the answer, they enrolled 160 prospective coronary bypass patients (average age 66 yrs) in an open-label, randomized, controlled trial. Patients with a history of atrial fibrillation or valvular surgery were excluded. One group of patients received about 2 g/day n-3 LC-PUFAs with usual care for at least 5 days prior to surgery. The control group received usual care without supplementation. Monitoring for atrial fibrillation continued during the hospital stay and length of hospitalization was compared for both groups.

The main finding was a significant 54% reduction in the occurrence of atrial fibrillation in patients treated with n-3 LC-PUFAs compared with controls. Atrial fibrillation occurred in 15.2% of patients (12 of 79) treated with n-3 LC-PUFAs and in 33.3% of untreated patients (27 of 81, P for difference between groups=0.013). When it occurred, atrial fibrillation in treated patients was for a shorter time compared with controls, but the differences were not statistically significant (15.5 ± 15.8 vs 23.9 ± 15.3 hours). Time of occurrence was similar in both groups, 3.1 vs 3.4 days in treated and control patients, respectively.

An additional finding from this study was shorter hospital stay for patients treated with n-3 LC-PUFAs compared with controls. Mean hospital stay for supplemented patients was 7.3 ± 2.1 days compared with 8.2 ± 2.6 days for controls (P=0.017). Occurrence of atrial fibrillation lengthened hospitalization for both supplemented and control patients (9.6

± 3.1 vs 9.3 ± 2.9 days, respectively) compared with patients who did not develop the condition (6.9 ± 1.6 vs 7.6 ± 2.2 days for supplemented and controls, respectively).

The authors commented that the 54% reduction in the occurrence of atrial fibrillation with n-3 LC-PUFAs compares favorably with reductions achieved with conventional drug therapies such as beta-blockers or sotalol. n-3 LC-PUFAs have the advantage of virtually no adverse effects in low to moderate doses. Simple and effective, they are just what the doctor should order.

Caló L, Bianconi L, Colivicchi F, Lamberti F, Loricchio ML, de Ruvo E, Meo A, Pandozi C, Staibano M, Santini M. N-3 Fatty acids for the prevention of atrial fibrillation after coronary artery bypass surgery: a randomized, controlled trial. J Am Coll Cardiol *2005;45:1723-1728.*

Fish Oil Reduces Heart Muscle Damage After Cardiac Surgery

The importance of inflammatory processes in exacerbating cardiovascular disease and mortality has stimulated investigations into the effectiveness of reducing inflammation in patients with cardiovascular disease. Data on the long-term consequences of this strategy are not yet available. Because omega-3, long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) have various favorable effects on the abnormalities accompanying cardiovascular disease, Charman and colleagues at Queen Margaret University College, Edinburgh, Scotland wanted to know whether reduced neutrophil activation would be one of them. Neutrophils, the most abundant white cells in blood and our primary line of defense against infection, are activated in response to cardiopulmonary bypass surgery. It is thought that they may be involved in damage to heart cells as well.

Because treatment with fish oil reduces neutrophil activation under laboratory conditions, Charman and colleagues wondered whether treatment with fish oil prior to cardiopulmonary bypass surgery would reduce neutrophil activation and possibly cardiac damage following the operation. They undertook a pilot study. Forty patients undergoing coronary artery bypass surgery were randomly assigned to consume fish oil (8 g/day) or placebo (short-chain fatty acids) for 5 to 7 weeks prior to surgery. Immediately following surgery and 6 weeks later the investigators measured several indices of neutrophil activation such as superoxide anion production,

the extent of neutrophil cell death, and troponin I, a protein released from heart muscle following heart damage.

During the treatment period prior to surgery small, but not statistically significant alterations were observed in both groups for neutrophil activation and cell death. Immediately after surgery, neutrophil cell death was reduced from 32 to 14% in both groups, but responses in the two treatment groups did not differ.

Release of troponin I significantly increased in all patients immediately following surgery and values were highly variable among patients. At 24 hours post surgery, troponin I fell significantly in the fish oil group ($46 \pm 23\%$ of post operative values, P=0.0002), but remained elevated in the placebo group ($107 \pm 72\%$ of post operative values, P=0.013) until 120 hours following surgery, when values had fallen to normal levels. These differences are suggestive of a protective effect of fish oil on cardiac damage, but the large variation in individual values makes it difficult to be conclusive on this point. Questions about the high dosage used in this study can be raised, as some evidence suggests that release of inflammatory mediators may be increased by doses of n-3 LC-PUFAs greater than 2 d/day. Much work remains.

Charman A, Muriithi EW, Milne E, Wheatley DJ, Armstrong RA, Belcher PR. Fish oil before cardiac surgery: neutrophil activation is unaffected but myocardial damage is moderated. Prostaglandins Leukot Essent Fatty Acids 2005;72:257-265.

Maternal and Infant Health

Enhanced Growth and Development in Premature Infants Fed Formula with LC-PUFAs

Although the long-chain (LC) polyunsaturated fatty acids (PUFAs) docosahexaenoic acid (DHA) and arachidonic acid from algal and fungal oils, respectively, are now commonly added to infant formula in the U.S., questions linger about using fish oil as an alternate source of omega-3 (n-3) LC-PUFAs in formula. In addition to DHA, fish oil contains eicosapentaenoic acid (EPA) and small amounts of n-3 docosapentaenoic acid. The majority of studies have reported equivalent growth in infants fed LC-PUFA-supplemented formula and control infants. However, in one study, premature infants fed formula containing DHA, EPA, and arachidonic acid had significantly reduced weight and length compared with breast-fed infants.

Further evaluation of the efficacy and safety of LC-PUFA-supplemented formula for premature infants was conducted in this randomized, double-blind, controlled clinical trial of 361 infants born at or less than 35 weeks postmenstrual age. Infants were stratified by birthweight into three groups: <1000 g, 1000 to 1500 g and >1500 g and assigned randomly to one of three formulas. The control formula had no added LC-PUFAs and two LC-PUFA-supplemented formulas contained DHA from algal oil (17 mg/100kcal) and arachidonic acid from fungal oil (34 mg/100 kcal) or DHA from tuna oil and arachidonic acid from fungal oil in the same concentration. The study included a reference group of breast-fed term infants. Each group was given premature, discharge, and term formulas that differed in having progressively reduced caloric density, ranging from 24 kcal/oz. in the premature formula to 20 kcal/oz. in the term formula. Formula was the sole source of nutrition for the infants until 57 weeks postmenstrual age (4 months after term) and the primary source of nutrition until 92 weeks postmenstrual age, when formula was discontinued. Infants were observed at intervals through 118 weeks postmenstrual age.

The study was divided into two phases, the first lasting until infants reached 40 weeks postmenstrual age, and the second continuing from 40 through 118 weeks postmenstrual age. Sixty infants who completed the first phase did not continue with the second.

At enrolment, the fish-DHA group had significantly lower mean birthweight and head circumference compared with the control and algal-DHA groups. Their mean gestational age at birth was also significantly lower than the control group (28.8 vs 29.6 weeks). Of the 361 infants at enrolment, 56 discontinued the study before 40 weeks postmenstrual age because of intolerance to the formula, medical complications, or parental request. Another 60 infants did not continue past 40 weeks postmenstrual age because of insufficient formula intake, birthweight over 1500 g, formula intolerance or parental/physician elected withdrawal. Thus, in the second phase of the study, 245 infants continued past 40
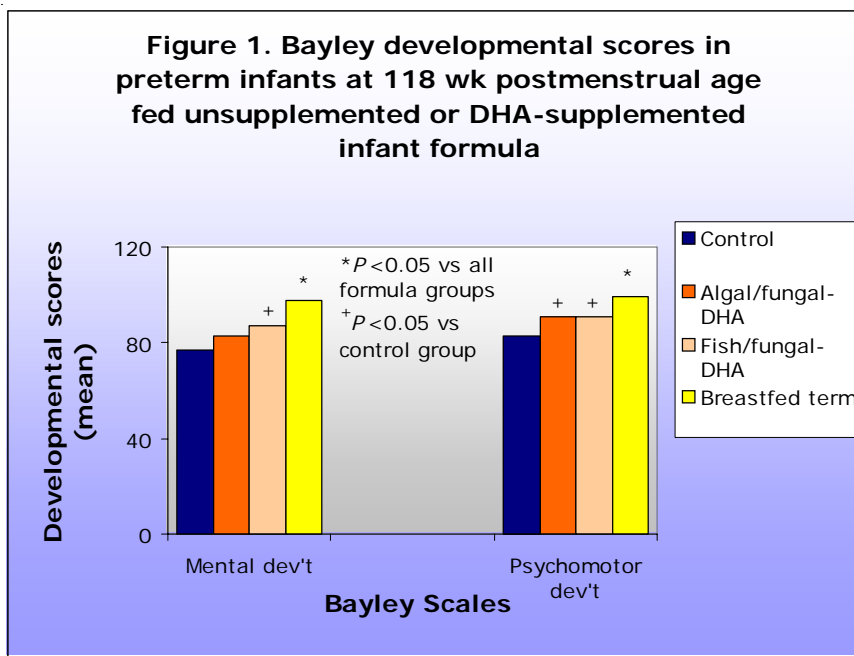
weeks with 179 completing the study through 118 weeks postmenstrual age.

At completion of the first phase and at the beginning of the second phase, mean weight, length, head circumference, and gestational age at birth did not differ among the three pre-term formula-fed groups through 40 weeks postmenstrual age. Reference breast-fed infants born at term had greater mean weights than all pre-term groups until 118 weeks, when the weights in the algal-DHA group became similar to those in the breast-fed group. Among the three pre-term groups, the algal-DHA group had greater mean weights than the unsupplemented group, beginning at the 66th week postmenstrual age and continuing through week 118. By week 118, the algal-DHA group also weighed significantly more than the fish-DHA group.

Mean body length was greater in the breast-fed term infants than all formula groups through 66 weeks postmenstrual age, and from week 79 to 118, greater than the fish-DHA and control groups, but not the algal-DHA group. Small differences in head circumference among the groups emerged at the 79th week, and by week 118, mean head circumferences in the breast-fed infants were greater than the control and fish-DHA groups. At the end of the study, the algal-DHA group did not differ from any other group.

The investigators assessed mental and psychomotor development of the groups using Bayley's Scales of Infant Development at 118 weeks postmenstrual age. Breast-fed term infants had significantly higher scores on both tests than any of the pre-term groups as expected (Figure 1, $P<0.05$). Both algal-DHA and fish-DHA groups had significantly higher scores on both tests than the unsupplemented control group, but results between the algal-DHA and fish-DHA groups did not differ from each other.

Two clinical observations from the study were noteworthy. One was the lower incidence of intraventricular hemorrhage in the algal-DHA group compared with the unsupplemented and fish-DHA groups (14 vs 32 and 33 cases, respectively, $P<0.01$) by 40 weeks postmenstrual age. Because the fish-DHA group weighed significantly less than the other groups at enrolment in the first phase of the study, they would be

Figure 1. Bayley developmental scores in preterm infants at 118 wk postmenstrual age fed unsupplemented or DHA-supplemented infant formula

expected to be at higher risk for intraventricular hemorrhage. The other significant difference was the increased occurrence of unspecified adverse events in the nervous system of control infants compared with those on the fish-DHA formula (control 16% vs fish-DHA 6%, $P=0.04$).

These findings add credence to previously documented benefits to growth and development in premature infants who receive supplementary DHA and arachidonic acid. A strength of the study was its inclusion of infants of widely varying birthweights, which increased the chance of detecting any potential adverse effects among the most vulnerable infants. Those consuming DHA and arachidonic acid from algal and fungal organisms, respectively, eventually gained as much weight as breast-fed term infants ($11,030 \pm 282$ vs $11,363 \pm 225$ g body weight at 118 weeks, respectively) although it took until 118 weeks postmenstrual age for differences between the two groups to disappear. In contrast, unsupplemented control infants and those fed fish-DHA had significantly lower total body weights at the end of the study ($10,177 \pm 282$ vs. $10,409 \pm 250$ g, respectively)*.

Both algal-DHA and fish-DHA groups performed significantly better than unsupplemented infants on widely used measures of mental and psychomotor development. There were no significant differences between the two supplemented groups in either mental or psychomotor scores.

It is puzzling that growth in the fish-DHA group lagged that

of the algal-DHA group, as the amounts of supplemental fatty acids were similar. However, the fish-DHA group received 0.1% EPA that the algal-DHA group did not. Others have suggested that EPA may be associated with lower weight gain. The fish-DHA group also consumed less formula than the algal-DHA group at 48 weeks, though intakes were similar at other ages. Overall, the study confirmed the growth and development-enhancing effect of DHA and arachidonic acid from single-cell organisms in premature infants.

*Data kindly supplied by the authors.

Clandinin MT, Van Aerde JE, Merkel KL, Harris CL, Springer MA, Hansen JW, Diersen-Schade DA. Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. Pediatr 2005;146:461-468.

ALA-Enriched Infant Formula: Analysis of Controlled Trials

A certain pizzazz surrounds the usually tranquil world of infant formula due to the addition of long-chain polyunsaturated fatty acids (LC-PUFAs) for improved infant growth and neurodevelopment, particularly in the U.S. Now, most formula for premature and term infants includes docosahexaenoic acid (DHA) and arachidonic acid, the LC derivatives of alpha-linolenic acid (ALA) and linoleic acid, the respective parent fatty acids of these substances. Several studies have indicated that preformed DHA is more effective in enhancing DHA status than its precursor ALA. However, adjusting the blend of ALA and linoleic acid in formula may have its own merits for infant development and DHA status.

To evaluate the literature on the modification of ALA and linoleic acid in infant formula, Udell and colleagues at Flinders University, Australia, conducted a detailed analysis of the published randomized, controlled trials addressing this question. They identified five trials in term infants and three in pre-term infants. The main outcomes were weight, length, head circumference, neurodevelopmental indices, and in some studies, visual function. What makes this analysis more rigorous than an interpretive review is the inclusion of unpublished data from the original studies.

In the five studies of term infants, three added ALA to infant

formula without changing the linoleic acid content and two increased both ALA and linoleic acid content, keeping the ratio of the two fatty acids the same as the unenriched formula. Assessments were performed from 2 to 24 months of age. Results indicated that ALA enrichment had no effect on infant growth except at 12 months of age, at least four months after supplementation ended. At one year, ALA-supplemented infants were heavier and longer than unsupplemented control infants. There were no differences in visual function or neurodevelopmental scores. DHA content in plasma and red blood cell phospholipids was significantly increased with ALA enrichment at 2 and 4 months of age, respectively, at the expense of diminished arachidonic acid content. By 8 months, arachidonic acid concentration was no different from control values. Increases in DHA did not reach the DHA levels of breast-fed infants. The authors noted that the data did not support an association between lower weight and altered blood EPA or arachidonic acid levels in the ALA-supplemented infants, as there were no differences in weight at the times when fatty acid levels were altered.

There were three randomized, controlled studies in pre-term infants, all examining the effects of additional ALA (2.0 to 3.2 wt%) compared with unsupplemented formula (0.5 to 1.0 wt%). ALA supplementation had no significant effects on weight, length or head circumference in premature infants, but significant improvements in visual development were observed at 36 weeks postmenstrual age. However, by 57 weeks, these differences in visual responses disappeared. None of the studies measured neurodevelopment. One study measured red cell phospholipids and reported significantly increased DHA and EPA and unchanged arachidonic acid concentration at 57 weeks postmenstrual age. Two non-randomized studies not included in the analysis reported that lowering the linoleic acid content of the formula alone increased red cell DHA above that observed with unsupplemented formula, but levels did not reach those in breast-fed infants.

The key finding from this analytical review is the dearth of effects on growth and development of term and preterm infants supplemented with ALA. However, term infants consuming additional ALA showed increased weight and length at 12 months of age, 4 months after the discontinuation of the supplemental formula. Pre-term infants had a short-lived improvement in visual function that disappeared at 57 weeks post-menstrual age. The importance of these observations

is not clear. A significant increase in red blood cell DHA and EPA occurred in term infants at 2 months post-menstrual age, persisting through 8 months, and was observed in one study in pre-term infants. These findings suggest that ALA-supplemented formula can improve infant DHA status without compromising arachidonic acid levels.

Udell T, Gibson RA, Makrides M, PUFA Study Group. The effect of a-linolenic acid and linoleic acid on the growth and development of formula-fed infants: a systematic review and meta-analysis of randomized controlled trials. Lipids 2005;40:1-11.

Cod Liver Oil in Early Pregnancy Linked to Heavier Babies

Readers who remember protesting their daily dose of cod liver oil in childhood may now be enthusiastically consuming fish oil for its health benefits. Cod liver oil is still widely consumed in Scandinavia and Iceland to provide vitamin D during winter. It is also a rich source of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). Because high intakes of marine foods rich in n-3 LC-PUFAs have been associated with higher birthweights, and Icelandic birthweights are among the highest in the world, Olafsdottir and colleagues at the Public Health Institute of Iceland investigated the possible relationship between cod liver oil and consumption in pregnancy and birthweight in Icelandic women.

In this observational study of 436 pregnant Icelandic women, Olafsdottir noted that nearly half of the women were consuming cod liver oil in either liquid or capsule form. Fish consumption, assessed by food frequency questionnaire in early and late pregnancy, averaged 28 g/day in all women, but tended to be greater in women who consumed liquid cod liver oil in early pregnancy compared with those who did not (35.6 vs 28.3 g/day, *P*=0.056). The investigators measured gestational age, birthweight and length, and head circumference, and obtained descriptive information about the mothers' diet, lifestyle, and clinical variables. Sixty-three women reported taking liquid cod liver oil from

early (11th to 15th week) pregnancy on. Outcomes in these women were compared with the remaining 373 women, of whom 134 took cod liver oil capsules. Cod liver oil capsules provided only one-tenth as much n-3 LC-PUFAs as liquid cod liver oil (0.18 vs 1.8 g/daily dose).

The chief observation in this study was the significantly higher birthweights (mean 139 g) among mothers taking liquid cod liver oil compared with the other mothers (Table 1). There was no difference in gestational age, birth length, head circumference, or weight gain during pregnancy between the two groups. In addition, birthweight was not related to the consumption of cod liver oil capsules or fish. After adjusting for confounding variables, healthy women were 11 times more likely to give birth to an infant weighing 4,500 g or more if they used liquid cod liver oil. The authors attributed these findings to the increased intake of n-3 LC-PUFAs from the liquid cod liver oil. Women taking liquid cod liver oil were also significantly older (29.6 vs 27.8 yr, *P*=0.008) and less likely to be delivering their first child (31.7 vs 49.2%).

In contrast, alcohol consumption, which was more than double in those using liquid cod liver oil, was negatively associated with birthweight after adjustment for multiple confounding variables. An unexpected finding was significantly increased energy, fat (all classes), and protein intake in women using liquid cod liver oil without significant additional weight gain. This observation remains explained. After adjustment for these and other relevant confounding variables, liquid cod liver oil intake from early pregnancy on was still significantly and positively correlated with birthweight (*P*=0.023).

Table 1. Pregnancy outcomes and maternal characteristics (means) of 436 Icelandic women consuming liquid cod liver oil in early pregnancy or not

Outcome/maternal characteristic	Liquid cod liver oil		*P*
	Yes (n=63)	*No* (n=373)	
Birthweight, g	3,898	3,759	**0.026**
Birth length, cm	52.5	52.0	0.089
Head circumference, cm	35.9	35.8	0.584
Gestational age, days	282.2	282.9	0.525
Maternal age, yr	29.6	27.8	**0.008**
Maternal weight gain, kg	13.5	13.7	0.695
Energy intake, kJ/day	9,417	8,521	**0.032**
Fat intake, g/day	85.4	74.4	0.079
Alcohol intake, g/day	1.8	0.8	**0.005**
First child	31.7	49.2	**0.036**

The intriguing findings in this study imply that liquid cod liver oil, a concentrated source of n-3 LC-PUFAs, may be an especially useful dietary supplement from early pregnancy on, especially for women at increased risk of low birthweight. The authors pointed out that the liquid cod liver oil available in Iceland, is a good source of vitamins A, D, and E and is purified to control for organic contaminants such as PCBs and dioxins. Since completion of this study, the vitamin A content of Icelandic cod liver oil has been reduced from 2,400 to 460 mcg/10 mL to guard against excessive intake of vitamin A. The caveat to read the label before using any fish oil product is especially important for pregnant women.

Olafsdottir AS, Magnusardottir AR, Thorgeirsdottir H, Hauksson A, Skuladottir GV, Steingrimsdottir L. Relationship between dietary intake of cod liver oil in early pregnancy and birthweight. BJOG* 2005;112:424-429.

Mental Health

Fish Oil and Childhood Aggression: Is There a Link?

Considerable hope attaches to the use of fish oil and various long-chain polyunsaturated fatty acids (LC-PUFAs) for the reduction of undesirable social behaviors associated with attention deficit hyperactivity disorder, aggression and other forms of hostility. The rationale comes from the fact that docosahexaenoic acid (DHA) is concentrated in the brain and children with various social and learning problems have reduced tissue omega-3 (n-3) LC-PUFAs. Some reports claim behavioral benefits from PUFA supplementation. Suggestive, but inconclusive and insufficient, data characterize the field.

The research team of Tomohito Hamazaki at the Toyama Medical and Pharmaceutical University, Toyama, Japan, has been studying these questions for a decade. They reported in 1996 that consumption of DHA-enriched fish oil providing 1.5 to 1.8 g/day DHA was protective against aggression resulting from the increased stress of exams in 19 young university students. Another study reported that 1.5 g/day DHA for 2 months reduced aggression against others in 50 to 60-year-old Thai university employees. In the study described here, Hamazaki's group investigated the effect of DHA-rich fish oil in 166 Japanese school children aged 9 to

12 years (mean age 10 yrs).

Children were stratified according to age, gender, and body mass index, and randomly assigned to consume 4 fish- or control-oil supplemented foods in their customary diet for 3 months. Fish oil supplementation provided 514 and 120 mg/day DHA and EPA, respectively. Psychological, physical, and blood tests were administered on the first and last days of the study. A Japanese version of the Buss-Perry Aggression questionnaire and a modified psychological test originally developed by Rosenzweig and Rosenzweig were used to assess hostility-aggression. Attention deficit hyperactivity disorder was assessed by the children's parents according to diagnostic criteria (DSM-IV) accepted for this condition.

Compliance with dietary interventions was reflected in the fatty acid composition of the red blood cell phospholipids. Those consuming the DHA-enriched foods had no significant change in concentrations of DHA (7.1 vs 6.6 area %, P=0.06) and arachidonic acid (11.8 vs 12.6, P=0.08), but a significant increase in EPA (1.4 vs 1.2 area %, P=0.0009) compared with the control group. The greatest change occurred in the ratio of EPA to arachidonic acid in the fish oil group versus the control group (0.117 vs 0.098, P=0.0001). Linoleic acid was significantly greater in the control group compared with the fish oil group.

Gender had a significant effect on the outcomes of the psychological tests. Scores on physical aggression in female children increased significantly in the control girls compared with fish oil-treated children (scores 13 vs 15, P= 0.008), but were unchanged in boys. When scores for all children were combined, there was no difference between the groups in verbal aggression, anger, hostility, and impulsivity (Table 1). Results for the diagnostic questionnaires for attention deficit hyperactivity disorder assessed by parents were not different between the two groups, with the exception that impulsivity scores in girls consuming fish oil declined significantly compared with girls in the control group.

Scores on the test for aggression against others increased significantly in the fish oil group compared with the control group, whose scores remained unchanged (score change 4 to 5 in fish oil group vs no change in the control group, P=0.02 for intergroup comparison). This unexpected finding remains unexplained, but the authors noted that scores for the fish oil group at baseline were significantly lower than

Table 1. Psychological test scores (median, 25-75 percentile) in children aged 9 to 12 yr who consumed fish oil- or placebo-enriched foods for 3 months

Test parameter*	Control		Fish oil		P inter-group[†]
	Baseline	3 mo.	Baseline	3 mo.	
Verbal aggression, total	13 (11-15)	12 (11-15)	13 (11-14)	13 (11-14)	NS
Physical aggression, total	14 (12-16.8)	15 (13-18)	14 (12-16)	13 (12-16.8)	**0.04**
Boys	15 (13-17)	14 (13-18)	15 (12.7-18.3)	14.5 (12-17)	NS
Girls	13 (10.3-15)	15 (13-18)	13 (11-15.5)	13 (11-16)	**0.008**
Anger, total	11 (9-14)	12 (10-14)	11 (9-13)	11 (9.25-13.9)	NS
Hostility, total	12 (12-14.8)	13 (11-15)	13 (10-15)	13 (12-16)	NS
Impulsivity, total	1 (0-2)	1 (0-2)	1 (0-2)	0 (0-2)	NS
Boys	1 (0-3)	1 (0-2)	1 (0-2)	1 (0-2)	NS
Girls	1 (0-2)	1 (0-2)	1 (0-2)	0 (0-1)	**0.008**

*Tests of aggression by Japanese version of Buss-Perry Questionnaire; anger, hostility, and impulsivity tested with the Japanese version of the Rosenzweig and Rosenzweig test
[†]Adjusted for gender

control group scores. In this study, unlike the only other two studies available, no outside stressor was included in the evaluation.

The investigators evaluated the relationship between the ratio of EPA to arachidonic acid and the change in physical aggression scores in girls. They observed a significant inverse correlation (R=0.53, P=0.01). However, impulsivity in girls was not correlated with this ratio or with the increase in red cell phospholipid linoleic acid that occurred.

This study provides some evidence that increased consumption of n-3 LC-PUFAs may thwart or dampen aggressive behaviors in girls, but had no effect in boys. On the other hand, fish oil consumption was associated with increased aggression toward others. While it might be expected that any effects of n-3 LC-PUFAs on aggression would be detected in boys who score slightly higher than girls, the study found no supporting evidence for such an expectation. Rather than being conclusive, these findings suggest that more extensive evaluations of behavior in larger groups of children might answer the unresolved questions and contradictions.

Itomura M, Hamazaki K, Sawazaki S, Kobayashi M, Terasawa K, Watanabe S, Hamazaki T. The effect of fish oil on physical aggression in schoolchildren—a randomized, double-blind, placebo-controlled trial. J Nutr Biochem 2005;16:163-171.

Low Red Cell Omega-3 Fatty Acids Increase Suicide Risk

Suicide is the ultimate triumph of despair. In China, suicide is the leading cause of death among young people aged 15-34 years and is higher in women than men. In the U.S., it is the third leading cause of mortality in the young, with rates about half those reported in China. Suicide in young American men is about five times greater than among women. These sharp disparities suggest widely different causes. However, a common link to lower rates of suicide around the world is higher fish consumption, the main source of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs). In Finland, fish eaters between 25 and 64 years of age were only 60% as likely to take their lives as those not eating fish. In Japan, the risk of suicide among those 40 years or older was 20% less among daily fish eaters compared with those eating fish less frequently. The question asked in this study was whether fish consumption was associated with risk of suicide among Chinese youth.

To find out, Tomohito Hamazaki's research team conducted a case-control study among patients affiliated with Dalian Medical University hospitals in Liaoning Province, China. One hundred patients aged less than 20 to more than 75 years, who were hospitalized for attempted suicide, were matched for age, gender, and smoking with 100 control patients injured at construction sites or in car accidents. There were 71 female and 29 male patients in the study. Catego-

ries of fish consumption were at least once a week, more than once in two months, and less than once in two months. Study participants did not differ in their frequency of eating fish. Fatty acids were measured in red blood cell phospholipids.

With the exception of stearic acid and n-3 LC-PUFAs, there were no significant differences between suicide patients and controls in red cell phospholipid fatty acids. Suicide patients had significantly lower n-3 LC-PUFAs compared with controls, but alpha-linolenic acid, the parent n-3 PUFA, was similar in both groups. Concentrations of eicosapentaenoic (EPA) and docosahexaenoic acids (DHA) in suicide patients compared with controls were 0.74 ± 0.52 vs 1.06 ± 0.62 ($P<0.0001$) for EPA and 4.4 ± 1.6 vs 5.3 ± 1.7 ($P=0.0003$) for DHA, respectively.

Odds ratios for the risk of attempted suicide were calculated according to quartiles of EPA and DHA red cell concentration. Significant inverse trends were observed with increasing red cell EPA and DHA concentrations (Figure 1). Risk of attempted suicide in the highest versus the lowest quartile of EPA was 88% lower in the highest group ($P<0.0001$) and 78% lower in the highest DHA group ($P=0.0003$). Odds ratios were not appreciably altered by adjustment for age, gender, or multiple other factors.

Interestingly, red cell n-3 LC-PUFA concentrations were not associated with test scores for depression by the Hamilton Rating Scale, suicide intent, or impulsivity. Small numbers of

Figure 1. Adjusted odds ratios for attempted suicide by quartile of EPA or DHA in red cell phospholipids

patients with high scores in these assessments may have been a factor. Neither was there an association between fish consumption, which was relatively low in this coastal population, and risk of attempted suicide. Infrequent fish consumption and the custom of sharing food at meals may have reduced the accuracy of fish intake estimates.

This study adds another link to the epidemiological chain connecting n-3 LC-PUFAs with lower risk of suicide. Lack of association with fish consumption is at odds with the link to DHA concentration in red cell membranes, and may reflect the difficulty in estimating intakes when consumption is low. In this study, the biomarker provided a clearer finding. Although it cannot be shown directly, the presumption is that red cell n-3 LC-PUFAs reflect concentrations in the brain. Considerable data from human observational studies and direct observations in animals indicate that n-3 LC-PUFAs are involved in the regulation of serotonin, an important neurotransmitter frequently linked to suicidal behavior, and other mental and behavioral conditions. There is evidence that DHA may suppress excessive activity in the noradrenergic neural communication system too, a condition associated with severe anxiety. There are plenty of leads to chase.

Huan M, Hamazaki K, Sun Y, Itomura M, Liu H, Kang W, Watanabe S, Terasawa K, Hamazaki T. Suicide attempt and n-3 fatty acid levels in red blood cells: a case control study in China. Biol Psychiat *2004;56:490-496.*

LC-PUFAs in Red Blood Cells Reduced at Onset of Schizophrenia

Changes in the polyunsaturated fatty acid (PUFA) content of neural membranes, often reflected in red blood cell phospholipids, have been associated with various psychiatric illnesses, including schizophrenia, bipolar disorder and depression. Populations eating little seafood have higher rates of various mental illnesses compared with those where fish is eaten regularly. Administration of omega-3 long-chain PUFAs (n-3 LC-PUFAs), especially eicosapentaenoic acid (EPA), is reportedly effective in reducing the severity of the symptoms of schizophrenia, but findings have been inconsistent and may be confounded by concomitant drug therapy (see following review article).

To clarify the reports on altered red blood cell membrane fatty acid composition in schizophrenic individuals, Ravinder
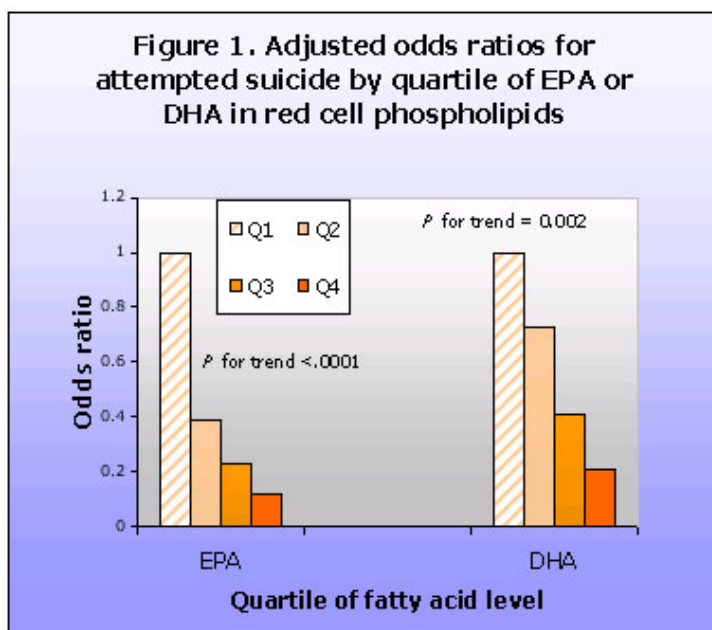
Reddy and colleagues at the Western Psychiatric Institute and Clinic in Pittsburgh, USA, compared the fatty acid profiles of red cell phospholipids in 24 patients at their first episode of schizophrenia with age and gender matched healthy controls. Patients (mean age 28 years) were recruited from the authors' psychiatric clinic and were not taking any antipsychotic agents. Thirty-one healthy volunteers (mean age 27 years) with no family history of psychosis were recruited from outside the healthcare system. Healthy controls had significantly more years of education (16 vs 13 years) and included fewer smokers (6% vs 46%) compared with patients. Blood samples were obtained from patients immediately prior to the initiation of antipsychotic agents.

Fatty acid analyses of the red cell phospholipids revealed no significant differences in total, saturated, or monounsaturated fatty acid concentrations between the two groups. Patients had 13% less PUFAs compared with controls and individual differences among LC-PUFAs were substantial. Arachidonic acid was lower by 18% (P=0.001), n-3 docosapentaenoic acid by 36% (P=0.002) and docosahexaenoic acid by 28% (P=0.003) in patients compared with controls.

These observations confirm previous reports of reduced PUFAs, especially n-3 LC-PUFAs, at the time of diagnosis of schizophrenia. Because dietary intake was not assessed, its contribution to red cell lipid fatty acids could not be gauged. Smoking is an important potentially confounding variable in this report, but could not be assessed without data on healthy smoking subjects. In the patients, fatty acid profiles were not significantly different between smokers and non-smokers. Whether there is an abnormality in PUFA metabolism in people who develop schizophrenia remains an unanswered question.

Reddy RD, Keshavan MS, Yao JK. Reduced red blood cell membrane essential polyunsaturated fatty acids in first episode schizophrenia at neuroleptic-naive baseline. Schizophrenia Bull *2004;30:901-911.*

Dietary Omega-3 Fatty Acids and Psychiatry: A Review

A burgeoning field of polyunsaturated fatty acid (PUFA) research is mental health and behavior. Research stems from the involvement of long-chain (LC) PUFAs, particularly docosahexaenoic acid (DHA), in the construction, maintenance, and function of the brain where these fatty acids are constituents of cell membranes, myelin, and nerve terminals. From the effects of omega-3 (n-3) PUFA deficiency in laboratory animals to the association between low DHA concentrations and several detrimental effects of aging, including dementia, n-3 LC-PUFAs are involved in diverse mental functions.

Bourre reviews the history and quality of data across the spectrum of clinical conditions ranging from stress to schizophrenia. Readers outside the field may be forgiven for some confusion about the nature of various mental illnesses and assessments such as cognitive function, "dementia," psychological test scores, depression, and a host of others. Information and "hard facts" are further obscured by studies with too few participants, weak experimental design, and contradictory findings. Thus, it is refreshing to find a critical review of this literature that assembles the information, puts it in context, and discusses the implications for dietary recommendations.

Jean-Marie Bourre of the Institut National de la Santé et de la Recherche Médicale in Paris, France was among the first to describe the effects of long-chain omega-3 PUFA deficiency in animal brain and different types of brain cells. His review covers childhood disorders such as autism, hyperactivity and dyslexia, as well as afflictions more common in adults, including mood disorder, depression, dementia, and cognitive aging. There is also a brief section on drug addiction, an insightful discussion of the actions of n-3 LC-PUFAs, and what remains to be determined before research can be conclusive. In his conclusions, Bourre points out that n-3 PUFAs have long-term actions on membrane composition and function, as well as short-term effects on activities such as signal transduction. With scientists eager to find effective treatments for the prevention and amelioration of psychological problems, Bourre notes that "rigorous clinical trials have yet to provide incontrovertible proof that capsules containing omega-3 fatty acids as fish oil extracts are effective."

Bourre J-M. Dietary omega-3 fatty acids and psychiatry: mood, behaviour, stress, depression, dementia and aging. J Nutr Health Aging *2005;9:31-38.*

Clinical Conditions

Visual Function

Seeing Clearly: Omega-3 LC-PUFAs Linked to Lower Cataract Risk

You have to hand it to American nurses. Since 1976, some 120,000 female registered nurses have been filling out questionnaires every two years, sending data on their diet and health to a team of Harvard epidemiologists to mine. The pay dirt has provided insights into such diseases as breast and colorectal cancer, cardiovascular disease, stroke, and type 2 diabetes and the consumption of substances as diverse as caffeine, fiber, trans fatty acids, polyunsaturated fatty acids (PUFAs), and carotenoids. Now the dietary habits of these women suggest a connection between consuming omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) and lower risk of cataract.
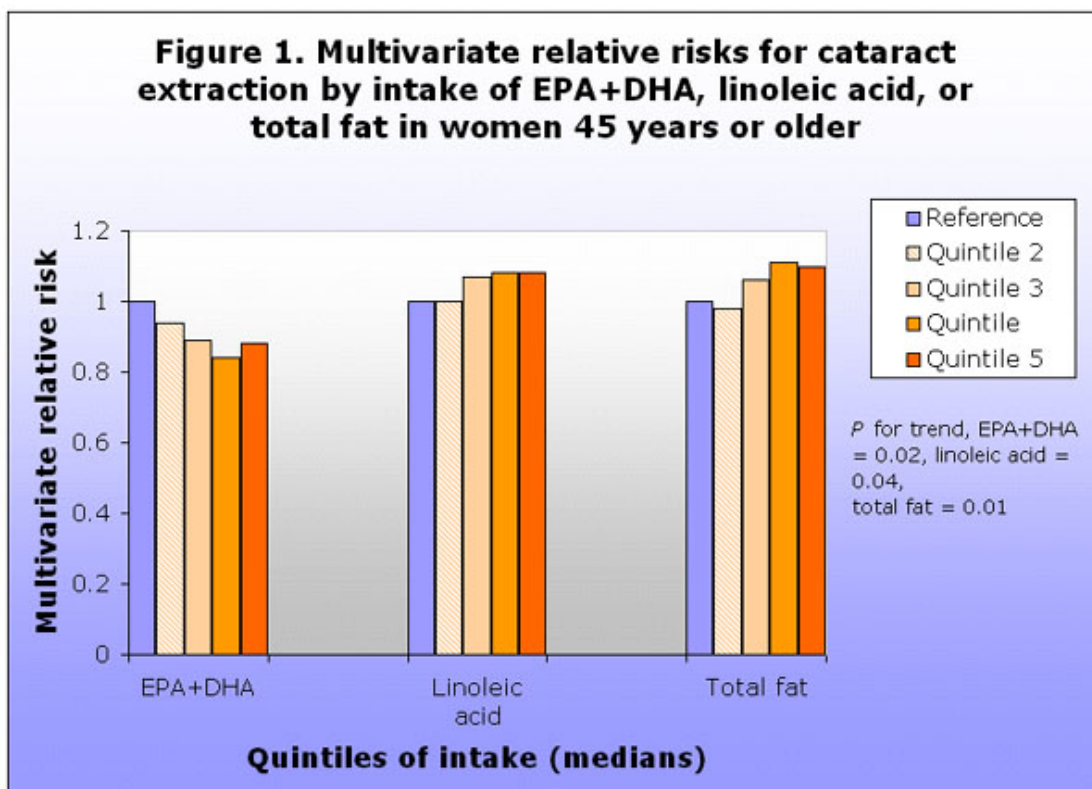
Cataracts — clouding of the normally clear lenses of the eyes — are one of the chief causes of impaired visual function and blindness in older people, but they can be treated by surgical removal. Their development is associated with certain diseases like diabetes, and some medications, such as corticosteroids. Although changes in fatty acid composition of the lens have been documented, diet has not been a critical consideration. That may change.

The consumption of n-3 LC-PUFAs has been associated with several benefits for visual function from increased retinal function in infancy to reduced risk of age-related macular degeneration and progression of retinitis pigmentosa. Animal and laboratory studies more than 10 years ago indicated that oxidation breakdown products from n-3 PUFAs damage the lens, but that diets rich in PUFAs delayed the onset of cataracts, but there have been few studies in people since then.

In the first report cited below, 71,083 nurses 45 years or older were followed for 16 years with repeated dietary assessments by food frequency questionnaire. Reports of surgical cataract extraction were confirmed by physicians for 90 percent of the participants. Those whose cataracts could be attributed to congenital origin, chronic use of steroids, various intraocular problems or glaucoma, and those who developed cancer, diabetes, or cataracts before age 45 were excluded from the analysis. There was a total of 4,196 cases. The epidemiologists used Cox proportional hazards models to determine the associations between dietary factors and rate of cataract extraction. Nutrient intakes as a percent of energy were divided into quintiles.

The two chief findings were increased risk of cataract extraction with high total fat intake (P for trend = 0.01) and a significant 12% lower risk with increased intake of n-3 LC-PUFAs (P for trend = 0.02), as shown in Figure 1. The analysis confirmed a similar inverse association with eating dark meat fish, but not other types of fish. Additional adjustment for vitamin use did not affect the relationship with n-3 LC-PUFAs. Women with the highest intake of n-3 LC-

Figure 1. Multivariate relative risks for cataract extraction by intake of EPA+DHA, linoleic acid, or total fat in women 45 years or older

PUFAs were older, less likely to smoke, and more likely to have hypertension. Omega-3 PUFA intake was related to alcohol, vitamin C, vitamin E, lutein and zeaxanthin, and lower energy intake.

Intakes of saturated, polyunsaturated, and trans fats were not related to risk of cataract development, but the trend with increased monounsaturated fat intake was statistically significant (P=0.04). Among individual PUFAs, only linoleic acid was associated with an increased risk of cataracts (8% greater risk, P for trend = 0.04), but the trend disappeared after further adjustment for other fats.

Because of extensive adjustments to the analysis for potentially confounding variables and the lack of any false positives in cataract surgery, the authors asserted that factors associated with lifestyle and other differences between those who developed cataracts and those who did not could not explain the observed associations. Further, this epidemiological team distinguished between total n-3 PUFAs and n-3 LC-PUFAs, a welcome refinement of their analysis.

In a second report from this group, 603 participants in the nurses' cohort living in the area of Boston, USA, who were free of cancer and had both lenses intact were recruited for assessment of lens status. Dietary assessments were obtained using a 131-item food frequency questionnaire and detailed eye examinations performed. Lens opacity was expressed for nuclear, cortical, and posterior subcapsular regions according to the Lens Opacities Classification System III. After exclusions for diagnosis of cancer, diabetes, elevated blood glucose, or missing data, 440 participants averaging 61 years of age remained.

The noteworthy finding in this cross-sectional study was the positive association between total PUFA consumption and risk of lens opacity (Odds Ratio=2.3, CI= 1.1-4.8, P=0.02, for highest vs lowest quintile of intake). When consumption of individual fatty acids was examined, a significant positive association was observed for the prevalence of nuclear lens opacity and both linoleic and alpha-linolenic acids (Odds Ratio for both fatty acids was 2.2 for highest vs lowest quintile of intake). No association was observed for opacity in either cortical or posterior subcapsular regions of the lens. There was no significant association for n-3 LC-PUFAs or arachidonic acid.

Certain findings from this "snapshot" of lens opacity are at odds with some of the findings from the prospective observations over 16 years in women in the same population group. For example, observations for n-3 LC-PUFAs, alpha-linolenic acid, and total PUFAs are inconsistent. The authors noted the limited number of studies in this field and the inconsistency among reports to date. This makes fertile ground for additional investigations.

On balance, it seems clear that a critical function of n-3 LC-PUFAs throughout life is the development and maintenance of visual function. From prenatal visual development through retinal cell signaling to protection against various degenerative eye diseases, n-3 LC-PUFAs are active. Keep mining the data.

Lu M, Cho E, Taylor A, Hankinson SE, Willett WC, Jacques PF. Prospective study of dietary fat and risk of cataract extraction among US women. Am J Epidemiol 2005;161:948-959.

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Prostate Cancer

Long-Chain Omega-3s Linked to Reduced Risk of Advanced Prostate Cancer

The cause of prostate cancer, the second leading cause of cancer mortality in men, remains unknown. The disease is most prevalent in the U.S. and Canada, and has been linked to various fatty acids. For example, long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) are reported to reduce risk, while alpha-linolenic acid, an 18-carbon n-3 PUFA, is associated with increased risk in some, but not all studies. This paradox has not been resolved. Further, the associations with n-3 LC-PUFAs have been observed for advanced prostate cancer, not with early stages of the disease.

To elucidate more clearly the effects of dietary PUFAs in prostate cancer, Vincent Freeman of Loyola University, Chicago, USA, and colleagues at other institutions examined the relationship between various PUFAs in the prostate of men with different stages of the disease. Of 485 men with histologically confirmed, organ-confined prostate cancer, who were scheduled for radical prostatectomy, 196 men 50

Table 1. Odds ratios* for advanced prostate cancer by stage according to prostate fatty acid concentration

Fatty acid (%)	Any advanced cancer		Extracapsular		Seminal vesicle	
	Odds ratio (CI)	P	Odds ratio (CI)	P	Odds ratio (CI)	P
Total PUFAs	0.93 (0.87-0.99)	**0.035**	0.97 (0.89-1.05)	0.39	0.86 (0.78-0.95	**0.003**
n-3 LC-PUFA	0.72 (0.52-1.01)	0.06	0.81 (0.56-1.17)	0.25	0.54 (0.32-0.92)	**0.02**
Arachidonic acid	0.95 (0.88-1.03)	0.22	1.00 (0.91-1.11)	0.85	0.85 (0.76-0.95)	**0.006**

*Calculated with reference to organ-confined disease

years or older finished the study with complete clinical and nutritional data. Mean age of participants was 63 years. Disease stages were determined from pathological examination of gland slices with pathology and grading done according to published recommendations. Fatty acids were determined in non-cancerous tissue to avoid possible effects of the disease itself.

The majority of participants (73%) had organ-confined disease, but the remainder (26%) were classified as having advanced disease according to the pathology findings. In about 10% of men, the disease had advanced to the seminal vesicle, the most advanced form observed in these men.

Tumor stage was inversely related to total PUFAs, n-3 LC-PUFAs (eicosapentaenoic plus docosahexaenoic acids), and arachidonic acid concentrations, but not to linoleic acid content. Men with extracapsular extension of the disease or seminal vesicle involvement had significantly lower n-3 LC-PUFAs than those with organ-confined disease (Figure 1). Significantly lower arachidonic acid concentration was observed only in men with seminal vesicle involvement.

The researchers calculated odds ratios for the risk of locally advanced cancer by stage of disease associated with PUFA concentrations, adjusting for initial clinical stage of the tumor, pre-surgical prostate-specific antigen level (a marker for prostate cancer) and Gleason sum, a score for histological evaluation (Table 1). Risk was

inversely related to total PUFA concentration (Table 1), largely attributable to the cases of seminal vesicle involvement. Risk of the latter was inversely and significantly related to n-3 LC-PUFA and arachidonic acid concentrations. It is worth noting that there was no association with alpha-linolenic acid concentration, a contentious issue with this fatty acid.

The authors noted that men with locally advanced prostate cancer have a significantly poorer prognosis than those with organ-confined disease. Invasion of the seminal vesicles carries the least favorable prognosis, so finding an inverse association between advanced prostate cancer and with n-3 LC-PUFAs, and to a lesser extent with arachidonic acid, suggests that these fatty acids may retard the progress of the disease. The investigators discussed potential mechanisms

Figure 1. Prostate fatty acid concentrations by disease stage
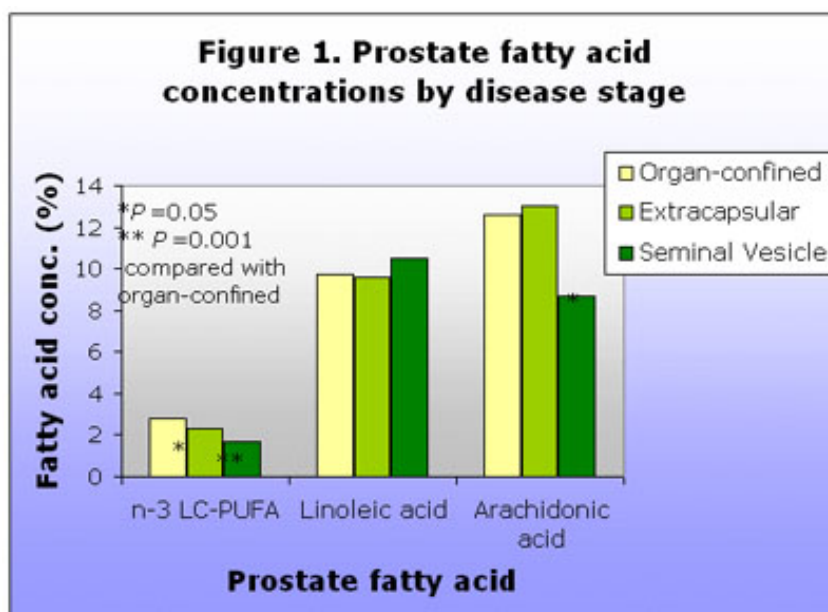
that might account for these effects, including increased production of eicosanoids from arachidonic acid. Enzymes involved in that conversion are reportedly overexpressed in prostate cancer. Randomized controlled trials with n-3 LC-PUFAs might provide useful insights.

Freeman V, Meydani M, Hur K, Flanigan R. Inverse association between prostatic polyunsaturated fatty acid and risk of locally advanced prostate carcinoma. Cancer 2004;101:2744-5274.

Letter

Biomarkers, Fatty Acid Database, & More

Editor:

The March 2005 PUFA Newsletter suggested an increased controversy concerning correlations between fish consumption and cardiovascular (CV) health and the relevance of the dietary omega 6 (n-6) to omega 3 (n-3) fatty acid ratio for CV health.

Most data rely on the assessment of n-3 PUFA intakes from fish or other dietary sources based on questionnaires. Although this is practically the only approach for large population groups today, I suggest moving to direct measurements of fatty acids in circulating lipids. This would address various problems: dietary levels are difficult to assess; fatty acid bioavailability depends on the form administered, e.g. fish or encapsulated preparations[1]; bioavailability of individual long-chain n-3 PUFAs may differ relative to lipid class, e.g. phospholipids or cholesteryl esters; correlations between intakes and circulating levels are largely unknown; and possible correlations between plasma or red blood cell levels and CV functions in large population groups are unknown.

We need innovative approaches to create a fatty acid database in large population groups, i.e., more than 10,000 analyses per year, as was done decades ago for serum cholesterol. We need to reduce costs, time, and involvement of specialized personnel from the collection of blood samples to innovative analytical strategies. For example, our laboratory developed an innovative procedure to evaluate fatty acid status from a drop of blood collected on an adsorbent followed by direct methylation without lipid extraction[2]. This approach simplifies the first steps in fatty acid analysis and with new methylating agents, automated sample handling, and new instrumentation, should reduce costs and time for fatty acid analysis.

Regarding the n-6 to n-3 PUFA ratio in the diet[3], this factor appears attractive and relevant at first glance, but examined more closely is not thoroughly convincing. Practically speaking, even raising n-3 LC-PUFA intakes to only a few grams/day (versus dietary n-6 linoleic acid of 12 to 15 g/day) has been reported in several studies to exert significant benefits, in spite of the large excess of n-6 fatty acid in the diet and in tissues. This suggests that the effects are somewhat independent of dietary n-6 fatty acids. To complicate the issue, unexpected complex interactions between different members of the n-6 fatty acid series may occur. For example, nearly 20 years ago, Tremoli et al. reported that increased intakes of linoleic acid in the population of North Karelia, Finland, resulted in elevated platelet linoleic acid and an unexpected reduction of platelet arachidonic acid (20:4 n-6) and thromboxane production[4]. This effect was attributed to the possible competition between the two n-6 PUFAs for incorporation into the 2-position in platelet phospholipids. It could also result from the well documented reducing effect of excess linoleic acid on its desaturation[5]. Further, excess linoleic acid from the diet would to some extent replace arachidonic acid in several cells by competing with it for the 2-position of glycerol. These considerations reinforce the need for large-scale measurements of fatty acid profiles in blood lipids in relation to dietary fats for the correct assessment of interactions between fatty acid classes and correlations with metabolic and functional processes in the CV system.

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