

Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6 and Omega-3 Fatty Acids

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Program and Abstracts

The Cloisters

National Institutes of Health

Bethesda, Maryland, USA

April 7-9, 1999

Sponsored by:

National Institute on Alcohol Abuse and Alcoholism-NIH

Office of Dietary Supplements-NIH

The Center for Genetics, Nutrition and Health

International Society for the Study of Fatty Acids and Lipids

National Institute of Child Health and Human Development

Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6 and Omega-3 Fatty Acids, The Cloisters, National Institutes of Health, Bethesda, MD, USA

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Background

Following the 3rd Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL) in Lyon, France, June 1-5, 1998, the ISSFAL Board of Directors agreed to convene a workshop on the essentiality of and DRIs for omega-6 and omega-3 fatty acids. An international group of experts will present reviews and new data in a round table format with ample time left for discussion. The participants will include speakers and discussants from the National Institutes of Health, other government agencies, academia, industry, non-profit organizations, the World Health Organization, the Food and Agriculture Organization, and the Food and Nutrition Board.

Venue

The Workshop will be held in the Mary Woodard Lasker Center for Health Research & Education (The Cloisters, Building 60) at the National Institutes of Health in Bethesda, Maryland, USA.

Conference Secretariat

The Center for Genetics, Nutrition and Health, 2001 S Street, NW, Suite 530, Washington, D.C. 20009 USA, phone: (202) 462-5062, fax: (202) 462-5241, e-mail: cgnh@bellatlantic.net.

Hotel Accommodations

We have selected *The Bethesda Ramada* in Bethesda, Maryland, as our hotel [8400 Wisconsin Avenue, phone: (301) 654-1000], since it is within walking distance of the National Institutes of Health (NIH). Parking on the campus of the NIH is very limited. The *Medical Center Metro stop* (Red Line) is on the NIH campus.

Conference Cochairs

Artemis P. Simopoulos, M.D. (USA)

Norman Salem, Jr., Ph.D. (USA)

Alexander Leaf, M.D. (USA)

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**Workshop on the Essentiality of and Dietary Reference Intakes (DRIs) for Omega-6
and Omega-3 Fatty Acids**

National Institutes of Health, The Cloisters

April 7-9, 1999

WEDNESDAY, APRIL 7, 1999

Welcoming Remarks - Enoch Gordis, M.D., NIAAA-NIH

Claudio Galli, M.D., President, ISSFAL

Session I. Principles to be Considered in Determining Essentiality and DRIs

Cochairs: Artemis P. Simopoulos, M.D.

Harald S. Hansen, Ph.D., D.Sc.

9:00 - 9:30 a.m. *Criteria for Determining Essentiality and Standards for DRIs*

Vernon R. Young, Ph.D., D.Sc.

9:30 - 10:00 a.m. *Essentiality of Omega-3 Fatty Acids*

Arthur A. Spector, M.D.

10:00 - 10:30 a.m. *Defining the Omega-3 Status in Mammals*

Andrew J. Sinclair, Ph.D.

10:30 - 11:00 a.m. Coffee Break

11:00 - 11:30 a.m. *An Evolutionary View of Diet Recommendations*

S. Boyd Eaton, M.D.

11:30 - 12:30 p.m. Discussant and General Discussion

Harald S. Hansen, Ph.D., D.Sc.

12:30 - 2:00 p.m. Lunch

Session II: Essential Fatty Acids and Central Nervous System Function

Cochairs: Norman Salem, Jr., Ph.D.

William C. Heird, M.D.

2:00 - 2:30 p.m. *Evidence for the Essential Nature of DHA for the Human and Rat Nervous System*

Norman Salem, Jr., Ph.D.

2:30 - 3:00 p.m. *DHA Supplementation of Breastfeeding Mothers: Effects on Maternal Plasma and Milk Fatty Acids, Infant Plasma Fatty Acids, Infant Visual*

Function and Infant Neurodevelopmental Status

William C. Heird, M.D.

3:00 - 3:30 p.m. *Functional Basis for the Importance of Omega-3 Fatty Acids in Retinal and CNS Development*

Martha Neuringer, Ph.D.

3:30 - 4:00 p.m. *Long Chain Polyunsaturates and Human Visual Development*

Eileen Birch, Ph.D.

4:00 - 4:30 p.m. Coffee Break

4:30 - 5:00 p.m. *The Effects of DHA on Hostility*

Tomohito Hamazaki, M.D., Ph.D.

5:00 - 5:30 p.m. *Omega-3 Fatty Acids in Mood Disorders*

Andrew L. Stoll, M.D.

5:30 - 6:30 p.m. Discussants and General Discussion

Peter Willatts, Ph.D.

Joseph Hibbeln, M.D.

7:30 - 10:00 p.m. Dinner at the Bethesda Ramada

THURSDAY, APRIL 8, 1999

Session III. Cardiovascular Disease

Cochairs: Alexander Leaf, M.D.

Raffaele De Caterina, M.D., Ph.D.

9:00 - 10:00 a.m. *Polyunsaturated Fatty Acids and Cardiovascular Disease*

Alexander Leaf, M.D.

10:00 - 10:30 a.m. *n-3 Polysaturated Fatty Acids Inhibit COX-2 Expression*

Raffaele De Caterina, M.D., Ph.D.

10:30 - 11:00 a.m. Coffee Break

11:00 - 11:30 a.m. *Alpha-Linolenic Acid in the Prevention of Cardiovascular Disease*

Serge Renaud, M.D.

11:30 - 12:00 p.m. *Omega-3 Long Chain PUFA and Triglyceride Lowering: Minimum Effective Intakes*

William S. Harris, Ph.D.

12:00 - 12:30 p.m. *Efficacy of n-3 PUFA and vitamin E in 11,324 post-MI patients: Results of GISSI-PREVENZIONE*

Roberto Marchioli, M.D.

12:30 - 1:00 p.m. Discussant and General Discussion

William E. Lands, Ph.D.

1:00 - 2:00 p.m. Lunch

Session IV: Relationship of Essential Fatty Acids to Saturated, Monounsaturated, and Trans Fatty Acids

Cochairs: Claudio Galli, M.D.

Andrew J. Sinclair, Ph.D.

2:00 - 2:30 p.m. *Relationships Between Saturated, Monounsaturated, Polyunsaturated Fatty Acids: Dietary Data vs. Data from Plasma Fatty Acid and*

Lipid Analyses

Claudio Galli, M.D.

2:30 - 3:00 p.m. *Nutritional and Metabolic Interrelationships Between Omega-3 Fatty Acids and Trans Fatty Acids*

Bruce J. Holub, Ph.D.

3:00 - 3:30 p.m. Coffee Break

3:30 - 4:00 p.m. *Choice of Monounsaturated, Trans and Omega-3 Fatty Acid-Rich Oils for the Prevention of Excessive Linoleic Acid Syndrome*

Harumi Okuyama, M.D.

4:00 - 5:00 p.m. Discussion

FRIDAY, APRIL 9, 1999

Session V. Dietary Recommendations and Omega-6:Omega-3 Ratio (LA, LNA, AA, EPA, DHA)

Cochairs: Peter R.C. Howe, Ph.D.

Bruce J. Holub, Ph.D.

9:00 - 9:20 a.m. *Intakes of Dietary Fatty Acid in the United States: Results from the USDA's 1994-1996 Continuing Survey of Food Intakes by Individuals*

Gary J. Nelson, Ph.D.

9:20 - 9:30 a.m. *World Health Organization/Pan American Health Organization (Status of EFA Worldwide)*

Manuel Peña, M.D.

9:30 - 9:40 a.m. *n-3 Fatty Acids: Food Supply, Food Composition and Food Consumption Data*

William D. Clay, Ph.D.

9:40 - 9:52 a.m. *BASF's Approach to Commercialization of Long Chain*

Omega-3 Fatty Acids

Herbert D. Woolf, Ph.D.

9:52 - 10:04 a.m. *Essential Fatty Acids and the Products of the Groupe Danone for Human Nutrition*

Dominique Lanzmann-Petithory, M.D.

10:04 - 10:16 a.m. *Advantages and Disadvantages of the Use of Flax Seed as a Source of Omega-3*

Paul A. Stitt, Ph.D.

10:16 - 10:28 a.m. *Omega-3 LC-PUFA ñ from a Health Concept to Foods in the Shelves*

Reto Muggli, Ph.D.

10:28 - 10:40 a.m. *Infant Formulas with no DHA or ARA.. Are They Causing Harm?*

David J. Kyle, Ph.D.

10:40 - 11:00 a.m. Coffee Break

11:00 - 11:12 a.m. *Clinical Safety Studies of LCPUFA Supplementation of Premature and Term Infant Formulas*

James W. Hansen, M.D., Ph.D.

11:12 - 11:24 a.m. *Omega-3 Long Chain PUFA ñ Closing the Nutritional Gap*

Jacques Boudreau

11:24 - 11:36 a.m. *OmegaTech, Inc.*

William R. Barclay, Ph.D.

11:36 - 11:48 a.m. *Safety of Omega-3 Products Based on Fish Oil as Starting Material*

Bjorn Rene

11:48 - 12:00 p.m. Other

12:00 - 1:00 p.m. Discussants and General Discussion

Bruce Holub, Ph.D.

Rebecca Costello, Ph.D.

1:00 - 2:00 p.m. Lunch

Session VII. Conclusions and Recommendations

Cochairs: Alexander Leaf, M.D.

Artemis P. Simopoulos, M.D.

2:00 - 5:00 p.m. *Roundtable Discussion*

ABSTRACTS

Wednesday, April 7, 1999

Session I. Principles to be Considered in Determining Essentiality and DRIs

Criteria for Determining Essentiality and Standards for DRIs

Vernon R Young, Ph.D., D.Sc.

Massachusetts Institute of Technology, Cambridge, MA 02139, USA

This introductory presentation to the workshop will begin with an initial, brief statement about the importance of knowledge on the quantitative needs for nutrients and the multiple uses of nutrient-based dietary reference values. From this introduction we will turn to (i) a consideration of the evolving conceptual and factual basis underlying the "essentiality" of nutrients and (ii) the definition and description of dietary reference intakes (DRIs). The latter include (following the structure proposed and applied recently by the US Food and Nutrition Board/Institute of Medicine/National Academy of Sciences):- Estimated Average Requirement (EAR); Recommended Dietary Allowance (RDA); Adequate Intake(AI) and Upper Tolerable Level (UL). The most useful DRI is the EAR, the reasons for which will be examined. Then a detailed discussion will follow with respect to the establishment of DRIs, including an emphasis on (a) the choice of the criterion (criteria) of nutrient adequacy chosen to establish a specific DRI and (b) the approach(es) that might be taken and data that are desirable to achieve this goal. The importance of seeking a congruence of evidence, where this is possible, in arriving at a DRI will be emphasized, by example. Finally some suggestions will be made with respect to the setting of DRIs for omega-3 and omega-6 fatty acids.

Essentiality of Omega-3 Fatty Acids

Arthur A. Spector, M.D.

Department of Biochemistry, University of Iowa College of Medicine,

Iowa City, Iowa 52242, USA

There is a growing consensus that omega-3 fatty acids are essential nutrients for humans. Much of the evidence is based on physiological measurements such as neurological development and visual acuity. To better understand why this class of polyunsaturated fatty acids is required, we must determine the biochemical basis for the essentiality. Of the eight fatty acids that comprise the omega-3 metabolic pathway, the two that are most likely to have essential biochemical functions are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

EPA can be converted to prostaglandins, thromboxanes and lipoxygenase products. However, no

essential role for these EPA-metabolites has been reported, and it seems unlikely that the formation of these products is the reason that omega-3 fatty acids are essential. When elevated amounts of EPA are available, the incorporation of arachidonic acid (AA) into cell phospholipids and its conversion to eicosanoid mediators is reduced. Thus, EPA acts as a competitive inhibitor of AA, and this probably accounts for some of the beneficial effects of omega-3 fatty acids in the treatment of cardiovascular and inflammatory diseases. While the possibility that EPA is essential in order to modulate the effects of AA cannot be ruled out, the amounts ordinarily present in the plasma and tissues probably are too low to competitively inhibit the actions of AA. Therefore, modulation of AA metabolism is more likely to be a pharmacological effect of omega-3 fatty acid supplements rather than an essential physiological function.

The basis for considering DHA as the biochemically essential omega-3 component is much more

compelling. DHA is the most abundant omega-3 fatty acid in most tissues, and it is present in large amounts in the brain and retina. DHA is the omega-3 fatty acid required for normal development of the nervous system and optimum visual acuity. Furthermore, when an omega-3 fatty acid deficiency exists, the body compensates by replacing it with the corresponding fatty acid of the omega-6 series, omega-6 docosapentaenoic acid (DPAn-6). These findings strongly suggest that DHA has an essential biochemical function. The most likely possibility is a membrane structural effect involving the packing of phospholipid head groups or the interaction of the lipid domains with membrane proteins. The lipids that contain the highest percentages of DHA are ethanolamine plasmalogen, phosphatidylethanolamine and phosphatidylserine. Therefore, it is likely that the function of DHA involves the metabolism, trafficking or physical properties of these phospholipids. Other possibilities that must be considered include the conversion of DHA to a lipid mediator, binding of DHA to a nuclear receptor that regulates gene expression, or formation of a DHA-centered free radical.

A central question concerning the essentiality of omega-3 fatty acids is why DHA rather than the

corresponding member of the omega-6 series, DPAn-6, fulfills this purpose. The usual Western diet contains 10- to 20-times more omega-6 fatty acid, and the same metabolic pathway is utilized by both fatty acid classes. One possibility is that DHA is utilized more efficiently than DPAn-6.

However, studies with neural cells in culture indicate that there is no appreciable difference in the uptake, retention or incorporation into phospholipids of DHA as compared with DPAn-6. While more detailed measurements may reveal a functional difference between DHA and DPAn-6, no such evidence is currently available. This suggests that DHA is utilized rather than DPAn-6 because it is more available to the tissues. Although the absolute amounts of these fatty acids in the plasma lipids are very small, there ordinarily is about five-times more DHA than DPAn-6. Furthermore, the main product formed by cultured astrocytes from omega-3 fatty acid precursors is DHA, whereas the main omega-6 product is AA. Astrocytes are the site where most of the polyunsaturated fatty acid precursors are elongated and desaturated in the brain. Thus, much more DHA than DPAn-6 appears to be available in the central nervous system.

These findings suggest the following hypothesis regarding the essentiality of omega-3 fatty acids.

Certain tissues, especially parts of the central nervous system, require a relatively large amount of a 22-carbon polyunsaturated fatty containing a 4,5-double bond for optimum function. The omega-6 metabolic pathway cannot satisfy this requirement because it operates primarily to produce AA for eicosanoid and inositol phospholipid synthesis. While some docosatetraenoic acid (22:4n-6) is made, it is primarily retroconverted to AA rather than proceeding down the pathway to form DPAn-6. Therefore, even though more omega-6 fatty acid precursors are available, the omega-6 pathway cannot produce enough DPAn-6 to satisfy tissue requirements. By contrast, the main product of the omega-3 pathway is DHA, not the 20-carbon intermediate. This fundamental

difference in the operation of the polyunsaturated fatty acid metabolic pathway is likely to be the biochemical reason why omega-3 fatty acids are essential.

(Supported by NIH grants HL49264 and CA66081)

Defining the omega 3 status in mammals

AJ Sinclair, Ph.D.

Department of Food Science, RMIT University, Melbourne, Victoria, Australia 3001

This talk examines the status of omega 3 polyunsaturated fatty acids (PUFA) as essential nutrients in mammals. The first issue to be addressed is the importance of having a surrogate champion which promotes the cause of a nutrient on a daily basis. The question, *what is your cholesterol level*, is a message which sustains the cholesterol-heart disease story. Clearly, despite the importance of anti-oxidants, fibre, folate and anti-platelet therapy in CHD, cholesterol has been a survivor. Do the omega 3 PUFA have such a champion? In other words, do we have the data to support the importance of these essential nutrients; the existence of the MRFIT data and the more recent secondary prevention data from France and India provide strong support for the essentiality of the omega 3 PUFA.

The history of the EFA reveals that the omega 3 PUFA were ignored by most for 40 years or more.

Why is this so? If their dietary absence was associated with more obvious clinical symptoms, there is no doubt there might have been an omega 3 champion. The rather subtle effects of the dietary absence (or low intakes) makes it hard to sell to the general public. For example, we know that effects of deficiency on the electroretinogram (ERG) amount to a loss of a- and b-wave amplitudes of say 30% with perhaps other more substantial losses in sub-components, however we cannot yet say what this might mean in terms of "vision" which is what the public relate to. Perhaps, we too often ignore the fact that in the EFA field there are substitute fatty acids which prevent complete absences of say 22 carbon PUFA in the retina (e.g. 22:5n-6 or 22:3n-9 substitute for 22:6n-3 in omega 3 and EFA deficiency, respectively). This argues for the importance of these types of PUFA in this tissue, however necessarily the availability of such substitutes reduces the physiological impact of a dietary deficiency. Perhaps, we should be looking for a tissue where it is possible to alter the DHA content without the substitute PUFA being present. Such a tissue is the guinea pig heart - with an ALA rich diet the level of DHA is less than 1% of the phospholipid fatty acids and it is only on the inclusion of DHA that the heart DHA level rises. Given the sound data showing the crucial role of omega 3 PUFA and DHA, in particular, on cardiac function in other species/situations, surely this tissue in this species might be a useful research tool.

In the early years, linoleic acid had a prominent role as an anti-cholesterol fatty acid, however since the 1970s the omega 3 PUFA have made a comeback in heart disease, vision and other diverse areas such as arthritis, bone development and neurological disorders. Where we currently stand is that we have much data on diet and the effect on tissue fatty acids, but relatively few data on exact intakes titrated against physiological function. This is especially true in the omega 3 and electroretinography field which was the first area where omega 3 PUFA (ALA!) were shown to have a specific physiological role. Furthermore, much of our research could be criticized because there are few studies where pure ALA has been used. It is surely no longer adequate to compare oil A (poor in ALA) with oil B (containing ALA) because of our ever increasing understanding of the potential actions of the many compounds found in the unsaponifiable fraction of

naturally-occurring oils. This highlights the need for pure ALA for research purposes.

Finally, we might be responsible for diluting the message of essentiality for the omega 3 PUFA because of the many arguments in house and in the public arena regarding the nutritional importance of ALA versus the long chain PUFA (EPA and DHA). I think it is instructive to recall that the research data show that ERG function is optimal in all animal models with dietary ALA and not dietary DHA and that the de Lorgeril and Singh data on secondary prevention indicate a role for ALA in the cardiac area.

An Evolutionary View of Dietary Recommendations

S. Boyd Eaton, M.D.

Emory University, Atlanta, Georgia, USA

Traditional Research - satisfactory for preventing classical deficiency

syndromes; less so for reducing chronic degenerative disease risk:

1. Clinical trials - generally focus on diagnosis or treatment, not prevention.

2. Mechanistic studies - limitless possible study subjects; limited funding, facilities, and investigators.
3. Epidemiology - conflicting results emphasized by media leading to public confusion and skepticism. Examples:
 - a. vitamin E, β carotene and lung cancer
 - b. fat and coronary heart disease
 - c. fiber and colon cancer
 - d. salt and overall mortality
 - e. calcium and osteoporosis
 - f. fat and breast cancer

Needed: Additional Approach - to focus future efforts, reconcile past investigative discrepancies, and provide solid theoretical basis for entire field. Viewing nutrition from the perspective of human evolutionary experience might achieve these ends.

Evolution and Nutrition

1. Basic Premise - current humans are genetic Stone Agers; cultural change since agriculture has exceeded capacity of genetic evolution to keep pace.
2. Essential goal - determine character of human nutrition during Stone Age experience.
3. Investigative approaches:
 - a. analysis of recent forager subsistence patterns
 - b. analysis of human skeletal remains - gross anatomy and radioisotopic
 - c. archaeological finds - animal remains, botanical residues, implements
 - d. nutritional analyses of game animals and wild plant foods - similar to those available before agriculture
4. Modeling
$$A(C^aX) + V(C^vX) = \text{daily energy intake}$$

A and V - mean energy content (kcal/g) of animal and vegetable foods

C^a and C^v - proportions of animal and vegetable foods, respectively

X - total number of grams of food required to provide daily energy

5. Previously reported results:

a. Protein: 30-35% total energy

b. Carbohydrate: 40-50%

c. Fat: 20-25%

Recently Revised Model Inputs

1. Hunter-gatherer subsistence patterns

a. old mean: 35% animal : 65% plant (by weight)

b. revised mean: 45% animal : 55% plant

2. Improved assessment of game nutritional properties

a. old view - based solely on muscle meats (i.e. "selected cuts")

b. new view - hunter-gatherers actually consume "total edible," hence fat content is 1.5 - 18%, not 1 - 5%.

New Estimates:

Mean macronutrient contribution (% total energy)

a. Protein 30-33%

b. Carbohydrate 31-34%

c. Fat 36%

General Fat Characteristics:

1. Energy contribution similar to Mediterranean (and current American)

pattern; unlike East Asian paradigm.

2. But character of fat is much different from U.S. pattern:

Paleolithic U.S.

% saturated less more

% C18 more less

% C14 + C16 less more

% monounsaturated more less

% polyunsaturated more less

% C20 + C22 more less

$\omega 6 : \omega 3$ lower higher

New Inputs Alter Essential Fatty Acid Retrojections:

Paleolithic Current

1998 Estimate 1999 Estimate American

Total C20 + C22 3.01 g/d 5.79 g/d 0.80 g/d

AA : $\omega 3$ LCP 1.68 1.43 5.6

Overall $\omega 6 : \omega 3$ 0.79 1.39 > 10.0

Wednesday, April 7, 1999

Session II. Essential Fatty Acids and Central Nervous System Function

Evidence for the Essential Nature of DHA in the Human and Rat Nervous System

Norman Salem Jr., Ph.D., Rebecca Greiner, Toru Moriguchi,

Jim Woods, Patricia Mena, and Ricardo Uauy

Laboratory of Membrane Biochemistry & Biophysics, NIAAA, National Institutes of Health, Rockville, MD, USA and the Institute of Nutrition and Food Technology, University of Chile, Santiago, Chile

A series of experiments were performed which demonstrate that diets that are low in n-3 fatty acids lead to low brain DHA and also lead to losses in nervous system function. Diets were constructed that varied only in the amount of alpha-linolenic acid intake derived from flax oil and adequate in linoleic

acid derived from safflower oil. No long chain (20C or more) polyunsaturates were present in these diets. Rats were raised for three generations on these diets and animals were tested at adulthood in the second and third generation. Brain and retinal DHA was markedly depressed in the second and third generations with increases in the long chain n-6 polyunsaturates, especially docosapentaenoate (22:5n6). Accompanying this "reciprocal replacement" of DHA were significant losses in performance on behavioral tasks related to learning and memory. The n-3 deficient rats acquired an olfactory discrimination task more slowly and made significantly more errors. This was significant as it extends the constellation of deficits described in n-3 deficiency to another sensory modality in addition to vision. In addition, n-3 deficient rats showed delayed escape latency in the Morris Water Maze task. This was more pronounced in the third generation where the DHA deficit was slightly greater relative to the second generation. Motor activity was not significantly different between groups. The swimming speed and distance traveled was greater for the n-3 deficient animals, yet they took a longer time to find the platform. In a subsequent memory test with the platform removed, the deficient animals made fewer crossings of the former position of the platform indicating that the n-3 adequate group better retained the memory of the position of the platform. This effect was particularly pronounced in the third generation. These experiments show that there are functional deficits associated with low brain DHA that may relate to sensory function, but it is more likely that they are due to losses in higher level functions related to information processing in the brain that are necessary for memory and learning.

In the second series of experiments, the focus was on the level of alpha-linolenate necessary to support nervous system DHA levels. An artificial rearing system was used to control the EFA content of rat pup diet from day 5-18 of life. At weaning ratios of linoleate to alpha-linolenate of 10:1 and even 1:1 did not produce the same level of brain DHA as a 1:12 ratio or that of dam-reared pups whose mothers were fed a diet containing 1.1% DHA as well as other LCPs, i.e., were well nourished. However, the 1:12 ratio led to a decrease in brain AA while the 10:1 ratio led to a slight increase over the dam-reared level. There was a similar picture in the retina, with the exception that even the extreme case of LA/LNA of 1:12 did not support the same level of retinal DHA as that of dam-reared animals. The high LNA diet (1:12) again led to a significant decrease in retinal AA. Thus it appears that increasing the level of alpha-linolenic acid in developing mammals is not an entirely adequate solution to the problem of supporting the neural DHA at a level comparable to that of a well nourished maternal reared individual. Raising the n-3 content to a 1:1 level does support a balanced EFA composition of the nervous system to a much greater extent than the 10:1 ratio, a ratio that is more typical of human infant formulas in North America.

The third issue to be addressed is the applicability of these studies to humans. Essential fatty acid metabolism was assessed *in vivo* in adults and in infants of various gestational ages and birth weights. A controlled trial in adults demonstrated conclusively that linoleic acid is converted to arachidonate and alpha-linolenate is converted to DHA. The rates of the n-6 metabolism appear faster than the n-3 conversions, in contrast to some previous findings. Increased levels of n-3 fatty acids associated with a fish-poultry based diet led to decreases in deuterium incorporation in DHA. Smoking and alcohol intake were associated with increased deuterium incorporation into DHA from linolenate. Infants are capable of LA to AA and LNA to DHA conversion *in vivo* within the first week of life even when born very prematurely (e.g., 1 kg BW). In fact, it was surprising that there was an inverse correlation of deuterium enrichment of DHA with gestational age. Although it is clear that premature and term infants express EFA metabolic activity, it must be understood that these are trace level studies; the metabolic activity towards DHA in particular is very limited and unlikely to be adequate to support rapid brain and organ DHA accretion during the first months of life.

Docosahexaenoic Acid (DHA) Supplementation of Breastfeeding Women: Effects on Maternal Plasma and Milk Fatty Acids, Infant Plasma Fatty Acids, Infant Visual and Neurodevelopmental Function and Indices of Maternal Depression

Craig L Jensen, Antolin M Llorente, Robert G Voigt, Thomas C Prager, J K Fraley,

Yali L Zou, Marcia C Berretta and William C Heird, M.D.

Children's Nutrition Research Center, Department of Pediatrics,

Baylor College of Medicine, Houston, Texas, USA

DHA, an important component of the structural lipids of brain and retina, is present in human milk but not in formulas currently available in the United States and it has been suggested that the better visual and cognitive development of breastfed infants is due, at least in part, to the presence of DHA in human milk. However, the DHA content of the milk of U.S. women, which is dependent on maternal plasma lipid DHA and, hence, intake of α -linolenic acid and/or DHA, is less than that of many other populations. Further, the DHA content of maternal plasma lipids decreases during lactation. Thus, it has been suggested that breastfeeding women and their infants might benefit from maternal DHA supplementation. Indeed, we and others have shown that maternal DHA supplementation prevents the usual decline in maternal plasma lipid DHA content and increases the DHA content of maternal plasma as well as that of milk and the recipient infants' plasma phospholipid. Based on these data, we hypothesized that maternal DHA supplementation also would result in better visual and neurodevelopmental status of the recipient infants and lessen the incidence of maternal depression which, in epidemiological studies appears to be higher in populations with low DHA intake.

To test these hypotheses, women were assigned randomly and blindly to receive either ~200 mg of DHA daily (n=80) or a placebo (n=65) for 120 days after delivery. Visual function of infants was assessed by transient visual evoked potentials (VEP) and visual acuity was measured by sweep VEP and the Teller Acuity Card Procedure at 4 and 8 months of age. Infant neurodevelopmental status at 12 months of age was assessed by the Clinical Adaptive Test/Clinical Linguistic and Auditory Milestone Scale (CAT/CLAMS) and the Gesell Gross Motor Developmental Quotient (GM DQ). Maternal depression was assessed by the Beck Depression Inventory (BDI), the Edinburgh Postnatal Depression Scale (EPDS) and the Structured Clinical Interview for Depression (SCID).

There were no differences at either 4 or 8 months in VEP latency, VEP amplitude, sweep VEP acuity or Teller acuity between groups whose mothers did or did not receive DHA. There also were no statistically significant differences in mean CAT (111.2 ± 11.0 vs. 107.3 ± 9.3) or CLAMS (101.5 ± 16.0 vs. 100.9 ± 13.9) scores of infants whose mothers did or did not receive DHA; however, the mean GM DQ of infants whose mothers received DHA was significantly greater than that of infants of mothers who did not (102.6 ± 13.3 vs. 95.2 ± 12.7 ; $p=0.03$). The incidence of postpartum depression as assessed by BDI, EPDS or SCID did not differ between groups and was lower than expected in both groups.

We conclude that maternal DHA supplementation maintains or increases the DHA content of maternal plasma lipid and increases the DHA content of both maternal milk and the lipids of infant plasma. However, in this study, these positive effects of maternal DHA supplementation were not

accompanied by better visual function, visual-motor problem-solving ability or language development of the recipient infant and also did not affect the incidence of maternal depression. On the other hand, maternal DHA supplementation resulted in the recipient infants having somewhat better indices of motor development at 12 months of age. These data, therefore, do not support our hypothesis that maternal DHA supplementation improves visual and neurodevelopmental status of the recipient infant or lessens the incidence of maternal depression. They provide little support for the concept that breastfeeding mothers require supplemental DHA.

Functional Basis for the Importance of Omega-3 Fatty Acids

in Retinal and CNS Development

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Infants fed standard infant formulas lacking DHA have low blood and tissue levels of DHA compared with those receiving pre-formed DHA or human milk. Whether dietary intake of omega-3 fatty acids has a substantial impact on CNS levels depends on the infant's stage of development and prior nutritional status. Preterm infants, who are at an earlier stage of brain development and of DHA accretion, are at greater risk than term infants of failing to achieve normal DHA levels in the retina and nervous system. However, animal studies have shown that low tissue levels are rapidly corrected once a dietary supply becomes available and, once incorporated into neural tissue, DHA is tenaciously retained. Therefore CNS levels in older children or adults are unlikely to be altered significantly by low dietary intake of omega-3 fatty acids.

For the purpose of defining essentiality, the more important question is whether a difference in fatty acid status during development is related to functional deficits. The most consistent effects of omega-3 fatty acid deficiency and supplementation have been on measures of visual system function. In monkeys and in preterm human infants, diets low in DHA's precursor, alpha linolenic acid, lead to poorer development of both visual acuity and the electroretinogram, a measure of retinal physiology. Furthermore, supplementation with pre-formed dietary DHA has been associated with enhanced visual acuity development in most studies of preterm infants and in some, but not all, of term infants.

It is assumed that these effects are mediated by differences in the fatty acid composition, and particularly the DHA content, of retinal and neural membranes. However, the underlying mechanisms for these effects, and the critical site(s) for these effects within the nervous system, are not clearly understood. Changes in the electroretinogram, which specifically measures retinal function, are hypothesized to be the result of changes in the biophysical properties of photoreceptor outer segment membranes, the site for the absorption of photons and their transformation into neural signals. These membranes contain the body's highest levels of DHA. Differences in visual acuity development, on the other hand, may be due to changes within photoreceptor membranes, other elements within the retina, the central visual pathway, and/or the visual cortex. Possible mechanisms include alterations in the development of the fovea, changes in retinal sensitivity, or changes in the synaptic connectivity or activity of the visual cortex.

Studies of DHA supplementation in human infants have reported differences in visual acuity primarily during the first few postnatal months and in one major study at one year of age. The longer-term implications of these differences in infant acuity still are unclear, due to the lack of studies with more extended follow-up.

However, it is known that restriction of visual input during early development can lead to lasting effects on visual function, so it will be important to examine this issue more closely.

Differences in visual development are of interest not only in their own right, but also because they may reflect a more general effect on neural, and perhaps cortical, maturation. Studies reporting an advantage in intellectual development in breast-fed compared with formula-fed infants have prompted speculation that the DHA present in breast milk is a critical factor. However, the difference in DHA content is confounded with many other compositional differences, as well as socioeconomic and parenting factors which are known to strongly influence intellectual development.

In monkey studies and in randomized human clinical trials, differences have consistently been found in one aspect of cognitive development, visual attention. In monkey infants fed low levels of alpha linolenic acid, and preterm human infants fed formulas without DHA, the duration of fixations to visual stimuli are prolonged compared to infants with higher DHA status. Developmental psychologists have interpreted increased look duration as indicating slower speed of processing the stimulus and encoding it into memory. It is also possible that this effect reflects a specific difficulty in shifting or disengaging attention, an ability which develops during the first postnatal year, or a difference in the intensity of the infants' responses to visual stimuli. This effect appears to be independent of effects on visual acuity, as the two outcomes are not correlated in either monkey or human infants. Longer look durations are moderately correlated with poorer achievement in later tests of cognitive development, including IQ tests at school age. Thus, as with the effects on visual acuity, the implications of this difference for later development are unclear but worthy of further study.

Both animal and human studies of the effects of omega-3 fatty acid status on behavioral development have focussed on possible changes in cognition and learning. Other aspects of behavior generally have not been examined but are of equal interest. There are good rationales to hypothesize effects of omega-3 fatty acid status on, for example, sleep and temperament. Preliminary findings in rhesus monkeys indicate changes in both sleep and responsiveness to environmental stimuli. Changes in eicosanoids or in neurotransmitter metabolism provide plausible mechanisms for such effects.

The range of functional effects of omega-3 fatty acid deficiency and supplementation during development and their impact on later vision, cognition and behavior are not completely understood, nor are the relationship of these effects to the dose of dietary DHA and the age and duration of dietary intervention. These issues can only be resolved by longer-term studies with a range of dietary treatments and functional outcomes.

Long Chain Polyunsaturates and Human Visual Development

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The Effects of DHA on Hostility

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Numbers of studies have indicated an association between Type A behavior pattern (TABP) and CHD. TABP is characterized by aggression, hostility, excessive competitive drive, and time urgency. Because TABP is a vague and complex mixture of behavior patterns, many researchers began to investigate components of the TABP construct. Hostility is the most popular factor among them. Actually it better predicts important adult diseases than TABP.

We have been investigating the effects of DHA on extraggression (aggression against others, EA) of students using P-F study originally created by Rosenzweig. In P-F study testees are asked to give comments to frustrating pictures. Those comments are judged if they are aggressive against others (EA), self or nobody. EA contains three categories: obstacle-dominance, ego-defense

(extrapunitive) and need-persistence. Comments are judged as ego-defensive, if comments contain hostile words to others, or aggressive denial or rejection against others' reproach or accusation. Thus, we regarded ego-defense as hostility in the following studies.

According to our previous three-month double-blind study, hostility was enhanced by final exams in control students, whose average intakes of DHA were about 200 mg/d, whereas hostility was not enhanced in students who took DHA capsules (1.5-1.8 g DHA/d); in another study we found that if there was no stressor like exams, hostility was not changed significantly in either the control or the DHA group. We also found that DHA administration significantly enhanced the ratio of plasma epinephrine to norepinephrine in the DHA group compared with the control group during continuous psychological stress (final exams for two months). This DHA effect was mainly due to norepinephrine reduction in the DHA group.

Those studies above were all done with young adults. But people over 50 are more susceptible to stress-related adult diseases. Consequently, we decided to perform a similar study with older subjects to investigate the effects of DHA on hostility.

Method. Twenty-two males and 18 females of 50-60 yr of age volunteered for the present

double-blind study. They were all healthy, and one half of them were farmers from suburban farming villages in Nakornpathom, Thailand. They were randomly allocated either to the DHA group (11 males and 8 females) or to the control group (11 males and 10 females). Subjects in the DHA group took 10 DHA capsules/d containing 1.5 g DHA as a total for two months, and those in the control group took 10 control capsules/d, each capsule containing 280 mg of mixed plant oil (47 % olive oil, 25 % rapeseed oil, 25 % soybean oil and 3 % fish oil). At the start and the end of the study, volunteers took P-F study. Just before they took P-F study at the end of the study, they watched a provoking videotape for 20 min as stressor. The videotape contained many cruel scenes from the real crimes and disasters.

Results. EA was significantly decreased in the DHA group ($32 \pm 15\%$ to $25 \pm 11\%$, $M \pm SD$, $p < 0.02$), whereas not in the control group ($27 \pm 16\%$ to $23 \pm 10\%$). Inter-group difference was not significant by ANOVA. Hostility was significantly decreased in the DHA group ($17 \pm 8\%$ to $11 \pm 7\%$, $p < 0.05$), whereas not in the control group ($16 \pm 11\%$ to $12 \pm 8\%$). Although the inter-group difference was not significant by ANOVA, the ratio of increment in hostility in the DHA group (2 out of 19) was significantly ($p < 0.05$) lower than in the control group (8 out of 21).

Discussion. We provoked subjects of both groups by videotape, but extra aggression or hostility did not increase in either group. The place where PF study was performed (Silpakorn University,

Nakornpathom) was not familiar to most of the volunteers. Consequently, there might be effects of becoming accustomed to the test in a very unfamiliar place at the end of the study. Although the effects were marginal compared with the case of young adults with natural stressor, it is likely that DHA influenced hostility of people even in their fifties. Taken into account that hostility is a risk factor of adult diseases, enough amounts of DHA (up to 1.5 g/d) might be beneficial.

Omega-3 Fatty Acids in Mood Disorders

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Omega-3 fatty acids may inhibit neuronal signal transduction pathways in a manner similar to lithium and valproate, two effective treatments for bipolar disorder. To examine this pharmacological similarity more closely, a study was performed to examine whether omega-3 fatty acids also exhibit mood-stabilizing properties in bipolar disorder. This was a 4-month, double-blind, placebo-controlled study, comparing omega-3 fatty acids (9.6 g/d) vs. placebo (olive oil), in addition to usual treatment, in 30 patients with bipolar disorder.

The results of the study revealed strong mood stabilizing and antidepressant effects of the omega-3 fatty acids. A Kaplan-Meier survival analysis of the cohort revealed that the omega-3 fatty acid patient group had a significantly longer period of remission than the placebo group ($p = 0.002$; Mantel-Cox). In addition, for nearly every other outcome measure, the omega-3 fatty acid group performed better than the placebo group. Omega-3 fatty acids were well-tolerated and improved the short-term course of illness in this preliminary study of patients with bipolar disorder.

The omega-3 fatty acids offer some unique benefits, should they prove to be truly effective mood stabilizers. The advantages of the omega-3 fatty acids as mood stabilizers include the apparent acute efficacy in both the manic and depressive phases of bipolar disorder, their lack of toxicity, as well as high patient acceptance. In addition, omega-3 fatty acids confer some health benefits during chronic use, such as possible reduction in the risk of a fatal myocardial infarction. In addition, the omega-3 fatty acids have no documented adverse drug interactions, and appear to be safe (and possibly beneficial) in pregnancy and in children.

The disadvantages of the omega-3 fatty acids include their low potency, which results in a relatively large number of capsules per day. This may effect compliance. In addition, at the high doses used in the pilot study, several patients treated with either olive oil placebo or omega-3 fatty acids developed mild gastrointestinal distress, generally loose stools. This was completely abolished by lowering the dosage slightly or dividing the dosage into 3 or 4 separate portions. There is also the theoretical risk of increased bleeding during high-dose omega-3 fatty acid treatment. However, no change was observed in bleeding times during the controlled trial in bipolar disorder.

We have also treated more than 20 bipolar patients with open-label flaxseed oil. Flaxseed oil contains alpha-linolenic acid, a shorter chain omega-3 fatty acid. Measuring the clinical response to an open-label treatment is unavoidably subjective. However, the majority of the bipolar patients treated with flaxseed oil appeared to benefit. Many of these patients have described a distinct mood elevating effect from the flaxseed oil, and most have elected to remain on the flaxseed oil for the long-term. As with fish oil, the flaxseed oil was used adjunctively, in that the flaxseed oil was added to whatever mood stabilizing medication the patient was already receiving. The flaxseed oil was generally better tolerated than fish oil. However, whether causally related or not, we have observed several cases of hypomania in bipolar patients treated with flaxseed oil.

Our results support other data suggesting that the mechanism of action of mood stabilizers in bipolar disorder is the suppression of aberrant signal transduction and inhibition of kindling processes. This is consistent with a model of abnormal signal transduction in the pathophysiology of bipolar disorder. If further studies confirm their efficacy in bipolar disorder, omega-3 fatty acids may represent a new class of membrane-active psychotropic compounds, and may herald the advent of a new class of rationally designed mood stabilizing drugs.

Thursday, April 8, 1999

Session III. Cardiovascular Disease

Polyunsaturated Fatty Acids and Cardiovascular Disease

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Coronary heart disease is the leading cause of death in the United States and in Western industrialized

countries. Many reports have appeared since the epidemiologic evidence of Bang and Dyerberg called attention to the low mortality from coronary heart diseases (CHD) among the Greenland Eskimos, which they attributed to potential antiatherosclerotic effects of the diet high in oil of marine vertebrates. Many studies have documented the effects of fish oils on a number of biochemical and physiologic factors that are believed to affect the atherosclerotic process. There are also a considerable number of experimental studies in animals which show a reduction in atherosclerosis when diets high in saturated fatty acids and cholesterol are supplemented with fish oils. Notably among these are the beneficial effects reported in swine and in nonhuman primates, but even in a nonhuman primate negative results have been reported.

What Bang and Dyerberg noted among the Greenland Eskimos has been largely confirmed among the Japanese. The Zutphen study by Kromhout and associates and the reanalysis of the Multiple Risk Factor Intervention Trial by Dolecek showed an inverse relation between fish intake and mortality from CHD, as have other studies. Dolecek analyzed the larger Multiple Risk Factor Intervention Trial dividing the 6000 subjects in the control group (Usual Care) for that Trial into quintals according to their mean ingestion of n-3 polyunsaturated fatty acids from 0 to 0.66 g daily and found significant inverse correlations between the ingestion of these fish oils and coronary heart disease, all cardiovascular diseases and all-cause mortality with the highest quintal having lowest mortality rates of some 40 to 50%. However, the rapid atherosclerosis-like processes that often cause restenosis following coronary angioplasty are not prevented by dietary fish oil supplements. There has been one prospective, randomized, placebo-controlled, secondary clinical trial which has reported a 29% reduction in all cause and cardiovascular mortality at 2 years follow-up in patients advised to eat oily fish 2 to 3 times per week compared with those not so advised. Another secondary, single blinded, clinical trial reported a remarkable reduction in all cause mortality at 27 months mean follow-up and recently again at almost 4 year follow-up in the same cohort of some 70% compared to controls in The Lyon Heart Study in which alpha-linolenic acid was considered the important dietary polyunsaturated fatty acid.

Reports by Charnock and McLennan have drawn attention to another aspect of coronary heart disease which the highly polyunsaturated fatty acids in fish oils seem to affect beneficially. They found that rats fed a diet high in a fish oil were protected from the fatal cardiac arrhythmias induced by experimental coronary artery ligation. We have confirmed their findings in dogs with Prof. George E. Billman, Ohio State University School of Medicine. We have then pursued the mechanism of the antiarrhythmic effect of the fish oil fatty acids. With isolated cultured heart cells we have produced arrhythmias with chemical agents which can cause fatal arrhythmias in humans. We have found in every instance that if we add the fish oil fatty acids to the fluid bathing the cells before we add the toxic agent, the arrhythmia is prevented. If we first induce the arrhythmia in the single cultured contracting heart cells and then add the fish oil fatty acids the arrhythmia is promptly stopped. This antiarrhythmic effect is due to stabilization of the excitability of every contracting cell in the heart. This in turn results from a modulating effect of the fatty acids on the ionic currents that initiate the heart beat. Further studies suggest strongly that the fatty acids interact with binding sites on the proteins of the ion channels thus affecting their conductivity to make the heart much less responsive to the electrical events that initiate fatal cardiac arrhythmias.

Once we had found that the polyunsaturated fatty acids modulate ion currents in an excitable tissue, the heart, we surmised that they must have a similar effect on all excitable tissues, since all utilize the same electrical communicating system and they do! We have reported that in the brain (hippocampal CA1 neurons) the voltage dependent Na^+ and the L-type Ca^{2+} currents are affected very much as are the same cardiac currents. One consequence of this action in the brain is that the

electrical threshold for inducing generalized seizure activity in the rat using the cortical stimulation model, is increased. So these fatty acids are anticonvulsants as well as antiarrhythmic agents. With the findings by some psychiatrists that these same fatty acids are apparently beneficial in the management of depression and bipolar behavioral disorders, the finding of an important effect of the fatty acids on the electrical activity of brain cells may have broader health implications than just to cardiovascular diseases. There remains much to be learned; we are probably just scratching the surface of the importance of polyunsaturated fatty acids to health and the prevention of diseases.

N-3 Polyunsaturated Fatty Acids Inhibit COX-2 Expression

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N-3 polyunsaturated fatty acids (n-3 FA), including docosahexaenoic acid (DHA) exert anti-inflammatory and anti-atherogenic properties, mostly ascribed to competition with arachidonic acid (AA) as substrate for cyclooxygenases and 5-lipoxygenase. A cytokine-inducible cyclooxygenase (COX-2) expressed at sites of inflammation permits high production of prostanoids and amplification of the inflammatory response. We previously showed that n-3 FA (particularly DHA) are inhibitors of cytokine-induced expression of adhesion molecules in vascular endothelial cells (EC). Since genes for adhesion molecules and COX-2 share consensus sequences for transcription factors and patterns of cytokine induction, we hypothesized that n-3 FA might be transcriptional regulators of COX-2 expression. We therefore measured changes in AA metabolism in cultured human saphenous vein EC following 48 h preincubation with 25 μ M DHA plus 24 h stimulation with IL-1 or LPS. We measured COX activity assessing 6-keto-PGF1a by RIA as a reflection of prostacyclin production. DHA decreased thrombin or AA-stimulated 6-keto-PGF1a to a greater extent in IL-1-stimulated EC than in the absence of IL-1, suggesting a greater inhibitory effect on COX-2 than on constitutively expressed COX-1. Inhibition of 6-keto-PGF1a production by DHA + the specific COX-2 inhibitor NS-398 was greater than inhibition by NS-398 alone, suggesting that DHA acted at a different level than on COX-2 enzymatic activity. Thus, COX-2 mRNA and protein expression were compared by Northern and Western analysis in control and DHA-treated EC stimulated with IL-1. DHA-treated EC showed a 50% inhibition of COX-2 expression at both mRNA and protein levels. Northern analysis of cells treated also with actinomycin D indicated that DHA exerted a transcriptional effect consistent with inhibition of NF-kB as assessed by electrophoretic mobility shift assays. These results show that treatment of EC with DHA reduces COX-2 protein expression and enzyme activity by transcriptional regulation likely to involve NF-kB activation, and offer a plausible alternative mechanism to many of the anti-inflammatory and anti-atherogenic effects of n-3 FA.

Alpha-Linolenic acid in the Prevention of Cardiovascular Diseases

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Cardiac mortality, especially sudden death, has been rarely prevented in dietary intervention trials to lower coronary heart disease (CHD). Only trials with an increased level of n-3 fatty acids (fish or fish oil) (DART Lancet 1989:2:757) have succeeded so far.

In Crete, cardiac death as shown by the seven country study is a rare event. In our duplication of the Cretan diet on 600 coronary patients (Lancet 1994:343:1454) cardiac death was reduced by 76 % and we did not observe any sudden death as compared to 8 in the control group with the prudent diet. Like the Crete population (Eur J Clin Nutr 1993:47:20), our subjects with the Cretan diet had a high level of oleic and alpha-linolenic acids in their plasma.

Studies have shown that arrhythmia of myocytes in culture, and ventricular fibrillation in dogs and rats are inhibited by n-3 fatty acids (Proc Natl Acad Sci USA 1997:94:4182). In rat reperfusion ventricular fibrillation was inhibited only by the alpha-linolenic acid rich canola oil but not by olive oil. (J Nutr 1995:125:1003)

In Crete it seems that it is through the consumption of walnuts, purslane and other greens as well as of snails, that a high intake of alpha-linolenic acid is achieved.

Recent prospective studies in USA (Harvard Public Health) and Europe (Euramic) indicate that the only fatty acid apparently inhibiting cardiac mortality in man is alpha-linolenic acid. Thus,

alpha-linolenic acid, in addition to regulating the level of prostaglandins and leukotrienes, may be the chief fatty acid protecting from the CHD clinical manifestations, cardiac death and coronary thrombosis.

Omega-3 Long Chain PUFA and Triglyceride Lowering: Minimum Effective Intakes

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<u>Author</u>	<u>Year</u>	<u>Source</u>	<u>ω3 FA (g/d)</u>	<u>Δ Trig*</u>
Ågren ¹	1996	Fish	1.05	-15% (PPL →)
Schaefer ²	1992	Fish	1.8	-7% (PPL →)
Silva ³	1996	Shrimp	0.81	-19%

Fahrer ⁴	1991	Fish	1.75	-19%
Jacques ⁵	1992	Fish	0.45	-8%
Gerhard ⁶	1991	Fish	1.95	-1%
Brown ⁷	1990	Fish	0.7	-7%
Ågren ⁸	1988	Fish	0.8	-16%
Fehily ⁹	1983	Fish	0.7	-7%
Brown ¹⁰	1991	Capsules	1.5	-25% (PPL →)
Oosthuizen ¹¹	1994	Capsules	1.6	-17%
Valdini ¹²	1990	Capsules	1.8	-16%
Gans ¹³	1990	Capsules	1.8	-33%
Beil ¹⁴	1991	Capsules	1.6	-20%
Radack ¹⁵	1990	Capsules	1.1	-10%
Roche ¹⁶	1996	Capsules	0.8	-21% (PPL →)
Demke ¹⁷	1988	Capsules	1.5	-24%
Schindler ¹⁸	1998	Capsules	1.1	-16-34% (depending on phenotype)
			(0.18 to 1.1 g/d)	
Saldeen ¹⁹	1998	Bread	0.3	-17%
Lovegrove ²⁰	1997	Multifoods	1.4	-4% (PPL →)
Sorensen ²¹	1998	Margarine	0.9	-12%

***Bold italic** = statistically significant. PPL = postprandial lipemia; → = lower on ω3 FA

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Efficacy of n-3 PUFA and vitamin E in 11,324 post-MI patients:

Results of GISSI-Prevenzione

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The protective effects of fish oil supplements and vitamin E have been long debated. Within Months of a myocardial infarction, 11,324 patients were randomized to an n-3 polyunsaturated fatty acid (PUFA) supplement (1g daily), a vitamin E supplement (300 mg daily), both, or neither. Baseline therapy included antiplatelet therapy in 90% of patients, beta blockers in 40%, and angiotensin converting enzyme inhibitors in 50%.

At 42 months follow up, patients who received n-3 PUFA had a significant 15% relative risk reduction in the combined rate of death plus nonfatal myocardial infarction and nonfatal stroke compared with those who did not receive n-3 PUFA (12.3% vs. 14.4% R= 0.001). By contrast, treatment with vitamin E caused a non significant 11% relative risk reduction in the combined endpoint. All of the beneficial effects of n-3 PUFA were due to 21% reduction in the risk of death.

There were no significant interactions between the two treatments. Both treatments were well tolerated. Gastrointestinal intolerance was the most commonly reported side effect.

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Thursday, April 9, 1999

Session IV. Relationship of Essential Fatty Acids to Saturated,

Monounsaturated, and Trans Fatty Acids

Relationships Between Saturated, Monounsaturated, Polyunsaturated Fatty Acids : Dietary Data vs. Data from Plasma Fatty Acid and Lipid Analyses

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Background

Plasma levels of individual fatty acids (FA), of FA classes and of single intermediates in the metabolic series, are the result of diversified processes : intake with the diet, transport, uptake by cells and tissues, with the additional influence of metabolic processes (*de novo* synthesis or precursor - product conversion and retroconversion in the FA series , i.e. n-9, n-6, n-3).

Determinants of plasma fatty acids

The relationships between major FA classes (saturates, SAT, monounsaturates, MUFA and polyunsaturated fatty acids, PUFA) in plasma lipids and the intake / synthesis from precursors, are rather different. In fact SAT and MUFA are synthesized *de novo*, besides being provided by the diet, whereas PUFA are exclusively supplied by the intake. In addition, long chain PUFA (LCP) of the n-6 and n-3 series in plasma and tissues represent a combination of amounts produced through the endogenous conversion of the short chain polyunsaturated fatty acids (SCP), linoleic (LA) and α -linolenic (ALA) acids to the LCP, and amounts provided directly by the diet. While the first pathway, i.e, synthesis from SCP, is the only source of LCP in strict vegetarians, the combined processes (intake + endogenous synthesis) take place in omnivorous subjects. It is however difficult to evaluate the relative contributions of these two components, due to the limited quantitative data on these "minor" individual LCP in foods. The assessment of LCP synthesis from SCP in individual subjects represents also a difficult task.

Additional factors which contribute to determine the final FA profiles in plasma lipids are : a. The different rates and degrees of esterification of individual FA into lipid classes (phospholipids, PL; cholesterol esters; CE; triglycerides, TG), as it emerges also from *in vitro* studies with cultured cells. b. The positional selectivity in the incorporation of different FA classes into glycerol. SAT are almost exclusively incorporated into the 1-position in cell PL and into the sn-1 and 3 position of TG, whereas MUFA are predominantly and PUFA almost exclusively incorporated into the 2-position. The 1-position is metabolically stable, whereas continuous replacement of FA takes place in the 2-position, through hydrolysis and reacylation processes. FA which in the 2-position should therefore be more readily modulated by changes in the relative availability of MUFA and PUFA. This should in turn result in a significant impact of the relative dietary intakes of these FA classes on the relationships between MUFA and PUFA in plasma lipids. In contrast, the intake of SAT should minimally affect their relative levels in circulating lipids.

Evaluations of FA relationships in plasma

We have measured several relationships between FA in plasma lipids, with the aim to establish possible correlations which could be of help in elucidating the processes governing the final plasma FA profile.

FA distribution in plasma lipids

The distribution of individual FA in plasma lipid classes (PL, TG, CE) in humans varies appreciably even among FA of the same class or metabolic series, as it is for instance shown in Table 1. Of the total circulating AA, the greatest proportion is associated with PL, followed by CE, and minimal amounts are found in TG, whereas LA is mostly associated with CE, followed by PL and TG. Marked differences are found also in the distribution of DHA (mainly associated with PL) and EPA (largely associated with CE). These differences may affect the relative incorporation and exchanges of individual FA with cell lipids.

Table 1. Concentrations and % levels of individual FA in plasma lipid classes in 20 women.

 % distribution

FA μ g/ml PL TG CE

18:2 640 \pm 125 34 \pm 6 10 \pm 5 56 \pm 9

20:4 148 \pm 39 67 \pm 6 4 \pm 2 29 \pm 7

20:5 9.6 \pm 3.9 57 \pm 12 11 \pm 10 31 \pm 12

22:6 28.8 \pm 9.8 85 \pm 5 7 \pm 7 8 \pm 3

FA correlations

Evaluation of the product/precursors relationships within the n-6 and n-3 FA series in Tanzanian populations on low fat diets (7-12 en%), strict vegetarians (VD) and fish eaters (FD) ingesting relatively high amounts of AA and DHA (typical in tropical fish) revealed the following : in VD only good correlations are present in the n-6 pathway (from LA to AA and especially between DHGLA and AA), and between ALA and EPA, in the n-3 series. In FD, significant correlations are found only between LA and DHGLA in the n-6 series, and between EPA and DHA in the n-3. These findings will be discussed in the context of the contributions of the exogenous supply of preformed LCP (FD and omnivores, in general) vs that of the endogenous biosynthesis exclusively. The correlations in omnivorous Italian populations (>30 en % fat), are somewhat intermediate between those in the two Tanzanian populations.

Evaluation of the correlations between SAT, MUFA and PUFA in the three populations at study, revealed that : a. there is no correlation between SAT and MUFA, weak but significant negative correlations between PUFA and SAT (SAT vs PUFA : $y=42.6-0.24x$, $r=0.535$, $p < 0.001$ in Italians; $y = 48-0.38x$, $r=0.62$, $p<0.001$ in VD Tanzanians; $y= 46.2-0.29x$, $r=0.47$, $p<0.001$ in FD Tanzanians), very strong negative correlations between MUFA and PUFA (MUFA vs PUFA : $y=58.4-0.75x$,

$r=0.89$ in Italians; $y=53.8 - 0.71x$, $r=0.80$ in VD and $y=51.9 - 0.62x$, $r=0.79$ in FD). These data obtained in populations on diets with quantitatively and qualitatively very different fat contents, fit and are in agreement with the hypothesis that PUFA and MUFA compete for esterification, whereas this does not occur between SAT and MUFA. Additional relationships which will be discussed concern those between n-6 and n-3 levels. These, in plasma, at difference with the situation in cellular lipids, do not appear to be reciprocally modulated.

In a controlled clinical study with subjects on isocaloric diets (25 en% fat) with defined FA proportions (prudent diet, olive oil based diet and corn oil based diet) we have evaluated the relationships between dietary SAT, MUFA and PUFA as en% and the same FA classes as % of plasma FA. It appeared that differences in dietary SAT between 5 to 9.6 en% result in no difference in plasma SAT (% of total FA), whereas differences in dietary MUFA and PUFA result in proportional changes in the corresponding plasma FA.

Additional evaluations on the relationships between levels of FA classes as well as of individual FA, on one side, and plasma cholesterol and TG, on the other, reveal that correlations are present only with TG.

In conclusion, the observation of selected correlations among plasma FA, based on detailed analytical data, facilitates the interpretation of the dietary and metabolic relationships between FA and plasma lipids.

Nutritional and Metabolic Interrelationships Between Omega-3 Fatty Acids and Trans Fatty Acids

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Much attention on the health concerns of current intakes of trans fatty acids (TFA) via fast and processed foods has focused upon the potential for dietary TFA to significantly increase the LDL-cholesterol level while lowering HDL-cholesterol as well as increasing triglyceride and lipoprotein(a) levels in some studies. Epidemiological studies have indicated that TFA represent a major dietary risk factor for cardiovascular disease (CVD) in the North American population. A lesser focus has been placed on the potential for dietary TFA to interfere with the convertibility of linoleic acid and alpha-linolenic acid (α -LNA) to their longer-chain metabolic products. There is also evidence that TFAs may impair early growth in humans by impeding desaturation/elongation reactions. Recent data from Health Canada (Ratnayake and Chen) has indicated that the mean TFA intake (as trans 18:1) in Canadian adults represents 3.7% of total daily energy which is above recent estimated intakes of TFA for the US population (Allison et al, J. Am. Diet. Assoc., 1999). Young males in Canada (age 18-34 years) have a mean trans-18:1 intake of 12.5 g/day with intakes as high as 39 g/person/day. One of the richest sources of TFA in the Canadian food supply is breast milk from mothers who show a mean content of total TFA representing 7.2% of total fatty acids (and up to 17.2%). Furthermore, the total TFA: α -LNA ratio in Canadian breast milk is 6.2 to 1. These high ratios reflect the very high ratio of TFA:n-3 fatty acids in the diet of pregnant and lactating women. We have analyzed a wide variety of processed and fast foods in Canada (showing very high ratios of TFA:n-3 PUFA) as well as a wide variety of baby foods (cereals and biscuits) which, in many cases, show extremely high ratios

of TFA:n-3 fatty acids. Foods containing hydrogenated vegetable oils which greatly compromise the n-3 fatty acids intake while enhancing the TFA consumption, as well as the potential for TFA to interfere with the convertibility of α -LNA to docosahexaenoic acid (DHA), likely accounts for the lower DHA status in humans consuming higher intakes of processed and fast foods containing TFA. Mandatory food labeling in North America for TFA and omega-3 fatty acids is needed to allow consumers to reduce the consumption of the former while increasing the latter. Such regulatory changes can be expected to enhance the physiological DHA status and related human health parameters beginning at conception.

**Choice of n-3, Monounsaturated and Trans Fatty Acid-Enriched Oils
for the Prevention of Excessive Linoleic Acid Syndrome**

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Excessive linoleic acid intake and relative n-3 deficiency syndrome

Animal experiments and epidemiological studies have revealed that excessive intake of linoleic acid (LA, n-6) is a major risk factor for cancers of western type, allergic hyper-reactivity, coronary heart disease (CHD) and cerebrovascular disease (CVD) (1). Although epidemiological studies performed in the USA failed to reveal a positive correlation between LA intake and breast cancer mortality, this is probably because the proximate marker for breast cancer is the proportion of n-6 eicosanoid precursors in phospholipids, which is saturated both in the high and low LA intake groups in the USA. Empirical equations presented by Lands indicate that both increasing the intake of n-3 fatty acids and decreasing that of n-6 fatty acids are necessary for effectively decreasing the n-6 eicosanoid precursors in phospholipids and thereby decreasing cancer mortality. On the other hand, high n-6/n-3 ratio but not hypercholesterolemia has been proved clinically to be a major risk factor for thrombotic diseases. Over-production of inflammatory lipid mediators of n-6 series has been shown to be a major cause for the rapid increase in allergic hyper-reactive patients in Japan.

President's Summary 1997 from the Japan Society for Lipid Nutrition

After discussion through several annual meetings of the Japan Society for Lipid Nutrition, Presidents Summary 1997 was published (in Japanese) as a review article (J. Lipid Nutr. 6:5-42, 1997), in which 20% as total fat energy was recommended for those with moderate physical activity. For healthy populations, saturated plus monounsaturated : n-6 : n-3 = 2.5 : ≤ 0.8 : ≥ 0.2 (n-6/n-3 ≤ 4) was recommended. For the primary and secondary prevention of those diseases described above, an n-6/n-3 ratio of 2 was recommended. The latter value was based on: 1) even the n-6/n-3 ratio of Danes was 3 in a well known epidemiology of Greenland natives; 2) the ratio of current Japanese is 4 but the incidence of cancers of western type has been increasing rapidly, and the ratio of 4 or above cannot be recommended; 3) animal experiments have shown the effectiveness of decreasing n-6/n-3 ratio to below 2 for the suppression of carcinogenesis and metastasis; and 4) the safety of n-6/n-3 ratio of 1 has been established in animal experiments and in a retrospective study on hunters and gatherers' foods.

In order to meet the recommendations described above, vegetable oils with n-6/n-3 ratios of 2 or below and those with very low n-6 fatty acid contents (e.g., high-oleic type) are useful. However, there was another criterion to be considered; the presence of minor components which affect animal physiology seriously.

Survival time-shortening and renal injury induced by some vegetable oils and partially hydrogenated oils in SHRSP rats

Using soybean oil as a control, some oils were found to prolong the mean survival time of SHRSP rats by ca 10% (e.g., DHA-rich fish oil, perilla seed oil, flaxseed oil) while some others shortened it dose-dependently by ca 40% (double-low rapeseed oil, evening primrose oil, high-oleate safflower oil, high-oleate sunflower oil, olive oil and partially hydrogenated rapeseed and soybean oil). When the rapeseed oil was lipase-treated, the resulting free fatty acid fraction was almost free of such activity, indicating that the survival-time shortening activity is due to minor components other than fatty acids in these oils. Free fatty acid fraction from partially-hydrogenated soybean oil exhibited a survival time between those of the original oil and soybean oil. It should be emphasized that lard, sesame oil and high-linoleate safflower oil were relatively safe for the SHRSP rats.

Those oils with survival-time shortening activity were found to cause renal injury; lesions in blood vessels, accelerated proteinuria, decreased platelet count and elevated gene expression for TGF β , fibronectin and renin.

Choice of n-3, monounsaturated and trans fatty acid-enriched oils

In order to decrease the n-6/n-3 ratio of our current foods to 2 or below, the intake of high- α -linolenate oils such as perilla seed oil and flaxseed oil as well as seafood and vegetables should be increased. High-linoleate oils are inappropriate for human use as foods. For deep-frying and preservation purpose, high-oleate vegetable oils are useful but all the high-oleate vegetable oils and hydrogenated vegetable oils we have examined so far exhibited the survival time-shortening activity,

and I cannot recommend people to have these oils in large quantities. Instead, lard was safe for this animal model, and could be used in quantities not to induce obesity; animal fats as well as a high-LA vegetable oil intake caused insulin resistance in a NIDDM model of rats.

Reference

Okuyama, H., Kobayashi, T., and Watanabe, S. (1997) Dietary fatty acids ñ The n-6/n-3 balance and chronic, elderly diseases. Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. *Prog. Lipid Res.* 35:409-457.

Friday, April 9, 1999

Session V. Dietary Recommendations and Omega-6:Omega-3 Ratio

Intakes of Dietary Fatty Acid in the United States: Results from the USDA's 1994-1996

Continuing Survey of Food Intakes by Individuals

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The USDA has been conducting biennial food intake surveys for many years. In the more recent surveys individual fatty acid intakes were estimated from food composition data accumulated and published in the USDA Handbook 8, "The Composition of Foods." In the compilation from the combined 1994 and 1996 surveys, the USDA has published data on 19

individual fatty acids in the US diet. Included are all the saturated fatty acids from C-4 to C-18, the monounsaturated fatty acids 16:1, 18:1, 20:1, and 22:1 and the polyunsaturated fatty acids 18:2, 18:3, 18:4, 20:4, 20:5, 22:5, and 22:6. The data is broken down by sex and age from less than 1 year to more than 70 and by percent of calories or grams per day. Although there are some age dependent trends in the consumption data, the majority of the population does not show significant differences between five and sixty years of age, and only slight differences due to sex. In the USA 11 percent of calories are consumed from saturated fat, 13 percent as monounsaturated fat, and 6 percent as polyunsaturated fat. Oleic acid is the preponderant monounsaturated fatty acid, and linoleic acid is the major polyunsaturated fatty acid. The USDA data for monounsaturated fatty acid presumably include trans isomers of monounsaturated fatty acids and are grouped together with 18:1 fatty acids. Current calculations using the best available estimated of trans fatty acid C-18 isomers in the foods consumed by the US population suggest that the actual consumption of trans configuration fatty acids is 3 percent and cis-monounsaturated fatty acids is about 10 percent. The saturated fatty acid category exhibits a broader distribution of fatty acids consumed than that observed for the unsaturated fatty acids; 12:0, 14:0, 16:0 and 18:0 all contribute significantly to the fat calorie intake in the US population. Palmitic acid accounts for 20 percent of fat calories and stearic acids about 9 percent. Of particular interest to this workshop is the intake of long-chain polyunsaturated fatty acids, especially those with twenty or more carbon atoms in the fatty acid chain. Unfortunately, the USDA data contain relatively little information on this topic. Due to the nature of survey information and the sparsity on information regarding long-chain polyunsaturated fatty acids in the food composition data from which the tables are prepared, no detailed view of the intake of omega-3 fatty acids can be made. The data do show that the US population consumes approximately 10 times the amount of omega-6 fatty acids as omega-3 (the ratio is 0.11 n3/n6),

but it is probable that the USDA data underestimated the omega-3 intake. In terms of grams per day the mean intake of linoleic acid plus arachidonic acid is 13.0 while the intake of α -linolenic acid plus docosahexaenoic acid is 1.5. The ratio of docosahexaenoic acid to arachidonic is, however, 1 (0.1 to 0.1 grams per day). As it is not possible to demonstrate an omega-3 fatty acid deficiency in the US population, the intake of 2 grams per day of omega-3 fatty acids must be at least the required daily intake when the intake of omega-6 is 20 grams per day. Of course, 13 grams per day of omega-6 fatty acids are likely to be considerably more than the required daily intake. Evidence from animals, and limited human data, suggests that the required daily intake is likely to be less than 5 grams per day (2 percent of calories). Whether the required daily intake of omega-3 fatty acids would be less if less omega-6 fatty acids were being consumed is unknown. If the total fat intake is reduced, it may be necessary to increase the intake of omega-3 fatty acids to avoid omega-3 fatty acid deficiency. Neither the USDA nor the federal Government have a recommendation for the DRI of polyunsaturated fatty acids presently. It is unlikely that a single amount could be recommended for all age ranges. The requirement for omega-3 fatty acids will probably be age dependent. Whether there is an absolute requirement for polyunsaturated fatty acids with twenty or more carbons in the chain remains to be determined.

World Health Organization/Pan American Health Organization

(Status of EFA Worldwide)

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The three main nutritional problems in the world are protein-energy malnutrition (PEM), micronutrient deficiencies (iron, vitamin A, iodine and folic acid) and overweight/obesity.

Stunting is the most common expression of PEM, but other forms of PEM are equally frequent among children below two years of age and child-bearing women.

Iron deficiency is the most widespread nutritional problem. Vitamin A and iodine deficiency showed a declining trend during the past years as a result of supplementation and fortification strategies, carried-out in countries.

Obesity is increasing worldwide at an alarming rate in both developed and developing countries. This situation is associated with rapid changes in dietary patterns and lifestyles.

Data from several countries show relatively high prevalence of obesity, particularly in women from poor urban areas. Furthermore, a sharp increase in morbidity and mortality rates due to nutrition-related non-communicable diseases has been reported.

One of the most important factors underlying this scenario among low socio-economic groups is the increase in energy intake associated with higher fat and refined carbohydrate consumption accompanied by a low iron, zinc, and folic acid intake.

Very little information exists on quality and composition of fats by low social-economic groups in the majority of countries.

Information in this area is of utmost importance to guide the selection and consumption of healthy diets as part of the Health Promotion strategy of PAHO/WHO.

n-3 Fatty Acids: Food Supply, Food Composition and Food Consumption Data

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Food supply data, food composition data, and food consumption data provide a fundamental basis for assessing the health and nutritional adequacy of individuals and populations. FAO has the UN mandate for these activities, and regularly produces Food Balance Sheets (FBS), which provide food supply data, including selected nutrient values. This paper will highlight estimates of available n-3 fatty acid containing foods from around the world. Most commonly, the nutrient data from FBS are expressed only in terms of energy, protein and fat. These international "default" nutrient values are being revised and the list of nutrients is now being expanded to include several micronutrients. In 1998, the cereals group was completed, and in 1999 the fish group will be revised. Under discussion is the possibility of including fatty acids and/or n-3 fatty acids as a special nutrient category for the fish group. Food composition activities in FAO come under the auspices of the FAO/UNU INFOODS project. We are providing assistance in all technical aspects of food composition. More and more frequently, fatty acids are included among the nutrients shortlisted by countries for inclusion in their national and regional food composition databases and tables. The inclusion of fatty acids is based on requests from the countries' users and potential users of food composition data, and

the countries' diet-related morbidity and mortality statistics. Commonly used laboratory instruments (gas chromatographs), well-defined analytical methodologies, and the availability of primary and secondary reference materials and standards, make analysis of n-3 fatty acids a routine activity for many laboratories. Data on n-3 fatty acids are now being generated, compiled and disseminated in many countries, including many developing countries. Sources and quantities of n-3 fatty acids will be presented. Food consumption data are routinely used, along with food supply data and food composition data, to establish food security at household, district and national levels. FAO prepares Nutrition Country profiles, which to date have not included assessment of n-3 fatty acids. However, now that acceptable quantities of high quality n-3 fatty acid data are becoming available from food composition laboratories, n-3 values can be incorporated into supply data, and food consumption studies will in the next few years be capable of reporting the n-3 fatty acid consumption in the assessments of food security.

BASF's Approach to Commercialization of Long Chain Omega-3 Fatty Acids

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The goal to deliver omega-3 fish oils without adverse taste, odor and to prevent oxidative degradation has been a formulation objective. Utilizing spray-cooling technology for microencapsulating highly refined, deodorized and stabilized fish oils has proven to be successful in regard to producing powdered products that can be used to formulate fish oils into most all conventional food forms. Formulated food products, such as pastas, cereals, and even beverages, can be formulated with microencapsulated fish oils at levels of about 100 mg LCPUFAs per 100 gram product without detection of their inclusion.

The powdered microencapsulated product has been evaluated in clinical investigations to confirm its equivalency to the bioavailability of oils. Studies are being conducted to establish the product's use in formulated food form to deliver meaningful amounts of omega-3 fatty acids to pregnant women and ultimately to their breast fed infants.

BASF is enhancing its activities to educate and promote the incorporation of long chain omega-3 fatty acids to the food industry in a variety of ways: participating in the scientific community by funding studies and providing test materials, developing educational materials for health care providers as well as retailers and the consumer, promoting the use of omega-3s through advertisements and public relation activities, and developing a trademark to help draw attention to the incorporation of a unique food ingredient.

BASF will continue to support trade and professional associations working towards the establishment of Dietary Reference Intakes and health claim allowances.

Essential Fatty Acids and the Products of the Groupe Danone for Human Nutrition

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Recent evaluations on the intake of fat in France show it to be in the range of 38 % of total calories : 50% of that fat is hidden in raw materials (27 % in meat and fish, 17 % in dairy products, 6 % in fruit and vegetables). The other 50% of the lipids consumed are added directly to the recipes; 30 % by the consumers themselves (butter, margarine, oil), 20 % by the food industries (meal, sausage, biscuits). Nevertheless, in recent years the intake of fat appears to be on the decline.

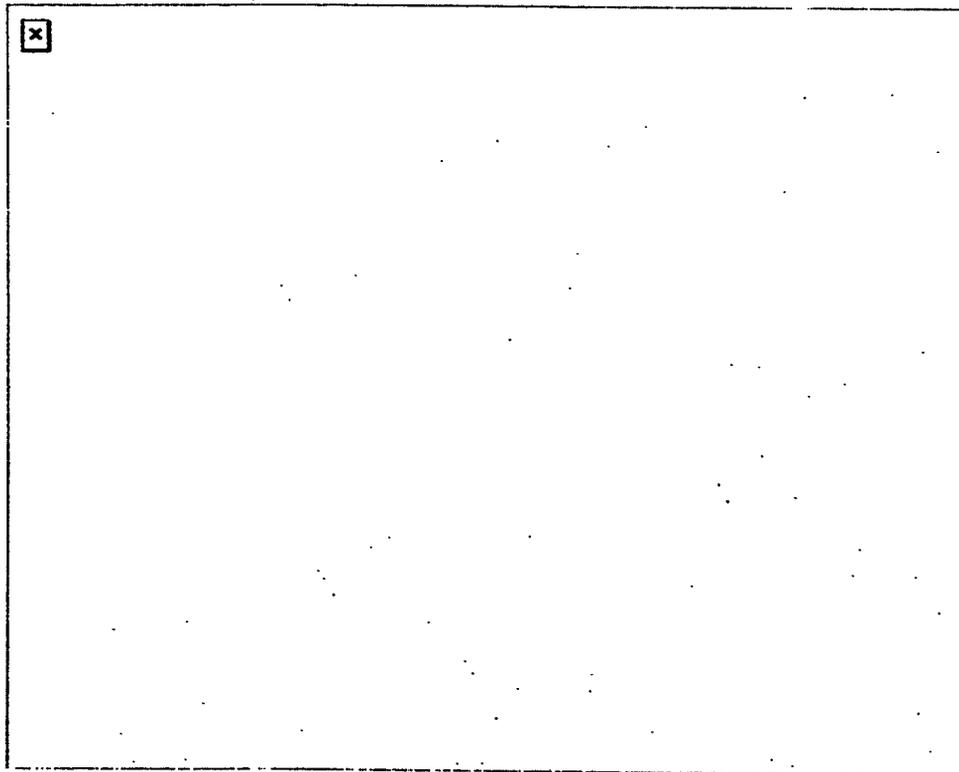
In an ongoing prospective study on 15,000 French people, it has been found that saturated fatty acids supply 17 % of calories, mono-unsaturated 14 % and polyunsaturated 6 % :

	Men	Women
g/day		
Total lipids	100.4	78.6
MUFA g/day	37.8	29.4
PUFA g/day	16.6	12.7
SFA g/day	44.8	35.6

Suvimax 1998

The ratio n-6/n-3 fatty acids is probably in the range of 15-20.

Over the last 30 years, recommendations have been changing in relation to the type of fatty acid to be included in the human diet and the total amount of fat suggested. The Danone group has been closely following this trend and has tried to adapt its products to the recent recommendations.



Trend in recommendations in fatty acids ratio : Danone approach

The present conclusion in France is that the population consumes too much saturated fat as well as too much n-6 polyunsaturated fatty acids.

Thus, the food industry has to change the fatty acid composition of its products to readjust the intake of fatty acids in the French population. Instead of using butter, tallow or different oils, canola can be utilized. Canola oil is a typical example of a fat containing a small amount of saturated fatty acids, and supplying both n-6 and n-3 polyunsaturated fatty acids in a proper ratio to counterbalance the fatty acid intake from meat, dairy products and other sources.

Thus, it seems fundamental that the experts in nutrition express clear recommendations in the field of fats and fatty acids, in relation to public health, since we, the food industry, will follow their recommendations.

Advantages and Disadvantages of the Use of Flax Seed as a Source of Omega-3

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Flax seed is presently being used worldwide as a source of Omega-3 in human and companion animals' diets. History of the use of flax seed as food for humans goes back 2,000 years. Flax seed has several distinct advantages and disadvantages as a source of Omega-3.

Disadvantages of flax seed include such factors as:

1. Presence of "Anti B-6" factor.

2. Presence of Cyanogenic Diglycosides.

3. Unstable after being ground.

4. Contains only short chain Omega-3.

Advantages of flax seed include such factors as:

1. High concentration of alpha-linolenic acid.

2. Presence of powerful anti-oxidants in some varieties.

3. Presence of high levels of soluble and insoluble fiber.

4. Presence of high levels of lignans that have anti-estrogenic properties.

5. FDA states, "no objection" as a food.

6. Desirable flavor in most foods.

Omega-3 LC-PUFA - from a Health Concept to Foods in the Shelves

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Incorporating long-chain polyunsaturated fatty acids (LC-PUFA) into the diet, continues to be a topic of interest among food manufacturers. Nutritionists believe that addition of omega-3 LC-PUFA - eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) - to the diet would result in better nutrition and assist in chronic disease prevention. General scientific opinion appears to be that as little as 200-1000 mg of EPA/DHA may achieve this dietary goal.

The refining technology for marine oils has advanced to the degree that, with due care, careful handling and proper precautions, it is now possible to produce a variety of foodstuffs fortified with omega-3 LC-PUFA that taste as good as similar, unfortified products.

At the forefront of developments are infant formula and baby follow-on food in Europe and the Far East. In addition, breads, margarines (or other low-fat spreads), UHT milks, yogurts, fruit juices and beverages have started to enter the mainstream in Europe. Niche products such as soups, salad dressings, mayonnaise, ice tea drinks, cakes, biscuits and the restoration of omega-3 LC-PUFA to canned seafood and tuna are being launched.

Despite of this growing list, many food manufacturers are still reluctant to develop products fortified with omega-3 LC-PUFA due to the following non-technical barriers:

Recommendations: There are no officially recognized intake recommendations for omega-3 LC-PUFA. The food manufacturer has no standard of reference for the nutritional value or dietary fortification levels of omega-3 LC-PUFA.

Claims: No product label or health claims are permitted by the FDA and other regulatory authorities, which makes it extremely difficult to market a food with omega-3 LC-PUFA.

Safety: There is an unwarranted fear of allergenicity and the possible effects of omega-3 LC-PUFA on bleeding and insulin resistance. There are numerous reports that such adverse reactions do not occur even at the maximum dosages of omega-3 LC-PUFA which are considered to be health beneficial (1-2g).

Awareness: The awareness about the health benefits of omega-3 LC-PUFA is generally poor among consumers. The closing of this knowledge gap is made difficult by the bewildering number of and sometimes complicated names for the family of omega-3 LC-PUFA and its members e.g. PUFA, HUFA, n-3 LCP, omega-3 LC-PUFA, EPA, DHA, eicosapentaenoic acid, docosahexaenoic acid.

To make omega-3 LC-PUFA a standard food ingredient it is imperative that food industry suppliers, food manufacturers and professional organizations (such as ISSFAL) work hand in hand to remove these obstacles by providing authorities, health professionals and the public with truthful, scientifically valid information about the health benefits of omega-3 LC-PUFA.

Infant Formulas with no DHA or ARA. Are They Causing Harm?

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Over the past twenty years there have been a large number of retrospective studies comparing the neurological outcomes of breast-fed and formula-fed infants. A recent meta-analysis of the most relevant of these studies has indicated that there is a consistent 3-4 IQ point advantage to the breast-fed infants even after the contributions of all other confounding factors had been removed. Breast-fed babies, however, are getting many nutrients from the breast milk in addition to docosahexaenoic acid (DHA) and arachidonic acid (ARA) and many have argued that the contribution of DHA and ARA is

inconclusive. One observation, however, is very clear and consistent. Infants who are provided standard infant formula have significant deviations in their blood and brain biochemistry relative to the breast-fed babies. Full term infants fed unsupplemented formulas have a circulating DHA status (as indicated by red blood cell or plasma phospholipid DHA levels) of less than one-half that of the breast-fed infant. Furthermore, the brain DHA levels of formula-fed infants are about one-third lower than those of

breast-fed infants.

Since some groups have also argued that such changes in the blood and brain biochemistry in the formula-fed infant is irrelevant, it has been critically important to more fully understand the function of DHA in the tissues of the body. Recent studies have revealed that DHA has many critical functions in the normal development and metabolism of neuronal cells. These include, but are not limited to, the following: 1) the control of normal migration of neurones from the surface of the ventricles of the brain to the cortical plate during development; 2) the control of the normal resting potential of the neurone by regulation of sodium and calcium channels; 3) the regulation of the density of certain membrane proteins such as rhodopsin in the retina and, possibly, 4) the regulation of levels of certain neurotransmitters such as serotonin. With such key roles in normal neuronal development and function, it is quite plausible that abnormally low levels of this primary nutrient during the development of the brain may be one cause of the long term neurological detriments observed in formula-fed infants relative to breast-fed infants.

The final proof of the importance of DHA in early infant nutrition, however, comes not from demonstrating that the long term neurological outcome of formula-fed infants is poorer than breast-fed infants, or that this poor outcome is correlated with a DHA deficiency early in life, but from interventional studies which demonstrate that when the DHA deficiency is removed, the neurological outcomes revert to normal. There have been at least 24 well-controlled studies involving over 2,000 infants in the last 15 years (12 studies with term infants and 12 studies with pre-term infants) which have compared outcomes of standard formula-fed infants with DHA-supplemented formula-fed infants. In every study the DHA status of the infants was returned to normal (as defined by the DHA status of the breast-fed infants) when the formulas were supplemented with DHA. In all of these studies, except where fish oil was used as a source of DHA, the ARA levels were also normalized because of the use of supplemental ARA in the formulas. In several studies, precursors such as gamma-linolenic acid (GLA) or alpha-linolenic acid (ALA) were added to the formulas in an attempt to elevate ARA or DHA levels respectively. Even when added in significant excesses over what is found in breast milk however, these precursors did not elevate the DHA and ARA levels to those of the breast-fed infant. That is, the precursors do not adequately substitute for the preformed DHA and ARA provided in mother's milk. Of all the trials completed with DHA/ARA supplementation, single cell oils (SCOIs) were used with the largest numbers of babies (45% with SCOIs, 35% with egg yolk; and 20% with various fish oils).

Of the 24 DHA/ARA supplementation studies mentioned above, only 12 looked for functional outcomes differences (i.e., visual, neurological, or developmental assessments). Seven of those 12 studies reported statistically significant deficits in standard formula-fed babies compared to breast-fed babies (the gold standard). In all 7 cases, those deficits were normalized with the DHA/ARA supplementation. Of the remaining 5 studies, no statistically significant differences could be found between formula-fed and breast-fed babies using the test metrics employed in those studies and, therefore, no effect of DHA/ARA supplementation was observed.

The totality of these observations provide strong evidence that DHA is a critical nutritional requirement for the newborn infant and that an early deficiency of DHA could lead to long term neurological deficiencies. Given our present state of understanding, it is quite possible that the lack of availability of DHA and ARA-supplemented infant formulas in the United States and Canada today may be putting formula-fed newborn babies at risk. Since the only way that newborn babies in the United States and Canada can get DHA and ARA today is from their mother's milk, we must use our best efforts to encourage new mothers to nurse their babies for as long as possible to avoid potential long term neurological deficits to the child.

Clinical Safety Studies of LCPUFA Supplementation of

Premature and Term Infant Formulas

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Introduction: Many studies support a need for long chain polyunsaturated fatty acids (LCPUFA), and particularly docosahexaenoic acid (DHA, 22:6n-3), for optimal retinal and neural development in early infancy. Human milk contains LCPUFA, including DHA and arachidonic acid (ARA, 20:4n-6), but U.S. infant formulas do not. We have now completed two of the largest clinical trials of LCPUFA supplementation, one with very low birth weight infants and a second with healthy full term infants.

Premature Infant Study: DHA supplementation has been shown to enhance visual development of preterm infants, but some studies found decreased growth when DHA was provided without ARA. **Objectives:** (1) To establish the safety of feeding DHA and ARA from single cell oils to preterm infants and (2) to determine effects on visual acuity. **Design:** In a double-blind, controlled, multi-center trial, 194 preterm infants were randomized to preterm formulas differing only in fatty acid content: no DHA or ARA (control), 0.15 % (of energy) DHA, or 0.14 % DHA + 0.27 % ARA. Preterm formulas were fed for at least 28 days; all preterm infants then received unsupplemented term formula. Ninety breast-fed term infants were enrolled as a reference group. **Results:** Growth suppression was not seen in the DHA or DHA+ARA groups; in fact, post-hoc analyses indicated that weight gain of DHA+ARA infants was significantly enhanced compared to control. Weight of DHA+ARA infants was not different from breast-fed term infants at 48 and 57 wk postmenstrual age (PMA), but weight of control and DHA infants remained significantly less than breast-fed term infants through 57 wk PMA. There were no significant differences between preterm groups in incidence of serious adverse events, NEC/suspected NEC, or sepsis/suspected sepsis. Visual acuity determined by Teller Acuity Cards (TAC) at 48 and 57 wk PMA did not differ among preterm groups. **Conclusions:** Single cell oils are safe for use in preterm infant formulas to provide DHA and ARA at human milk levels. Providing DHA plus ARA enhances catch-up growth of premature infants; however, supplementation for 28 days did not affect TAC acuity 3 and 5 months later.

Term Infant Study: Studies of LCPUFA supplementation of formula-fed term infants have shown equivocal effects on visual and cognitive development, but several recent studies with typical human

milk levels of DHA have found beneficial effects. Because term formulas may be fed for a full year, the safety of LCPUFA supplementation over this time period must be established. **Objectives:** (1) To establish the safety of feeding DHA from single cell and fish oil sources, each in combination with ARA from single cell oil, to term infants to a year of age and (2) to evaluate effects of supplemented formula on visual acuity and mental and psychomotor development. **Design:** In a double-blind, multi-center trial, 383 term infants were randomized to formulas differing in fatty acid content: no LCPUFA (control), 0.15% (of energy) DHA and 0.3% ARA from single cell oils, or 0.15% DHA from fish oil and 0.3% ARA from single cell oil. **Results:** Weight gain from day 14 to days 60 or 120 was not significantly less in supplemented groups compared with the control. Furthermore, post-hoc analyses indicated that supplemented infants had larger growth rates than control infants from 14 to 60 and 120 days. No differences were observed in mean weight, length or head circumference at 180, 270, or 365 days; in formula acceptance and tolerance; or in incidence of serious adverse events. No differences were observed in visual acuity (TAC) at 120, 180, and 365 days or in Bayley MDI and PDI scores at 365 days, although Bayley scores were somewhat higher in supplemented groups than in the control. **Conclusions:** DHA from single cell oils and ARA from single cell oil are safe for use in term infant formulas when fed at human milk levels for a full year. Supplementation with DHA and ARA increased early growth of term infants, similar to our findings with preterm infants, but did not significantly affect TAC acuity or mental or psychomotor development.

Overall Conclusions: Our large clinical trials, along with numerous other clinical and toxicology studies, demonstrate the safety of adding typical human milk levels of DHA and ARA to both premature and term infant formulas over the time periods these formulas are typically fed. While our trials did not find significant benefits of LCPUFA supplementation for visual and cognitive development, we did find increased growth in both premature and term infants supplemented with DHA plus ARA. This increased growth may be particularly important with regards to enhancing catch-up growth of infants born prematurely.

Omega-3 Long Chain PUFA - Closing the Nutritional Gap

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Significant research shows that the populations of many industrialized nations, including the U.S., consume significantly lower levels of omega-3 long chain PUFA than science shows is required for maintaining good health. There needs to be a concerted effort by industry, government and the scientific community to ensure that this nutritional gap is eliminated.

Trends - The Time to Act is Now

There is significant momentum and steam building that highlights the need for a cooperative effort in ensuring that the populations benefit from the improved science and manufacturing capabilities now in place.

Some of the important trends taking shape include:

- ? The improved manufacturing capabilities that permit the fortification of good tasting, stable food products as seen in many parts of the world;
- ? The ability to manufacture highly concentrated oils that can be delivered as adjunctive therapies;
- ? The increasing awareness (54% - Applied Biometrics, October 1998) of consumers with respect to the health benefits of omega-3 that now needs to be converted into usage;
- ? Improved collaboration between industry and science;
- ? Improved science showing the benefits of increased consumption of omega-3 LC-PUFA.

Steps to Success

In order to ensure that consumers benefit from the science, it is going to be essential that officially recognized intake levels are set for omega-3 LC-PUFA. Omega-3 LC-PUFA will not gain mass-market acceptance or incorporation into standard food channels until the manufacturers have an officially recognized reference point and/or the ability to make an approved health claim.

Key steps to success:

- ? Establish officially recognized intake recommendations for omega-3 LC-PUFA that manufacturers can reference on the label;
- ? An FDA-approved health claim for omega-3 LC-PUFA with reference to cardiovascular health and triglyceride lowering;
- ? A better understanding of the correct omega-6 to omega-3 ratios and the upper and lower limits based on age and health status;
- ? The standardization of analytical methods to ensure consumers and industry are able to make true product comparisons against the science;
- ? Quality standards enforced to ensure that consumers are not exposed to substandard product with contaminants or oxidative problems.

To make omega-3 LC-PUFA a standard food ingredient, the time to act is now. We need to form partnerships between industry suppliers, food manufacturers, professional organizations and the government. The goal is to utilize the present market conditions in an effort to ensure that consumers are given the best opportunity at better nutrition through the proper balance and total consumption of omega-3 LC-PUFA.

Safety of Omega-3 Products Based on Fish Oil as Starting Material

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Pronova is the largest producer of omega-3 products in the world today with products ranging from pharmaceuticals via medium concentrated food supplements to refined crude fish oil.

Convincing monitoring of safety is only possible in controlled clinical studies, preferably by so-called Good Clinical Practice studies. The present database of patients in controlled studies on active treatment comprises more than 9000 individuals mainly in long-term studies of more than one year; 60% more than 3.5 years. This study population consists of patients with chronic diseases related to the cardiovascular and renal system but also diabetics with age ranging from early adolescence up to 70 years and more.

Today we have no report of serious adverse effects, whatsoever. Even if bleeding time has been prolonged with omega-3 products, there are no reports of serious bleeding events even in patients on concomitant medication with Aspirin or Warfarin. Adverse effects are seen in 10-20% of the patients in studies mainly originating from the GI tract. Eructation of fishy taste is the most common finding. Interestingly, the frequency of eructation is the same in the placebo group receiving corn oil as in the active treatment group indicating that eructation is a function of ingesting oil in general. Studies including diabetics with a total number of approximately 1500 patients have not shown derangement of diabetic control. Patients with chronic renal disease, renal failure and even transplanted patients on chronic cyclosporin medication have not shown any systemic adverse effects but rather an improvement of renal function. In studies on pregnant women there have been no bleeding complications and the amount of bleeding during labour has not been significantly different from controls.

The regulatory authorities in countries like the US and several EU countries have examined the safety file of the pharmaceutical, Omacor, and there have been no major objections. Omacor is a registered pharmaceutical in several EU countries and an application for an NDA in the US is planned for later this year. At the recent American College of Cardiology meeting in New Orleans, the results of GISSI Prevention were presented. This is a study including 11,324 post-MI patients comparing 1g of Omacor, vitamin E and the combination with a control group. All patients were optimally treated with aspirin, beta-blockers, statins, etc. The Omacor group but not vitamin E showed a 20% reduction of mortality, and treatment was very well tolerated. Conducted by the prestigious Mario Negri Institute of Milan, Italy, this study is the most important documentation of efficacy and safety for any omega-3 product in the world today.

An interesting adverse report from one patient on omega-3 treatment in Houston, USA was an "urge to swim". We take this more as a joke but we would like to use this metaphor claiming that products using fish oil as starting material, and therefore containing both EPA and DHA, are the state-of-art today and based on a natural dietary principle and accepted by regulatory authorities as safe during long term use. Pure DHA products are expensive and the DHA content will readily be retro-converted to EPA in humans to meet metabolic needs. Mechanistic studies on separate effects of EPA or DHA will have to be conducted in *in vitro* systems but the results will have only minor impact on therapy traditions using omega-3 products introduced today.

In conclusion, Pronova, as the world's largest producer of omega-3 products using fish oil as starting material, holds the largest database on safety as well as efficacy in patients and healthy individuals today. These products are regarded as safe when used either as pharmaceuticals, food supplements, or

in fortification of food products.

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