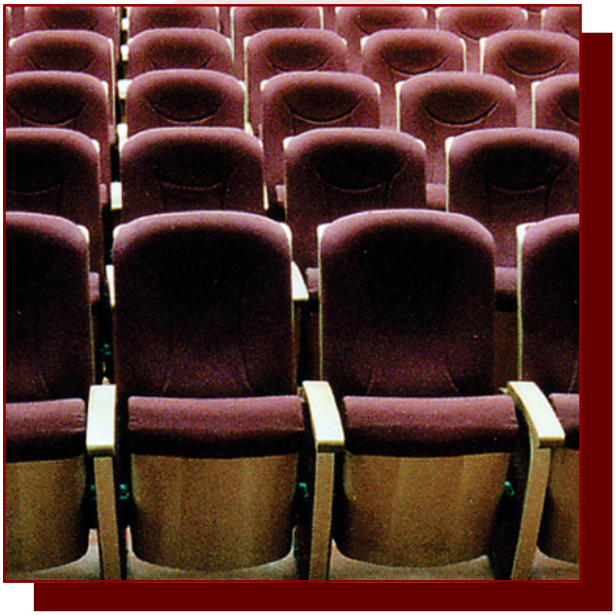
THE PHYSICIAN'S CONCISE GUIDE TO:

- 9 -

2002 First International Essential Fatty Acid (EFA) Conference

Shanghai, China



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Brian received an appointment as an Adjunct Professor at Texas Southern University in the Department of Pharmacy and Health Sciences (1998-1999). **The former president of the University said of his discoveries: "...His nutritional discoveries and practical applications through *Life-Systems* Engineering are unprecedented."** Brian earned his Bachelor of Science degree in Electrical Engineering from Massachusetts Institute of Technology (MIT) in 1979. Brian founded the field of *Life-Systems* Engineering Science in 1995. This field is defined as *The New Science of Maximizing Desired Results by Working Cooperatively with the Natural Processes of Living Systems*. To many, Brian is THE MOST TRUSTED AUTHORITY ON HEALTH AND NUTRITION IN THE WORLD.

Brian continues to be a featured guest on hundreds of radio and television shows both nationally and internationally. His sheer number of accomplishments during the last decade of the 20th century and into the 21st century are unprecedented and uniquely designate him as the #1 authority in the world of what really works and why. Forget listening to the popular press or most popular so-called health magazines. Their editors simply don't understand the complicated science that they write about – they merely "parrot" what everyone else says without independent scientific verification. Their recommendations often have no basis in reality of how the body works, based on its physiology.

Brian has dedicated his life to provide the truth – which is almost always opposite to what everyone says. Here's why Brian is the #1 man in America to listen to when it comes to your health.

Current International Research Situation on Fatty Acids and Government Policies in Western Countries

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In June 1985, the first major international conference on the "Health Effects of Polyunsaturated Fatty Acids in Seafoods" was held at the National Institutes of Health (NIH) (Bethesda, Maryland, USA). Research papers on fish oils [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] clearly suggested that EPA and DHA have profound beneficial effects on cardiovascular disease, hypertension, autoimmune and inflammatory diseases, and cancer, and are essential for the normal growth and development of infants, particularly the premature infant, due to their antithrombotic, anti-inflammatory and anti atherosclerotic effects and membrane function. Mechanisms involved focused primarily on eicosanoid metabolism, and the factors influencing membrane fluidity and intra- and intercellular metabolism. Five years later, in 1990, research had expanded exponentially and at the international conference held in Washington, D.C., USA, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) was established to expand research and education for professionals and the public. As expected, most of the research focused on cardiovascular disease and on infant development. The importance of n-3 fatty acids in these areas led to the development of statements by the Nutrition Foundation in England; the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) Consultative Group, ISSFAL, and the Child Health Foundation. ***Canada was the first country to provide separate dietary recommendations for n-6 and n-3 fatty acids.*** *The U.S. Food and Drug Administration (FDA) has given GRAS status (generally recognized as safe) to fish oils for inclusion in foods, and to Martek's products [DHASCO© (DHA oil) and ARASCOOO (AA oil)] for inclusion in infant formulas. Many countries (60) have included arachidonic acid (AA) and DHA in the infant formulas. Finally, this year (2002) two companies, Abbott and Mead-Johnson, have made available formulas containing AA and DHA in the U.S. In 1999, a group of research scientists, industry scientists and government representatives meet at the NIH, and after extensive deliberations,* ***they concluded that there is enough scientific evidence to recommend and define Adequate Intake (Al) for n-6 and n-3 polyunsaturated fatty acids for adults and for incorporation into infant formula, as follows.***

Introduction

In 1887 Deuel isolated alpha-linolenic acid (ALA;18:3 n-3) from hempseed oil (Deuel Jr., 1951). However, because the physical properties of the common unsaturated fatty acids are very similar, whether they are of plant or animal origin, lipids historically were difficult to separate and analyze until chromatographic techniques were developed about 50 years ago. Polyunsaturated fatty acids (PUFA) of vegetable origin were found to have a hypocholesterolemic effect when substituted for saturated fat in the diet. A series of papers beginning in 1954 by Ahrens (1954) and Keys et al. (1957), further confirmed the hypocholesterolemic effect of linoleic acid (LA) which led to the increased consumption of vegetable oils (corn oil, safflower, sunflower, cotton seed and soya oil), all high in n-6 fatty acids. In 1959 Ahrens used menhaden oil (rich in n-3 fatty acids) and concluded that the hypocholesterolemic effect was no more effective than that produced by the vegetable oils. As a result, further exploration of the effects of fish oil on cholesterol and other serum lipids was not pursued (Ahrens et al., 1959). Thus, historically, research on the role of PUFAs of the n-6 series has been emphasized. LA and its major metabolite, arachidonic acid (AA), are the most commonly ingested forms of these fatty acids, whereas ALA is ingested in limited quantities in the American diet and other Western countries and Northern Europe.

The important health-related roles of the n-3 fatty acids did not become apparent until the epidemiological investigations of Bang, Dyerberg and associates (Bang and Dyerberg, 1972; Dyerberg et al., 1975; Dyerberg et al., 1978; Dyerberg and Bang, 1979; Bang and Dyerberg, 1980). In the summer of 1970 these investigators studied 130 Greenland Eskimos in the Umanak district in the northern part of West Greenland. They attributed the scarcity of coronary thrombosis among Greenland Eskimos to the special Eskimo diet, which consisted of 400 g/day of meat from arctic animals (seal and whale) and some fish. This diet resulted in a daily intake of approximately 7 g of n-3 fatty acids. The discovery in 1979 by Needleman et al. that prostaglandins derived from eicosapentaenoic acid (EPA; 20:5 n-3) have different biological properties than those derived from arachidonic acid (AA; 20:4n-6) stimulated further research on fish oils and on the nutritional aspects of prostaglandins. Investigations by Hirai et al. in 1980 on Japanese fishing village inhabitants compared to farming village inhabitants, confirmed the findings of Bang and Dyerberg. Stimulated by these epidemiological findings of decreased coronary heart disease in fish eating populations, an increasing number of investigators in England, Denmark, Germany, Japan, Australia, the United States, and elsewhere began **to study the differential effects of the n-6 and n-3 fatty acids on plasma lipids and plasma coagulation parameters, particularly platelet and vessel wall composition and function**.

In 1983, practically 30 years after publication of the paper by Ahrens et al. (1954), Harris et al. published "The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: salmon oil versus vegetable oils". In the meantime, studies on fish consumption correlated with a lower breast cancer prevalence in both Greenland Eskimos and Japanese. Animal experiments indicated that fish oil reduces the growth of transplantable tumors in animals whereas LA increases the growth of tumors (Karmali et al. 1984). In 1985, Connor reported on his studies "Dietary n-3 fatty acid deficiency and visual loss: Evidence for a specific nutritional requirement."

It was the beginning of a series of studies by many investigators on the role of n-3 fatty acids in growth and development and in health and disease (Simopoulos, 1991). In 1984, a group of scientists met at the National Institutes of Health (NIH) in Bethesda, Maryland, USA, to discuss the need for an international conference to define the research on essential fatty acids and specifically, the role of n-3 PUFA in seafoods. The conference "Health Effects of Polyunsaturated Fatty Acids in Seafoods" held in June 1985 was a seminal conference that highlighted both the accomplishments of research and the need for additional research (Simopoulos et al., 1986).

The conference established the fact that n-fatty acids of marine origin, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) play important roles in prostaglandin metabolism, thrombosis and atherosclerosis, immunology and inflammation and membrane function (Simopoulos et al., 1986). The participants recommended:

- **The support of research on the role of n-3 fatty acids in growth and development and in health and disease, and the mechanisms involved; and**
- The establishment of a `test materials program' to specifically define nutritional requirements throughout the life cycle, dose, and type of n-3 fatty acid in intervention studies and in clinical trials.

This paper discusses the achievements of research and government policies in Western countries following the 1985 conference.

The Biological Test Materials Program

A major factor in the expansion of the research was the establishment of the "test materials laboratory" within the U.S. Department of Commerce, Division of Fisheries. The Biomedical Test Materials (BTM) program has been designed to produce a long-term, consistent supply of quality assured/ quality-controlled test materials to researchers, in order to facilitate the

evaluation of the role that n-3 fatty acids play in health and disease. The BTM program was established in December 1985 though the cooperation of the National Institutes of Health (NIH), the Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA), and the National Oceanic and Atmospheric Administration/Department of Commerce (NOAA/DOC). The objectives of the BTM Program were to develop and provide test materials necessary to attain a thorough understanding of the mechanisms and interactions of n-3 fatty acids; and to stimulate the conduct of well-designed clinical studies in order to assist in the interpretation of the action of n-3 fatty acids. By 1990, there were over 100 researchers throughout the world who used the BTM in animal and human studies as well as for *in vitro* studies. The Biomedical Test Materials (BTM) program terminated its activities effective October 15, 1997. Previous obligations for test materials were honored only for continuation of all approved studies.

The Period of 1985-1990

In December 1985 the U.S. National Institutes of Health (NIH) and the U.S. Alcohol, Drug Abuse, and Mental Health Administration (ADAMHA) developed the first program announcement on "Biological Mechanisms of n-3 Fatty Acids in Health and Disease States". Additional program announcements followed that provided the impetus and the funding for support of research in the field (Table 1).

Tablet RFAs and PAs by NIH, ADAMHA, December 6, 1985 to April 17, 1987

Date	Title	Type	Institute
Dec. 6, 1985	Biological Mechanisms of n-3 Fatty Acids in Health and Disease States	PA	NCC (NIAD-DK, NINCDS, MAID, NICHD, NIGMS, NEI, NIEHS, NIA, NIAAA, NIMH)
June 1986	Studies of n-3 Polyunsaturated Fatty Acids in Thrombosis and Cardiovascu-lar Disease	RFA	NHLBI
Aug. 22, 1986	The Role of n-3 Polyunsaturated Fatty Acids in Cancer Prevention	PA	NCI

April 17, 1987	The Role of n-3 Polyunsaturated Fatty Acids in Cancer Prevention (re-issued)	PA	NCI
Oct. 22, 1987	Fatty Acid Derived Mediators of Inflammation	RFA	NIAID

RFA = Requests For Applications PA= Program Announcements

From 1985 to 1990 over a period of five years, a number of conferences took place that further enhanced and expanded research in the field. Notable among them is the 1987 **NATO Advanced Research Workshop on "Dietary n-3 and n-6 Fatty Acids. Biological Effects and Nutritional Essentiality"** held in Belgirate, Italy (Galli and Simopoulos, 1989). The objectives of the workshop were to assess the comparative biological significance of n-6 and n-3 fatty acids in relation to the 1) dietary availability, utilization and metabolism of different members of these fatty acid series; and 2) roles of endogenous long chain members of the n-6 (e.g. 20:3 and 20:4) and n-3 (e.g. 20:5 and 22:6) series in different biological systems. The workshop was cosponsored by NATO, the Division of Nutritional Sciences-ILSI Research Foundation and the Nutrition Foundation of Italy, along with the U.S. Department of Commerce-National Marine Fisheries Service and industry groups.

In 1990, the second international conference on the "Health Effects of n-3 Polyunsaturated Fatty Acids in Seafoods" was held in Washington, D.C., USA. (Simopoulos et al., 1991 a). **The conference goals were to review the latest research data on n-3 fatty acid metabolism and their relationship to n-6 and n-9 fatty acids in relation to: (1) essentiality of n-3 fatty acids in growth and development, membrane structure and function; and (2) cardiovascular disease, hypertension, diabetes, cancer, arthritis, psoriasis, and other inflammatory and autoimmune diseases.**

A review of literature presented at this conference (Simopoulos et al., 1991b) showed the following. Prior to 1985, research focused on the modification of the eicosanoid system by dietary n-3 fatty acids. By 1990, there was research on essentiality, functions and mechanisms, including gene expression, and the role of n-3 fatty acids as an adjuvant to drug therapy. Review of the data showed that a number of randomized double-blind controlled studies have been carried out in disease states such as the following: angioplasty, rheumatoid arthritis, psoriasis, atopic dermatitis, Raynaud's phenomenon, ulcerative colitis, and bronchial asthma. Inconsistencies in the results of some of the above studies appear to be due to variations in the design and duration of the studies; selection of proper controls; failure to determine n-6

and n-3 fatty acid status along with the status of other dietary factors, e.g. vitamin E; and also failure to consider the genetic variations of multifactorial diseases and consequently the need to study subgroups for the various hypercholesterolemias, as well as hypertension, diabetes (whether NIDDM or IDDM), and arthritis; and failure to control for the amount of exercise and for body mass index.

The conference recommended that in undertaking studies it is therefore essential to know the initial phospholipid content of cells and to know about related factors that pertain to the disease under study. Also, genetic variations must always be considered in designing experimental protocols. Patients and controls must always be matched for age and sex since there is evidence that women respond to n-3 fatty acids differently than men. To illustrate, the same dose of n-3 fatty acids lowers thromboxane 2 (TXA_2) more in women than in men (Hansen et al., 1989). **Many of the inconsistent findings on the effects of n-3 fatty acids on LDL cholesterol in human subjects appear to be associated with inadequate dietary control of saturated and n-6 fatty acids during the control and intervention period**, as well as differences in life-long dietary intake of fatty acids, differences in disease state, differences in types of hyperlipidemia, and differences in the type of diabetes (NIDDM or IDDM) studied, amount of fish oil used, length of time administered, etc. Furthermore, it is essential to measure tissue levels of various metabolites, and other factors influenced by n-3 fatty acids, since plasma levels change faster after cessation of treatment than tissue levels.

Another evidence of the research interest in n-3 fatty acids and their relationship to n-6 and n-9 fatty acids is clearly seen in the large number of conferences and workshops that have been held since the memorable conference of June 1985 (Simopoulos et al., 1986). The growing number of conferences and workshops has resulted in bringing into focus important research findings, as well as setting research agendas for expanded research (Galli and Simopoulos, 1989; Bottinger et al., 1989). Some of the conferences were held explicitly on n-3 fatty acids, while at the same time numerous workshops were included as part of national meetings such as the American Oil Chemists Society (AOCS), the Federation of American Societies of Experimental Biology (FASEB), or as part of an international meeting like the International Congress on Prostaglandins. In 1989 there were at least 7 conferences and workshops on various aspects of n-3 fatty acids.

Therefore, in 1990, it was agreed that the time had come to establish a society.

International Society for the Study of Fatty Acids and Lipids (ISSFAL) 1991 - 2002

The International Society for the Study of Fatty Acids and Lipids (ISSFAL) was incorporated in The Commonwealth of Massachusetts, USA, on March 13, 1991 by Alexander Leaf, M.D., Artemis P. Simopoulos, M.D., and Carolyn Hintlian. **The purpose of the Society is to increase understanding through research and education of the role of dietary fatty acids and lipids in health and disease.** The aims of the Society shall be achieved through: (a) Sponsoring regional and international meetings; (b) Linking with other organizations which are national, regional or international, and joining efforts to promote the aims of the Society; (c) Promoting research on fatty acids and lipids; (d) **Educating professionals and the public about fatty acids and lipids in human nutrition; and (e) Serving as a clearinghouse for the media in disseminating facts regarding fatty acids and lipids in human nutrition.** The Society is educational in its mission to serve as a focus for communication among interested scientists - working in nutrition, physiology, pathology, biochemistry, cellular and molecular biology and clinical medicine - who are studying the biological effects of fatty acid and lipid metabolism in health and disease. It is believed that improved communication across these different branches of medical and biological sciences will stimulate new research and increase knowledge of nutrition. The Society will assist in interpreting the new facts into sound nutritional advice for the public.

The first meeting of the Board of Directors of ISSFAL took place in Pisa, Italy, with Alexander Leaf, M.D. (Boston, MA, USA) as President, and Artemis P. Simopoulos, M.D. (Washington, D.C., USA) as Secretary/ Treasurer. For the first seven years of its operation, ISSFAL operated out of The Center for Genetics, Nutrition and Health in Washington, D.C. In 1998, at the 3rd Congress of ISSFAL held in Lyon, France, the ISSFAL secretariat was transferred to the United Kingdom with Ray Rice, Ph.D. as the Secretary/ Treasurer.

ISSFAL has contributed enormously to the expansion of the research interest and understanding of the importance of essential fatty acids (Galli et al., 1994a; Galli et al., 1994b; Salem et al., 1996; Lagarde et al., 1999; Hamazaki and Okuyama, 2001), and the fact that our food supply consists of excessive amounts of n-6 fatty acids while it has been depleted of n-3 fatty acids due to agricultural practices.

1st International Conference on the Return of n-3 Fatty Acids into the Food Supply: I. Land-Based Animal Food Products and Their Health Effects – 1997

Studies on the evolutionary aspects of diet suggest that major changes have taken place in our food supply since the agricultural revolution 10,000 years ago. The change in animal feeds that came along with the domestication of animals changed the composition of meats, particularly the content of essential fatty acids (n-6 and n-3 fatty acids). The meat of animals in the wild has less total fat, less saturated fat and more polyunsaturated fat with a ratio of n-6 to n-3 fatty acids of 2:1. The change became even greater with the advent of modern agricultural practices and agribusiness. Using grains to feed cattle instead of grazing and eating grass has led to increases in the n-6 fatty acids and decreases in the n-3 fatty acid content of meat. Similar changes have occurred in the composition of eggs, poultry, and in fish from aquaculture (Simopoulos, 1991). Because wild plants have a ratio of linoleic acid to alpha-linolenic acid of less than 1, **the overall ratio of n-6 to n-3 was less than 2:1 prior to the agricultural revolution.**

These changes have been widely reported in the scientific literature. Industry has recognized the need to alter animal feeds in order to reverse this change. Today one can find products consistent with the evolutionary aspects of diet in the American market, such as n-3-enriched eggs with the n-6 to n-3 ratio closer to eggs under completely natural conditions. The n-3 enrichment was accomplished by adding to chicken feed the n-3 fatty acids in the form of fish meal, or flaxseed or docosahexaenoic acid (DHA) produced from algae. This change is not limited to the US market. n-3 enriched eggs can be found in various parts of the world, in Canada, Brazil, Australia, Israel, Greece, Germany, and other European countries.

As a result of the above developments, and the recognition of the important role of n-3 fatty acids in growth and development and in health and disease, it was thought timely to hold the 1" International Conference on the Return of n-3 Fatty Acids into the Food Supply: I. Land-Based Animal Food Products and Their Health Effects, at the NIH in Bethesda, Maryland, USA, September 18-19, 1997 (Simopoulos, 1998a). The conference, organized by The Center for Genetics, Nutrition and Health (Washington, D.C.) was cosponsored by two institutes of the NIH, NIAAA and NICHD, and national and international industry groups. This conference was the first to bring together scientists from academia, government and industry to discuss the return of n-3 fatty acids into the food supply and to redefine the concept of food safety (Simopoulos, 1998b). As a result of the conference

many companies have produced n-3 enriched products that are part of the markets worldwide today, and they are expanding exponentially (Simopoulos, 1999).

Dietary Recommendations/Statements

Ever since the first conference in 1985, the need to include an n-3 fatty acid dietary recommendation was recognized. Burr and Burr (1929; 1930) had recognized that both LA and ALA are essential for growth and development. **A number of international organizations, foundations and scientific groups have developed statements on n-6 and n-3 essential fatty acid requirements.** Table 2 lists chronologically the organizations involved in the development of health statements and dietary recommendations.

ESSENTIAL FATTY ACIDS IN MATERNAL AND INFANT NUTRITION

C.Gopalan

In many developing countries of the world and more especially in the countries of South Asia, the major nutritional problems that are now being encountered on a public-health scale are those related to mothers and infants. Maternal mortality rates in these countries range from 140 to 540; almost a third of infants are of low birth weight (<2.5 kg) and over 50% of under-fives are "stunted" **(Table 1)'.** The latest National Family Health Surveys data shows a maternal mortality rate of 540 in India. Thus more than 100,000 women in India die each year due to pregnancy related causes.

Low birth weight is multifactorial in its causation. Factors such as low energy intake, low weight gain in pregnancy, low pregnancy weight, short maternal stature, anaemia, infections and smoking have all been identified as possible factors. The studies of Barker et al' have revealed disturbing long term implications of intra-uterine growth retardation and low birth weight - namely predisposition to chronic degenerative diseases in adulthood. Retardation of growth and development at the intrauterine stage and in early infancy are perhaps the most urgent public health nutrition problems confronting many developing countries today.

Household diets of poor communities in these developing countries are deficient in a wide range of nutrients and stand in need of overall improvement. That good maternal nutrition status - at the preconceptional and post-conceptional stages, is important for maternal health, and fetal development, is now well recognized. However, precise information as to the crucial nutrients involved in ensuring optimal nutrition in pregnancy, lactation and in infant/child development could provide practical leads to public health agencies as to the specific directions in which dietary improvement must be attempted. It is in this context that the emerging knowledge on the importance of essential fatty acids in maternal and infant nutrition is of great practical relevance.

In this presentation we will briefly review the current knowledge regarding the role of essential fatty acids in maternal and infant nutrition. We will then consider the current state of maternal and infant nutrition in poor communities. **We will finally address the question of how the emerging knowledge with respect to essential fatty acids could be usefully applied towards the improvement of maternal and infant nutrition.**

Essential Fatty acids:

Polyunsaturated fatty acids (PUFA) include the parent essential fatty acids namely linoleic acid LA (n6) and a-linolenic acid ALNA (n3) and their long-chain more unsaturated derivatives. **The parent essential fatty acids LA and ALNA cannot be synthesized in humans and therefore need to be consumed as part of the diet.** They are converted into their desaturated derivatives as indicated in **figure-1.** Both n3 and n6 fatty acids have common enzymes in their metabolic pathway; n3 fatty acids usually have higher affinity for the enzymes than the n6 fatty acids. The rate limiting enzymes in the desaturation process is the A-6 desaturase. This enzyme is under the control of many dietary and hormonal factors.

The most important PUFA of the n6 series are dihomogammalinoleic acid (DHGLA) and arachidonic acid (AA) and these are the precursors of the eicosanoids *of `1'* and `2' series respectively. The important PUFA *of* the n3 series are eicosopentanoic acid (EPA) and docosahexaenoic acid (DHA). EPA (to which DHA can be reconverted) is the precursor of the eicosanoids of `3' series.

Eicosanoids derived from these precursors are highly active compounds. The eicosanoids cascade consists of (a) cyclic products generated by cyclo-oxygenase - such as prostaglandins, prostacyclin and thromboxane (b) lipoxygenase products such as leukotrienes and (c) products of cytocrane P400 activity (figure 2). The wide-ranging functions of the eicosanoids are indicated in table 2. AA (of the n6 series) and EPA (of the n3 series) compete for the same enzymes, cyclo-oxygenase and lipoxygenases for conversion to eicosanoids (prostaglandins thromboxanes and leukotrienes). Eicosanoids derived from AA and from EPA have opposing metabolic properties. **Therefore it is important to ensure a balanced dietary intake of n6 and n3 PUFAs.**

Pregnancy:

There are a number of observations pointing to the role of essential fatty acids in pregnancy[3,4]. According to some estimates[5] the average total accretion of essential fatty acids during normal pregnancy of a well-nourished woman could amount to 600g. During gestation the placenta preferentially selects arachidonic acid (of the n6 series) and docosahexaenoic acid (DHA)(of the n3 series); and this is reflected in substantially higher proportion of these acids in foetal circulation at mid-term[6] and term[7]. Such beneficial effects that these nutrients may have on birth weight and neonatal development may be denied in varying degrees to preterm infants. Deficits of arachidonic acid

and DHA have been reported in the circulation of low birth weight newborns[8-12] and there is also evidence of vascular pathologies in the placenta of low birth weight babies[13,14]. Essential fatty acid deficiency is believed to contribute to poor vascular growth and consequent rapture and coagulation in blood vessels leading to infarctions in the placenta that are believed to result in impaired placental functions and low birth weights[13,14] Arachidonic acid is essential for the structural integrity of the vascular endothelium. Arachidonic acid is the precursor for the synthesis of prostacyclin, which prevents thromboxanes filtration[15.] **Through a proper balance between n3 and n6 fatty acids in the endothelium, vasoconstrictors and thrombogenic activities are influenced[16,17].** Low birth weight and prematurity are associated with a high incidence of neuro developmental disorders and disabilities.[8-22]

A positive correlation between arachidonic acid level in circulation and birth weight as also between duration of gestation and DHA level have been demonstrated[12]. Reduced levels of arachidonic acid in maternal and cord blood phosphoglycerides are associated with low birth weight, low head circumference and low placental weight. High fish oil intakes have been shown to be associated with longer gestation and higher birth weight. Intervention studies with fish oils have also indicated that long-chain n3 fatty acids have an important role in ensuring normal birth weight [24]. **These observations point to the need for ensuring adequate nutritional status with respect to essential fatty acids in pregnancy. Indeed attention to adequate intake of essential fatty acids in proper could be as important as attention to iron and folic acid.** We will revert to this aspect later in this paper.

Lactation:

Mature human breast milk is a good source of essential fatty acids of both the n6 and n3 series. A normal well-nourished woman puts forth adequate amounts of these acids needed for her infant in her breast milk as shown in data in **table 3.** However, there is need for more information on the adequacy or otherwise of fatty acids in human milk of undernourished mothers. In a normal well nourished woman the fat-stores accumulated during pregnancy by themselves could provide a significant proportion of the essential fatty acids of milk at least during the first 3 months of lactation[26]. Undernourished woman may be expected to have accumulated relatively less fat-stores during pregnancy[27,28.]. Fat output in milk is variable and depends on maternal nutrition and prolactin secretion. It has been estimated that the mother's diet should provide 3 to 5g of essential fatty acid

daily to ensure adequate concentration of essential fatty acids in milk[29]. In the absence of adequate intake of foods rich in essential fatty acids including fish, green leafy vegetables and pulses, **essential fatty acids in breast milk of undernourished mothers could fall short of the requirements for normal infant growth and development. It is estimated that as many as six to ten thousand synaptic connections between neural cells are made in the postnatal period and early infancy. The basic materials required for this major operation are the essential fatty acids of human milk. The child's eventual state of mental development may well depend on the adequacy of supply of the needed fatty acids at this crucial stage of development.**

Neural development:

Almost two-thirds of the structural material of brain is lipid - composed of cholesterol and phophoglycerides rich in arachidonic acid and DHA. It is therefore understandable that dietary supply of EFA is limiting for brain growth. In the rods of the retina, DHA accounts for 50-60% of the phosphoglycerides embedding rhodopsin and the G-Protein. DHA is therefore central to receptor and neural transmission system on which brain function depends.

Studies on rhesus monkeys[32-35] subjected to n3 fatty acids deficiency revealed that progressive depletion of docosahexaenoic acid from neural and retinal phospholipids followed by significant impairment of visual acuity, abnormalities in the electroretinogram and polydypsia.

Evidence indicates that both n3 and n6 fatty acids are limiting for brain growth and that neural integrity can be permanently disturbed by deficiency of both n6 and n3 essential fatty acids.

Intrauterine growth retardation caused by n6 and n3 deficiency in pregnancy may thus affect both physical and mental development of the infant.

The above observations point to the important role of essential fatty acids in ensuring maternal nutrition, good pregnancy outcome and optimal growth & development of the offspring in the intrauterine phase and in the infancy.

Low birth weight and intra-uterine retardation in poor communities:

Probably the highest incidence of low birth weight deliveries in the world occurs in poor communities in South Asia[40] (including India, Pakistan, Bangladesh, Nepal, Srilanka, and Maldives). There have been several reported

studies on birth weights from these countries. The major conclusions that emerge from these studies are:

1) The gestational age of live born infants in poor communities appears to be significantly shortened as compared to those in the developed countries. It will thus be seen **(**table 4**)** that the infants born prior to 37 weeks of gestation is just 5% in developed countries (USA[49] and Norway[50]) as against 12-14% births in India.

2) The incidence of low birth weight in full term deliveries in South Asian Countries is significantly much higher than the incidence reported from the developed countries Thus in a study involving over 20000 full term deliveries, the incidence of low birth weights was as high as 25% as against 6-7% in developed countries. The results of a study[53] by the Nutrition Foundation of India in 1998, which involved nearly 15000 births that took place in a major hospital in Delhi catering to the poorest segments of the population are set out in table 5. This would show that the high incidence of low birth weight deliveries even in full-term infants born after the 37 weeks of gestation in poor communities is to a considerable extent, a reflection of relative shortening of the gestational period. It will be seen that nearly 34% of all deliveries had taken place 37-38 weeks of gestation and 44% after the 39th week of gestation **(**Table 5 & Figure 3**)**

3) On the other hand, even in developing countries among the affluent sections of the population the incidence of low birth weight deliveries is apparently of the same order as observed in the developed countries. Thus a study by Nutrition Foundation of India showed that only 6% of the infants among the affluent sections of Indian population were of low birth weight, indicating clearly that socio-economic factors, particularly under nutrition play the determining role.

As was pointed out earlier, low birth weight is probably of multifactorial origin. The outstanding common feature in poor communities associated with low birth weight is, however, maternal malnutrition.

Essential fatty acids in Indian diets:

The important question from the point of view of present discussion is how adequate are the diets of poor pregnant women in India with respect to a essential fatty acids content. Pioneering studies on the essential fatty acids

content of habitual Indian diets have been carried out by Ghafforunissa[63] and Achaya[64] and these studies provide a fair picture of the essential fatty acids intakes in Indian diets.

Diets of the poor Indian communities are largely cereal and pulse based. Vegetable oil extracted from oil seed used as cooking fat is the major source of (visible) fat in the diet. The intake of such vegetable oils hardly exceeds 10gms per head per day. While in most poor households a single type of oil is used, in some others different types of vegetable oil may be employed depending on the food to be cooked. Apart from vegetable oils other sources of vegetable fat like meat and fish do not figure largely in the diets of poor households. **Moreover, a considerable proportion of the Indian population is vegetarian.** Milk intake is also marginal in poor households. **All this may suggest that the intake of the essential fatty acid is highly inadequate in poor Indian diets.**

However, careful analytical studies in recent years have shown that this is not the case. The picture with regard to an overall essential fatty acids intake in poor Indian diets is reasonably bright in the light of recent studies.

The major components of the Indian diet like cereals, pulses, tubers and vegetables are good source of invisible fat (meaning fat, which is an integral part of grain). Invisible fat was earlier not recognised because it was poorly analysed and detected for the reason that structural tightly bound lipid is not easily extractable by conventional methods. Achaya [64] had computed that 10-15 en % (a level of fat that would provide as much as 10-15 % of the total energy value of the diet) is present in the invisible form. Invisible fat is a good source of LA and ALNA. For this reason Achaya considered poor Indian diets were reasonably adequate with respect to meeting essential fatty acids requirements in a normal subject. Using accurate method of extraction and gas-chromatography analysis Ghafforunissa [63] found that the cereals contain 3 % and pulses 2 % of invisible fat and that on an average cereals provide 1.3 % LA and 0.5% ALNA, pulses 1.2 % LA and 0.25 % ALNA. With an intake of around 500 gms of cereals, diets could thus provide 7 en % of invisible fat 2.2 % of LA and 0.16 en % ALNA. Ghafforunissa [63], however, argues that while the present level of essential fatty acids even in poor Indian diets would thus meet normal requirements, they could be inadequate in pregnancy and lactation. She had computed that over and above the invisible fat present in the diet an intake of vegetable oil of the order of 30 gm in pregnancy and 45 gm in lactation would be necessary to fully meet requirements. On the basis of this computation it could be

concluded that poor Indian diets are deficient in essential fatty acids despite the substantial contribution of invisible fat from cereals, pulses, tubers and vegetables in the case of pregnant and nursing women.

Studies on effect of dietary supplementation to mothers on pregnancy outcome:

Several studies on the effect of dietary supplementation on pregnancy performance and delivery outcome has been carried out in the developing countries. Some of the results have been briefly reviewed below.

Energy supplement:

In most of the earlier studies on the effect of dietary supplementation to mothers during pregnancy on pregnancy outcome, the basic hypothesis had been that maternal energy intake could be a major determinant of intrauterine growth and foetal nutrition. Thus, Iyengar [65] had shown that dietary supplements providing 500 additional calories per day even in the last 6-8 weeks of gestation to pregnant women habitually subsisting on low energy intake could bring about significant improvement in the birth weight of their offspring. Following on this several studies on the effect of dietary energy supplementation had been carried out in India[66], Srilanka[G7], Thaiwan[68], Guatemala[69], USA[70], Mexico[71], Canada[72] Indonesia[73] and Gambia[74]. The results of these studies have been confusing and contradictory. While some studies have reported increase in birth weight of offspring ranging from 40-320 gms, following on energy supplementation others have reported negative results. Kusin[73] had shown and in malnourished mothers with very low energy intake dietary energy supplementation during pregnancy contributed to increased weight gain in the mother but had no significant effect on the birth weight of the offspring. In situations of extreme under nutrition there could be a competition between maternal and feotal tissues for dietary energy supplements. Studies in Gambia[74] had shown that heavy physical activity resulting in increased energy expenditure could have a significant impact on maternal and infant birth weight and supplements to such women with such marked caloric deficit resulted in greater weight gain to the mother with little impact on the birth weight of the offspring. The results of studies on the effect on dietary energy supplementation to the mothers on birth weight of their offspring have been equivocal and contradictory. That protein supplementation to maternal diets in pregnancy had no beneficial effect on the birth weight of offspring was shown by studies of USA[70] and Guatemala[69].

Most of these studies on macronutrient supplementation had not fully taken into consideration, the multiplicity of factors, which could affect the birth weights of infants such as maternal anaemia, infections and lack of physical rest in the last trimester of pregnancy.

Apart from these considerations in some of these earlier studies where dietary energy supplementation was attempted, the food sources of the so-called energy supplement could have actually provided apart from energy, quite a few micronutrients including essential fatty acids. If for example - the so-called caloric supplement was largely based on cereals, the invisible fat in such cereals supplement could have provided essential fatty acids. It is possible that the absence of uniformity in the nature and the food source of supplement could account for the contradictory and equivocal results obtained.

Iron /Folic Acid /Zinc and Copper:

The diets of poor pregnant woman are deficient in a multiplicity of nutrients. **In predominantly cereal based diets on which poor communities subsist the bioavailability of iron and zinc, for example, are low because of the high phytate content of cereals. Iron deficiency anearnia is therefore common in pregnant woman of the poor communities of South Asia.** Aneamia is recognized to be a major factor associated with high maternal morbidity and mortality and routine administration of iron / folate in the last months of pregnancy is major public health approach in prevention of aneamia in pregnancy.

In this context it is important for us to determine to what extent iron / folate deficiency apart from anaemia, also accounts for intra-uterine growth retardation resulting in low birth weight. Leela Iyengar[75] had shown that the birth weight of infants born to mothers receiving 200-300 micro gms of folic acid were significantly higher than those of mothers who did not receive such supplement thus showing a positive effect of folic acid supplementation on the birth weight of infants. Prema had shown that with iron supplementation, there was not only increase in hemoglobin levels of the mothers but also in the birth weight of the offspring 76,77

The relationship between maternal haemoglobin levels and birth weight of offspring's has also been demonstrated in the form of a U curve with a progressive drop in the incidence of low birth weight deliveries with increase in haemoglobin levels from 8-11 gms and an actual increase in low birth weight deliveries from that point with an increase of haemoglobin beyond 11 gms. This latter increase may be due to hemoconcentration and consequently poor placental perfusion.

Striking changes in maternal serum zinc and maternal serum copper levels has been demonstrated [80i]n pregnancy. There is a slow but steady increase in the maternal copper levels and a corresponding slow fall in maternal serum zinc levels [81]. Maternal-serum zinc levels reach lowest point at the time of delivery [82]. It is only after six weeks of delivery that maternal serum zinc levels rise to the pre-pregnancy concentration.

The rise in maternal serum copper levels is due to estrogen induced synthesis of ceruloplasmin [83] and fall in zinc is due to altered binding affinity of zinc proteins. The fact that cord serum zinc levels are higher than maternal serum zinc levels is believed to indicate active transport of zinc across the placenta during pregnancy. However, the claims[84] that in low birth weight infants cord plasma zinc levels are higher as compared to infants with normal birth weights has not been substantiated.[85,86]

It is thus clear that iron, folic acid, zinc and copper are micronutrients, which are actively involved in pregnancy.

The role of micronutrients and fatty acids - a final common pathway

The claims with regards to the positive effect of iron and folate supplementation on the birth weights and those regarding the effect of essential fatty acid supplementation need not necessarily be contradictory. There is evidence now that iron, folic acid, zinc and copper could all play a part in A^6 and A^9 desaturases system, and in the cyclogenases and lipoxygenases system that convert arachidonic acid to eicosanoids.

Studies [87] on rats fed with fat free iron deficient diets on the one hand and studies[88] on rats where iron deficiency was induced during pregnancy have indicated that conversion of 18:2 n-6 to their long chain derivative's may be blocked in iron deficiency and that the cycloxygenases and lipoxygenases which convert arachidonic acid to eicosanoids are inhibited **(Figure 4).** Thus there is evidence in literature indicating an interrelationship [87,88] between iron status and eicosanoid metabolism.

There is also similar evidence to suggest that zinc and copper may play a role in essential fatty acid and eicosanoid metabolism, zinc and copper being integral components of the A^6 desaturase system[89](Figure 4). **Accumulation of 18:2 n-6 fatty acids in tissues of zinc deficient rats has been demonstrated [90]. Copper has also been shown to play a part in the eicosanoid metabolism. It has been reported that folic acid administration increases the n-3 PUFA in plasma lipid fractions, in platelet, erythrocytes and intestinal phospolipids [91]** (Figure 5).

It would seem reasonable to propose that the micronutrients like iron, copper, zinc and folic acid on the one hand and essential fatty acids on the other do not act discordantly or independently of each other. But that, they contribute to a part of a part well coordinated and orchestrated metabolic process. So we may be dealing with a well-coordinated symphony and not discordant solos.

On going studies at Nutrition Foundation of India:

In order to elucidate these aspects, the Nutrition Foundation of India is now engaged in extensive studies on low birth weight. In the ongoing study on low birth weight, the effects of supplements of iron and folate to one group of pregnant women, and iron, folate plus soya oil (a source of essential fatty acids - n-6 & n-3) to another are being investigated. In this study three groups of pregnant women belonging to the poorest community are being investigated. They are:

1. A group of women attending the centers and receiving routine attention is the control group.
2. A second group of women receive iron (100mg) and folate (500mcg) supplement regularly from the 20‴ week of gestation.
3. The third group of women in addition to iron and folate as above, receiving 15 ml soya oil from the 22" ^{d} week of gestation. The fatty acid composition of the soyabean oil used is as shown in table 6.

The supplements are being delivered to the subjects by specially trained appropriate health persons who visit the subject's home in order to ensure the intake of the supplement. The study is ongoing and we have not yet gathered large enough number of patients, which will enable us to draw final conclusions. The preliminary results indicate the following:

1. With iron and folic acid supplementation alone, there is a significant increase in birth weights and reduction in the incidence of low birth weight deliveries.
2. The addition of soya oil along with iron and folate brings about a further reduction in the incidence of low birth weight deliveries.

The study will continue for two more years during which period data with respect to fatty acid content of breast milk and growth and development of the infants will be gathered.

These investigations will help to decide whether the beneficial effects of iron/folate are mediated through better utilization of essential fatty acids by facilitating their conversion into active form as has been earlier proposed in this paper. It may thus be possible to explain the seemingly confusing claims with regards to positive effects of iron and folate on one hand and essential fatty acids on the other on the birth weights.

These considerations underscore the fact that in undernourished population living on diets deficient in a multiplicity of nutrients including iron, folate, zinc, copper and essential fatty acids, it may be unwise to depend on either essential fatty acid supplementation alone or on iron and folate supplementation alone in improving the pregnancy outcome. Indeed, it had been shown in the context of low iron intake, essential fatty acid administration could aggravate anaemia. Our objective must be to ensure adequacy with respect to iron and folate on one side and essential fatty acids on the other. Green leafy vegetables like spinach fortunately are not only rich in iron and folate but also in essential fatty acids*. Thus the major approach towards combating of low birth weight among under nourished population may lie in the promotion of dietary diversification resulting in the increased intake of green leafy vegetables, pulses, milk and probably fish and not on isolated synthetic supplements.

*Unfortunately, the iron and other mineral content of green leafy vegetables are not available to humans because, as you already learned, they are bound to the phytate. A human being could not possibly eat enough green leafy vegetables to obtain significant EFAs from them. That's why animals, like a cow with four stomachs, has to be eating all of the time.

Long Chain Polyunsaturated Fatty Acids: The Requirement In Early Life

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Abstract

Long chain polyunsaturated fatty acid, LCPUFA, is defined as the fatty acid holding >18carbon atoms and more ethylene bonds in cis-form, such as arachidonic acid (AA, n-3), eicosapentaenoic acid (EPA, n-3), docosahexaenoic acid (DHA, n-3), etc. It is the membrane component of all kinds of cells and plays some physiological function.

LCPUFA is gotten form elongation and desaturation of less long polyunsaturated fatty acid in body and by eating. Human body can form double bond in n-9 site of fatty acid, **but cannot in n-6 and n-3site, so the n-6 and n-3 unsaturated fatty acids must be gained from food, but not always from LCPUFA ones. The n-6 and n-3 LCPUFA can be synthesized in body using linoleic acid and c -linolenic acid called essential fatty acids.**

Only few free fatty acids of LCPUFA occur in food. LCPUFA combines mainly with other component to form complex lipids, phopholipid and glycosyl lipid. In the human body, except consisting in all parts of plasma lipoprotein and participating in synthesis and metabolism of prostanoids, LCPUFA in form of phospholipid composes cellular framework mainly and be oxidized with other lipids in catabolism. **Brain, retina and sperm are rich in LCPUFA.**

Fetus develops the enzyme system to synthesize LCPUFA very slowly. **For fetal development, LCPUFA source is from its mother mostly.** There is a particular mechanism in transportation of LCPUFA in placenta. A group of proteins located in capillary region, which prefer to combine and LCPUFA in placenta. A group of proteins located in capillary region, which prefer to combine and take and transport long chain and polyunsaturated fatty acids. **Maternal deficiency of LCPUFA is the one cause of worse development of fetus.** In premature, in 0 to 3 or 4 weeks after birth, the enzyme system in elongation and desaturation of fatty acid is not fully developed, hence, breastfeeding favors to reinforce LCPUFA and fortification of LCPUFA in infant formula is supported. Future research is needed to determine whether LCPUFA fortification will have functional benefits for infants born in term and in preterm after I month. LCPUFA as a conditional essential nutrient is exploring. The requirement of LCPUFA for fetus, infants and children is

waiting for detailed description. **And it is emphasized that the balanced n-6 and n-3 essential fatty acids intake, the balance, between all kinds of fatty acids and the reasonable proportion of fat in total energy source are the basic principle in nutrition.**

LCPUFA content in ordinary foods is not rich. Recently, the development of microbial oil rich in LCPUFA is a bright point in food industry.

n-3 FATS AND PREVENTION OF ARTHRITIS

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Dietary essential requirements for n-3 and n-6 polyunsaturated fatty acids (PUFA) are modest. In the case of n-6 fats, intakes are well above essential requirements. In modern Western diets, n-3 intakes are low relative to those for n-6 PUFA. Tissue levels of n-6 and n-3 PUFA are determined by dietary intakes. **Since n-6 and n-3 PUFA compete for the same metabolic pathways, the balance of these PUFA is, in practice, as important nutritionally as the minimal absolute amounts each consumed.** This balance, particularly in Western diets, is skewed strongly toward n-6. The ratio of intakes can be modulated by making choices in food processing and cooking that avoid visible fats, which are very rich in n-6 PUFA and by opting instead for ingredients that have a significant n-3 content. The latter can be coupled with monounsaturate fats, since the latter are neutral with regard to n-6 / n-3 balance and, in contrast to saturated fats, have no adverse health effects (see reference 1 for review).

n-6 and n-3 PUFA and monounsaturates (i.e. n-9 fatty acids) are present in a number of vegetables and seed oils. The latter, by virtue of their high fat content, are an especially important source of PUFA. PUFA, like all fatty acids, are metabolised for energy. However, a proportion is incorporated into the phospholipids of cell membranes. The cellular PUFA can be elongated and further desaturated. Linoleic acid (LA) C18:2 n-6 is converted to arachidonic acid (AA), 20:6 n-3. This fatty acid contributes substantially to PUFA at the n-2 position of cell membrane phospholipids. Conversion of the n-3 precursor alpha-linolenic acid (ALA), 18:3,n-3 to the n-3 C20 PUFA, eicosapentaenoic acid (EPA), C20:5, n-3 appears less efficient than is the case for conversion of LA to AA. Dietary EPA can displace AA from cell membrane phospholipids and this is best achieved by introducing EPA directly in the diet. Docosahexanenoic acid, C22:6, n-3 also has anti-inflammatory effects, appears to act synergistically with EPA and is best introduced directly in the diet (2). Fish, fish oils and other marine seafood are especially important sources of EPA and DHA . There are now prospects for the production of oils rich in EPA and DHA through land based agriculture of genetically modified organisms. Currently in Australia, average daily intakes of EPA and DHA are a modest - 220mg daily for men and 150 mg daily for women.

Daily ALA intakes are 1.4g for men and 1g daily for women. By contrast, daily n-6 intakes, mostly as LA, are 13g for men and 9g for women (P. Howe and A. Sinclair, unpublished).

AA and EPA are structurally very similar, differing only in the presence of the n- double bond in EPA. They are metabolised to eicosanoids (eiosa=20, refers to the number of carbon atoms in these molecules), which regulate metabolic activities in many cells. Certain enzymes responsible for eicosanoid synthesis are upregulated at sites of inflammation, eg. the cyclooxygenase (COX) isozyme, COX-2 (3). The eicosanoid products of AA and EPA, like their parent fatty acids, differ only in the presence of the n-3 double bond in the n-3 eicosanoids. The respective activities of n-6 and n-3 eicosanoid homologues can differ substantially, with the n-3 leukotriene B_5 ($LTB_{S)}$ and thromboxane A_3 (TxA3), having little activity relative to their n-6 counterparts, LTB_4, a potent chemotaxin for leucocytes, and TXA_2, a platelet aggregator and vasoconstrictor.

The result is an overall anti-inflammatory and anti-thrombotic effect of diets rich in long chain n-3 PUFA relative to n-6 PUFA dominant diets (1). Fish oil supplemented diets have also been shown to reduce IL-I and TNF production (4) perhaps due to reduced synthesis of TxA2, which upregulates the synthesis of these cytobines (5). Because dietary n-3 diets down regulate inflammation, they warrant consideration as preventives of inflammatory diseases, including arthritis.

To be used as a preventive for a disease at a community level, an intervention must meet a number of criteria. These include a biochemical rationale, lack of important unwanted effects, collateral health benefits, support from data in relevant animal models and epidemiological studies. Known therapeutic effects can also be an indicator of a likely favourable prophylactic effect. The candidacy of dietary n-3 fats in the prevention of rheumatoid arthritis, the prototypic inflammatory arthritis, will be assessed against these criteria.

The issue of biochemical rationale has been discussed above. The anti-thrombotic effects of dietary n-3 fats appear to be an advantage against a background of diets poor in n-3 fats. With extreme n-3 dominant diets, such as those consumed by circumpolar Inuits, who, in their aboriginal state, consume a diet of sea mammals and fish, adverse effects may be seen as a result of antithrombotic actions, eg. increased bleeding tendency and cerebral haemorrhage. An increase susceptibility to infection remains an unproven possibility. These effects are not likely to occur with n-3 fortification of diets containing a broad mixture of agricultural and marine products.

In animal studies, preventive effects of n-3 fats on the emergence of inflammatory diseases have been shown. For example, in mouse strains that are genetically susceptible to systemic lupus, an inflammatory disease in which arthritis is a common feature, feeding a fish oil diet from weaning has a striking effect on survival (6). This effect of prophylactic treatment exceeds the benefit seen when the fish oil diet is introduced at the first sign of overt disease. However, dietary fish oil has not been protective in all models of inflammatory disease and thus disease specific effects may exist (7).

Population studies support the hypothesis that dietary n-3 fats may have a preventive effect in rheumatoid arthritis. For example, the Japanese, whose diet is rich in seafood, have a comparatively high frequency of the rheumatoid arthritis susceptibility `shared epitope' conferred by certain HLA-DRBI alleles compared to Western populations (8). By contrast, the prevalence of rheumatoid arthritis in the Japanese appears to be only one third that of their Western counterparts (9). Among Greenland Inuits, who feed on sea mammals and fish, the frequency of the rhematoid suceptibility allele HLA-DR(3I 0401 is similar to that of Europeans (10), but rheumatoid arthritis is rare (11). There is an important caveat regarding these population studies. Genetic predispositions are multifactorial and other genetic factors, unrelated to HLA, may militate against the occurrence of polyarthritis.

More direct support for a preventive effect of dietary n-3 fats comes from the Seattle Women's Health Study. In this case control study, women consuming two or more servings of fish per week had an odds ratio of 0.32 for development of rheumatoid factor positive rheumatoid arthritis compared to subjects consuming less than one serving of fish per week (12).

Collectively, animal, population and case control studies suggest a preventive effect of dietary n-3 fats on rheumatoid arthritis that could be as high as a two-thirds reduction in disease occurrence. This equates to a very substantial reduction in disease burden imposed by a disease that affects more than 1 % of the population in countries that have a high fat intake, comprising much n-6 and little n-3 fat. In China, where average fat intakes are lower, the prevalence of rheumatoid arthritis appears to be about one third of 1% and the impact of n-3 fat intakes on disease prevalence may differ (13). The amount of n-3 fat required for a preventive effect may be less than that used to achieve anti-inflammatory effects in established rheumatoid arthritis. The evidence for the latter will be discussed below.

The feasibility of randomised, controlled trials to assess therapeutic effects of dietary n-3 PUFA in rheumatoid arthritis has allowed the gathering of more direct evidence of benefits from therapy than is the case

for prevention. There have now been at least 13 randomised controlled trials that show benefit from fish oil supplements in rheumatoid arthritis (reviewed in reference 14). A common feature of the studies has been reduction in symptoms and tender joint count. Reduced requirements for analgesic anti-inflammatory drugs were seen in each of the three studies that addressed this issue. Meta-analysis and mega-analysis (i.e. combined analysis of the primary data) have established decreased tender joint count and duration of morning stiffness as beneficial effects (15).

In applying dietary fortification with fish oil in the management of rheumatoid arthritis, there are a number of practical strategies that can reduce cost and enhance effectiveness. Firstly, inexpensive, good quality formulations of fish oil should be chosen. Encapsulation of the oil is an unnecessary expense.

When instructing patients it is important to explain that overt clinical benefit is not immediate and nay have a latency of two months or more. This delay is similar to that observed with the long acting antirheumatic drugs. Adjustment of the background diet to increase n-3 PUFA and reduce n-6 PUFA intakes can increase levels of n-3 fats achieved in tissues (16).

The following is a summary of findings in a number of dietary intervention studies in healthy human volunteers. EPA content in peripheral blood mononuclear cells is increased modestly by substituting flaxseed oil, which is rich in n-3 ALA, for sunflower oil, rich in n-6 LA, in dietary visible fats such as oils and spreads. The most effect on EPA levels was seen with the fish oil supplement, even with the n-6 rich background diet. The combination of the flaxseed and fish oil diet yielded the greatest increase in cellular EPA (16).

An anti-inflammatory effect of fish oil has been observed with daily doses of 2-6g long chain n-3 PUFA (EPA plus DHA) (14). We typically prescribe a dose that delivers 4g of EPA plus DHA daily. Such a dose, when taken as gelatin encoated capsules, can cost as much as $A850 (equivalent to US$ 420) annually at normal retail prices. By contrast, unencapsulated fish oil can deliver the same dose of long chain n-3 PUFA at one-tenth the cost. The fish oil is floated on juice and taken as a single gulp with care to avoid contact with lips to avert an unpleasant taste experience. This is followed immediately by juice from a separate glass, which is distributed about the mouth thoroughly before swallowing. Using this method, the dose can be taken without experiencing the taste of fish oil, which most find unpleasant.

It is best to take the dose with a meal and without extra fluid to prevent a repeating taste due to reflux. We have prepared a written instruction sheet, which details this technique, as well as ways of increasing n-3 PUFA and avoiding unnecessary n-6 PUFA in the background diet.

Considering the known health benefits of dietary n-3 fats and the documented inverse correlation between erythrocyte EPA levels and risk for sudden cardiac death (17), it is surprising that so little attention has been given to the development of a standard laboratory index of n-3 nutrition. We have explored the feasibility of using an assay to guide preventive and therapeutic treatments with n-3 fats. For convenience of sample processing, we have chosen to assay non-fasting plasma phospholipid EPA levels, having established that there is little diurnal variation in levels, no evident relationship with meals and a close correlation with cellular cell EPA levels (Cleland LG, James MJ, unpublished). More specifically, plasma phospholipid EPA correlated very closely peripheral blood mononuclear cell EPA levels with r = 0.97. While desirable levels need to be validated in appropriately constructed clinical studies, as a starting point, we have chosen a notional target level. This value is based on the correlations observed between mononuclear cell EPA levels and inhibition of synthesis of the inflammatory cytokines IL-1 $_{R}$ and TNFa ex vivo in human volunteers given diets fortified with n-3 PUFA (18). Substantial inhibition of production of these cytokines was seen when mononuclear cell levels of EPA were equal to or greater than 1.5% of total cell phospholipids fatty acid. This correlates with a plasma phospholipid EPA level equal to or greater than 3.2%.

Using this assay we have shown sustained plasma phospholipid EPA levels in patients with rheumatoid arthritis, who are prescribed fish oil supplements. In our Early Arthritis Clinic, which has been set up to allow early diagnosis and treatment of rheumatoid arthritis, dietary fish oil is part of our protocol of treatment with combination therapy. The assay has helped us to monitor the effect on EPA levels of advice to take unencapsulated fish oil on juice. Occasional baseline values above the target level suggest commencement of fish oil prior to capture of the baseline blood sample. Some patients chose not to take fish oil and their EPA levels do not change. In terms of continuation rates, that for fish oil compares favourably with those of most of the drugs in the combination regimen. The non-steroidal analgesic antiinflammatory drugs (NSAIDs) have no long-term, favourable effect on outcomes in rheumatoid arthritis. For this reason, we recommend NSAIDs be used on an as required only basis for pain relief so they do not divert from use of longer acting treatments that can reduce arthritic tissue damage in the long term. At 12 months as few as 25% of patients were still taking NSAIDs. In line with results of previously published

studies, which have shown that fish oil supplements reduce NSAID requirements in rheumatoid arthritis, patients achieving the target EPA level tended to higher discontinuation rates of NSAIDs. In view of the foregoing, we believe that non-fasting plasma phospholipid EPA will prove to be a useful assay to support the use of dietary n-3 supplements in this and other therapeutic and preventive situations.

In terms of future applications, one needs to consider the different situations, in which the preventive effects of dietary n-3 fats may prove useful. Primary prevention involves the prevention of arthritis in those who display no signs of arthritis. Secondary prevention involves an attempt to prevent joint damage in those who present early with polyarthritis before joint damage has occurred. Tertiary prevention involves containment of joint damage once it has commenced. Investigations into primary prevention for rheumatoid arthritis could be focused on family members, who are at special risk for the disease, particularly when they can be shown to express one of the HLA-DRI3 susceptibility alleles. Secondary prevention can be assessed through the early arthritis clinic approach and this is focus of our continuing investigations. Containment of joint damage needs to be assessed in established rheumatoid arthritis and osteoarthritis. Whereas the symptomatic benefit of fish oil has been established in rheumatoid arthritis, there are no reports of clinical studies of fish oil in osteoarthritis. A rationale for the latter is provided by in vitro studies, which show that exogenous n-3 fats down-regulation release of degradative enzymes by cultured articular cartilage chondrocytes (19).

When using fish oil in established rheumatoid arthritis, there can be advantages in reducing collateral risks, such as the increased frequency of occlusive cardiovascular events associated with this disease (20, 21). Reduced activity and damage from extra-articular inflammation is a further advantage that has been shown in inflammatory bowel disease, psoriasis and 1gA nephropathy (1). Favourable drug interactions can be a further advantage, which can involve reducing drug associated risks, e.g. reducing drug associated cardiovascular risks and ameliorating effects of fish oil on the hypertension and nephrotoxicity seen with cyclosporin A. On the other hand, fish oils potentially can fortify the action of certain therapies, e.g. the untested combined effects of reduced TNFa and IL-I (3 synthesis by fish oil and biological agent therapies that block the actions of these inflammatory proteins.

In summary, there is a strong biochemical rationale for a putative preventive effect of dietary n-3 fats in arthritis and support from animal, epidemiological and clinical data. *However, there remains a need for more direct studies of dietary enrichment with n-3 fats in the prevention of arthritis.*

DIET AS PREVENTIVE MEDECINE IN CARDIOLOGY

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1. ABSTRACT

Because the main cause of death in patients with established coronary heart disease (CHD) is sudden cardiac death (SCD), physicians should develop specific strategy, including dietary changes, to prevent it. In the long term, reduction of the diet-dependent chronic risk factors of CHID, hypercholesterolemia, hypertension and diabetes, is also important.

The association of the direct and specific cardioprotective effects of the Mediterranean diet (through various mechanisms, likely including the prevention of sudden cardiac death [SCD] with those expected from the reduction of blood lipids, blood pressure and a better control of the diabetes (and in addition to its gastronomic appeal) renders this dietary pattern extremely attractive for the prevention of coronary heart disease (CHD). **Experimental and epidemiological studies, and randomized trials, clearly demonstrated that n-3 fatty acids, when they are part of the Mediterranean diet, reduce the risk of SCD in CHD patients.** Their use is now encouraged either as supplements or by including them as part of a Mediterranean type of diet.

2. INTRODUCTION

Hypercholesterolemia, diabetes and hypertension are the main classical risk factors of coronary herat disease (CHD), along with smoking. They are dependent on personal dietary habits and may, in theory, be modified by dietary changes. When looking at the causes of death in CHD patients, it appears that sudden cardiac death (SCD) is the most frequent one. Thus, when aiming at improving the survival of CHD patients, it is wise to primarily focus the preventive intervention on the SCD endpoint. However, the priority in primary prevention should be given to the long-term reduction of the classical risk factors, because the absolute and immediate risk of death, and of SCD in particular, is low in these patients. Whether the reduction of the classical risk factors of CHD or the adoption of a cardioprotective diet might also reduce the risk of SCD is therefore a crucial question for the cardiologists.

3. A NEW PERSPECTIVE ON CHOLESTEROL AND DIET

Cholesterol is a determinant of CHD mortality, and its blood level is regulated, at least partly, by diet. However, few epidemiological studies have prospectively included the analyses of the dietary habits of the studied populations in the evaluation of their risk (1). In the Seven Countries Study, marked differences in CHD mortality, dietary habits and cholesterol distribution were observed in the different cohorts (1). Cholesterol levels were high in Northern Europe and in the USA (an average level of 7 mmol/L), and low in rural Japan (an average of 4 mmol/L in average), and population cholesterol levels were positively associated with CHD mortality. Secondary prevention trials with statins in Northern Europe (2) and Australia (3) confirmed the importance of cholesterol by demonstrating a reduction by 25-30% of the relative risk of CHD death in patients taking the drugs. Whether the effect of the statins was entirely related to their effect on cholesterol remains unknown. Actually, simvastatine was shown to reduce the risk of SCD, but not pravastatin (Table 1), which suggests specific cardioprotective effects of each drug beyond their common effect on cholesterol.

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Table 1. A summary of the main results regarding mortality in recent secondary prevention trials. Results are expressed as reduction of the relative risk (RRR) or as reduction of the absolute risk, expressed as the number of lives saved per 1,000 patients treated per year and the number of sudden cardiac deaths (SCD) prevented.

Reference	LIPID (prava) (3)	4S (simva) (2)	GISSA (omega-3) (51)	GISSI (vit E) (51)	Lyon trial (Med diet) (13-15)
Total mortality (RRR %)	22	30	21	10*	60
Number of lives saved (per 1000/ yr)	5	6	6	4*	8
Cardiovascular mortality (RRR %)	25	35	30	18	65

Non-sudden cardiovascular	34	29	10*	0*	74
Sudden cardiac death (RRR %)	13*	42	45	35	64
Number of SCDs prevented (per 1000/yr)	1*	3	5	3.5	3.5

**Statistically non-significant.*

(RRR %) means Reduction of the Relative Risk, in percentage. SCD means sudden cardiac death.

A major (and often underestimated) finding of the Seven Countries Study was the large difference in absolute risk of CHD death at the same level of serum cholesterol in the different cohorts. At a cholesterol level of about 6 mmol/L, for instance, CHD mortality was 3 times as high in Northern Europe as in Mediterranean Europe (18% vs. 6%). This suggested that factors other than cholesterol were playing an important role. *Because of the similarity of the other traditional risk factors and the large differences in the dietary habits of the cohorts (4), it was proposed that the difference in CHD mortality between populations was mainly related to their dietary habits, and through biological effects independent of cholesterol (5).* ***This was the basis of a new "diet-heart hypothesis" in which cholesterol was not the central issue*** *(5,6). In fact, the first dietary trials designed in the secondary prevention of CHD were based on the hypothesis that a cardioprotective diet should primarily reduce cholesterol (6).*

While the investigators succeeded in reducing cholesterol, they failed to reduce CHD mortality (6). *This was mainly attributed to an insufficient effect of the tested diets on cholesterol and the conclusion was that cholesterol-lowering drugs should be preferred. However, none of the diets tested in these old trials was patterned from the traditional diets of populations known to be protected from CHD (e.g. vegetarian, Asian or Mediterranean), although these diets are associated with low cholesterol (1,4). Also, no trial was aimed at testing the cholesterol-lowering effect of a Mediterranean diet, probably because this diet was (and still is) mistakenly regarded as a high fat diet, allegedly not appropriate to reduce cholesterol and weight. However, studies investigated the effect of the main lipid-related features of the Mediterranean diets, for instance diets low in saturated and polyunsaturated fat but relatively high in monounsaturated fat (7-12). The aspects of the Mediterranean diet not related to lipids were not investigated although they influence lipid metabolism. Nonetheless, the consensus now is that a diet low in saturated and polyunsaturated fat but rich in oleic acid results in a significant reduction in total and LDL cholesterol, and also has*

an effect on triglycerides and a small positive or no effect on HDL cholesterol (7-12). It is not certain whether these results can be completely reproduced in patients with established CHD, as none of these studies were conducted in such patients. Finally, the Mediterranean diet was shown to strongly reduce the risk of CHD complications in secondary prevention (13-15). In the Lyon trial, the lipid-lowering effect of the Mediterranean diet was not different from that of the prudent Western diet (as defined according to the American Heart Association criteria) followed by the control group, because lipid-lowering drugs were widely used in the two randomized groups. This suggested that the Mediterranean diet was cardioprotective (Table 1) through biological effects independent of its effect on cholesterol. In particular, data from the Lyon trial suggested that the Mediterranean diet may prevent SCD (Table 1).

4. BLOOD PRESSURE AND DIET

Blood pressure is also related to CHD mortality, and hypertension is a common problem in many Western countries. Survey data indicate that only 50% of the US adults have an optimal blood pressure and 25% have hypertension (16). In fact, the relations between CHD and blood pressure is continuous and there is not abrupt increase in risk at levels of blood pressure regarded as criteria for hypertension (17). This suggests that efforts towards the prevention of the blood pressure-related diseases should be focused both on hypertensive and on non-hypertensive persons. Traditional approaches to control the epidemic of blood pressure-related CHD have largely concentrated on drug therapy in persons with hypertension. However, because of the many side effects, the rate of discontinuation is high with these classes of drugs (18). Clearly, a non-drug therapy, including lifestyle modifications, may have an important and expanding role that complement drug therapy, especially in the long term. Another point refers to the importance for outcome of even small differences in blood pressure. For instance, a 5 mm Hg reduction in diastolic blood pressure has been shown to result in a 35-40% lower risk of stroke (19). Regarding the influence of diet on blood pressure-related CHD complications, data from the Seven Countries Study again provide major information (20). **CHD mortality varied greatly among populations at each level of systolic or diastolic blood pressure.**

At a diastolic blood pressure level of 90 mm Hg, CHD mortality was 3 times as low among the Mediterraneans as among the populations from the USA and Northern Europe. On the same reasoning as for cholesterol, **it is presumed** that the protective factor is the diet of the Mediterraneans. Another question is whether dietary factors influence blood pressure

(21,22). Whether the Mediterranean diet pattern may influence (decrease?) blood pressure is unknown. In the Lyon trial, the extensive use of blood pressure lowering drugs in both groups prevented from becoming apparent an effect on blood pressure. However, recent research has emphasized the powerful role of total diet in hypertension (22,23). An adequate intake of minerals (sodium, potassium, magnesium and calcium), rather than the sole restriction of sodium, was proposed to be the focus of dietary recommendations (23). Other studies suggested that dietary n-3 fatty acids could lower blood pressure in subjects with hypertension (24). The responses were proportional to the changes in phospholipid n-3 fatty acids whereas n-6 fatty acids had no effect, which suggests that the effect did result specifically from the n-3 family. These data implied that in addition to their benefits through mechanisms such as the prevention of ventricular arrhythmias, n-3 fatty acids might be helpful in modulating (endothelial) factors regulating blood pressure. Another trial, the Dietary Approaches to Stop Hypertension (DASH), tested the effect on blood pressure of either a diet rich in fruits and vegetables or a "combination" diet rich in fruits, vegetables, and low-fat dairy products, and with reduced saturated and total fat (25). Although this "combination" diet was, not a typical Mediterranean diet, its main characteristics can be included among those recommended in a Mediterranean diet trial. In DASH, sodium intake was kept constant and the "combination" diet decreased systolic blood pressure by 5 to 6 mm Hg in subjects with normal blood pressure; in those with mild hypertension, the blood pressure reduction was twice as great, about 12 mm Hg. Reduction of this magnitude are similar to those observed with antihypertensive medications, but they are obtained at a very much lower cost, particularly in terms of side effects. In fact, DASH confirmed the meta-analyses as well as earlier indications from observational studies suggesting that dietary factors other than sodium markedly affect blood pressure (23).

5. THE DIETARY APPROACHES OF DIEABETES MELLITUS

Type 2 diabetes mellitus is associated with a three- to four-fold increase in the incidence of CHD (26,27), and the risk of CHD death is as high in diabetics without CHD as in non-diabetic patients with established CHD (28). The decline in CHD mortality in most Western populations has been mainly attributed to reduction in risk factors, owing to dietary changes in particular. The smaller decline in CHD mortality among diabetics, particularly women, may be due to less effective changes in risk factors for those people (29). As a matter of fact, apart from calorie restriction, the composition of the

diet of patients with type 2 diabetes remains controversial. The emphasis currently is on a diet low in saturated fatty acids. A reduction in the total fat intake is also suggested when weight loss is a primary issue. Thus, as most type 2 diabetics should lose weight, a low fat diet is commonly prescribed. **However, many physicians still remain hesitant in recommending such a diet, saying that a diet high in monounsaturated fats improves metabolic control in these patients better than a low fat, high carbohydrate diet, and should be preferred (30).**

On the basis of a meta-analysis, it is clear that high monounsaturated fat diets improve the lipoprotein and glycemiac profiles and also lower blood pressure (30). This type of diet may also reduce the susceptibility of LDL particles to oxidation and thereby reduce their atherogenic potential; in addition, it does not induce weight gain, provided that energy intake is controlled. Thus, in theory, diets low in saturated fatty acids but rich in monounsaturated fats (two of the main characteristics of the Mediterranean diet) are advantageous for the prevention of CHD in diabetics. Curiously, any diabetic diet has never been tested in this way for the prevention of CHD.

An important message from UKPDS and other recent trials is that in the prevention of CHD in type 2 diabetics, it is unwise to focus on single risk factors (31). All known risk factors should be tackled simultaneously, including hyperlipemia and hypertension. Also, because of a high risk of SCD in diabetics (32,33), specific recommendations aimed at preventing SCD should be given (see below). Classical risk factors fail to explain the excess of CHD rate in Indian Asians as compared with Europeans although the high prevalence of diabetes in this population may play a part (34). When exploring the contribution of dietary fatty acids in Indian diabetics, large differences in phospholipids fatty acids were noted, with lower concentrations of n-3 fatty acids among Indians (35), suggesting an explanation for their high CHD mortality. Considering all of these observations, it seems that the optimal diabetic diet may be a low-calorie Mediterranean diet. Not only does this diet protect the heart, improves lipid profiles and reduces blood pressure, but certain components of the Mediterranean diet (n-3 fatty acids in association with vegetables and legumes) have also been shown to improve glucose tolerance and prevent the apparition of overt diabetes (36,37). These human data confirm animal research that showed the importance of n-3 fatty acids in the action of insulin in various experimental models (38-40). Thus, although further studies are required, in particular regarding the physical structure of foods to modulate glucose metabolism and insulin resistance

(41), it is clear that diabetics should be instructed in the basic principles of the Mediterranean diet.

6. DIETARY PREVENTION OF SCD

SCD is defined as death from a cardiac cause occurring within one hour from the onset of symptoms (42) and is currently attributed to ventricular fibrillation (VF). The magnitude of the problem is considerable as SCD is the most common manifestation of CHD and accounts for about 50% of cardiovascular mortality in developed countries (42). The hypothesis that n-3 fatty acids may protect against SCD is derived from the results of a secondary prevention trial, the Diet and Reinfarction Trial (DART), which showed a significant reduction in total and CV mortality (both by about 30%) in patients who consumed at least 2 servings of fatty fish per week (43). The authors suggested that the protective effect of fish may be due to preventing VF since no benefit was observed on the incidence of nonfatal AMI. The hypothesis was consistent with experimental evidence suggesting that the n-3 fatty acids, the dominant fatty acids in fish oil and fatty fish, have an important effect on the occurrence of VF in various animal models (44-49).

Support for the hypothesis of a clinically significant antiarrhythmic effect of n-3 fatty acids came from two randomized trials testing the effect of ethnic dietary patterns, i.e. a Mediterranean type of diet and an Asian vegetarian diet, in the secondary prevention of CHD (13-15,50).

The two experimental diets included a high intake of essential alpha-linolenic acid, the main vegetable n-3 fatty acid. Whereas the incidence of SCD was markedly reduced in both trials, the number of cases was quite small and the antiarrhythmic effect cannot be entirely attributed to alpha-linolenic acids as these experimental diets also included high intakes of other nutrients with potential antiarrhythmic properties. The GISSI-Prevenzione trial was aimed at addressing the question of the health benefits of foods rich in n-3 fatty acids (51). The primary efficacy endpoint was the combination of death and nonfatal AMI and stroke. Secondary analyses included overall mortality and SCD. **Treatment with n-3 fatty acids significantly lowered the risk of the primary endpoint** (the relative risk decreased by 15%). Overall mortality was reduced by 20% (Table 2). However, it was the effect on SCD (45% lower) that accounted for most of the benefits seen on mortality. There was no difference across the treatment groups for nonfatal cardiac events, a result comparable to that of DART (43). So far, in CHD patients, no drug treatment has ever been shown as effective as the n-3 fatty acids for the primary prevention of SCD. Their prescription is now encouraged as

supplements (52,53), although the best way is likely to include them as part of a Mediterranean type of diet.

Table 2 Clinical efficacy of n-3 fatty acids in the GISSI-Prevenzione Trial

Death, non-fatal AMI and stroke	0.85
(0.70-0.99) Overall mortality	0.80
(0.67-0.94) Cardiovascular mortality	0.70
(0.56-0.87) Sudden cardiac death	0.55
(0.40-0.76) Non-fatal cardiovascular events	
0.96 (0.76-1.21) Fatal and non-fatal stroke	
1.30 (0.87-1.06)	

Relative risk (95% CI)

Reprinted from reference 51 CI = Confidence Interval

Michel de Lorgeril n-3 Fatty Acids in Inflammation and Autoimmune Diseases

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Among the fatty acids, it is the n3 polyunsaturated fatty acids (PUFA) which possess the most potent immunomodulatory activities, and amongst the n3 PUFA, those from fish oil [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] are more biologically potent than a-linolenic acid (LNA). Some of the effects of n3 PUFA are brought about by modulation of the amount and types of eicosanoids made and other effects are elicited by eicosanoid-independent mechanisms, including actions upon intracellular signaling pathways, transcription factor activity, and gene expression. Animal experiments and clinical intervention studies indicate that n3 fatty acids have anti-inflammatory properties and therefore, they might be useful in the management of inflammatory and autoimmune diseases. Coronary heart disease, major depression, aging, and cancer are characterized by an increased level of interleukin I (IL-1), a proinflammatory cytokine. In other words, these chronic diseases have a proinflammatory component. Similarly, arthritis, Crohn's disease, ulcerative colitis, and lupus erythematosis are autoimmune diseases characterized by a high level of IL-I and the proinflammatory leukotriene LTB_4 produced by n6 fatty acids.

The anti-inflammatory properties of n3 fatty acids, especially EPA, are due to competition with arachidonic acid (AA) as a substrate for cyclooxygenases and 5-lipoxygenase. **The eicosanoids from the n6 and n3 fatty acids have opposing properties.** The eicosanoids are considered a link between PUFA, inflammation and immunity. In addition to their effects on prostaglandins, thromboxanes and leukotrienes, n3 fatty acids suppress the production on interleukin I (IL-1(3) by Suppressing the IL-1 $_{R}$ mRNA, as well as the expression of Cox2 (cytooxygenase) mRNA that is induced by IL-1 (3. Cox2 is overexpressed in colon cancer cells. Both LNA, and EPA and DHA are involved in immune function. The precise effect of LNA depends on the level of linoleic acid (LA) and total PUFA content of the diet. A high dose of LNA (about l5g/day) will suppress human IL-I and TNF (tumor necrosis factor). It is unclear whether LNA itself exerts these effects, or whether they are the result of its conversion to EPA. Excessive intake of n6 fatty acids characteristic of Western diets produces an imbalance of n6 to n3 PUFAs which leads to an overproduction of the proinflammatory prostaglandins of the n6 series and cytokines. Supplements of LA rich vegetable oils increase IL-I and TNFa. Humans given n3-rich flax seed oil or fish oil supplements have sharply reduced stimulated production of IL-1, IL-2 and TNFa, as well as suppressed mononuclear cell proliferation and expression of IL-2 receptors.

Thus, in humans, LA increases proinflammatory cytokine secretion, whereas fish oil reduces proinflammatory cytokine secretion. There have been a number of clinical trials assessing the benefits of dietary supplementation with fish oils in several inflammatory and autoimmune diseases in humans, including rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, lupus erythematosus, multiple sclerosis, and migraine headaches. Many of the placebo-controlled trials of fish oil in chronic inflammatory diseases reveal significant benefit, including decreased disease activity, and a lowered use of anti-inflammatory drugs.

n-3 Fatty Acids In Prevention of Cardiovascular Disease

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Interest in n-3 polyunsaturated fatty acids (PUFAs) began 30 years ago, the effects of n-3 PUFAs on cardiovascular disease (CHD) have been shown in hundreds of experiments in animals, humans, tissue culture and clinical trials. The strongest evidence obtained from Crosse-cultural comparisons, prospective studies and Intervention studies is an inverse relation between of n-3 fatty acids in diet and in blood and tissues and occurrence of CHD and complications. The role of n-3 fatty acids in reducing CVD risk, its possible mechanisms of actions, and safety concerns and dietary recommendation of n-3 PUFAs will be discussed in this review paper.

The evidences of n-3 fatty acids and CVD from published paupers can be summarized as following:

1. Greater effects of increased fish eating on reducing CVD are displayed in communities that normally have low habitual intakes of fish.
2. The effect of moderate increase in n-3 fatty acids through fish or fish oil is shown in people who's intake is usually low, and there's no additional protection from eating large amounts.
3. The effects of fish eating are meanly due to EPA and DHA in fish or fish oil, but other protective components of fish may attribute as well.

A number of Possible mechanisms of n-3 PUFAs on CVD prevention have been proposed: 1) inhibit platelet aggregation and blood to clot by promoting formation of TXA3 & PG-I_3, 2) modify dyslipidemia by lower triacylglycerols and Cholesterol, increase in HDL2-C, 3) improve arterial function, inhibit monocytes migration into plaque, and cytokine, PDGF and it's mRNA production, relax blood vessels and reduce BP by stimulate endothelial production of NO, 4) anti arrhythmias by altering cell membrane fatty acids composition and electrophysiological function, reduce vulnerability to ventricular fibrillation, and less risk of cardiac arrest & sudden death 5) anti-inflammation by reducing production of TXB2, PGE2 and interleukin 1.

Very high intake of PUFAs may carry risk of lipid oxidation which is thought to be an important mechanism involved in pathogenesis of inflammation, cancer, and atherosclerosis. However, whether high intake n-3 PUFAs increase LDLox is controversial, and the assessment methods for lipid oxidation in vivo have limitations in reflecting the processes in the arterial intima. Regardless of the influence on LDL oxidation, there seems to be a net beneficial effect on clinical outcomes by enrichment with dietary PUFAs. It's reasonable to encourage a high intake of antioxidants through habitual diet along with a PUFA-rich diets.

Current intake of total n-3 PUFAs is 1.6g/d in US (1999), 1.8g/d in UK (1995), and g/d, the majority comes from plant n-3 fatty acid (alpha-linolenic acid), EPA and DHA intake only about 0.1-0.2g/d. There's no published data about national dietary intake of n-3 PUFAs in China yet. Average total fat intake accounts 26% of energy, P/S ratio is 2:1, n6/n3 ratio 1.2-4:1, the relatively high n-3 PUFAs intake within a low-fat regimen are contributed by high consumption of fish, shellfish and also edible vegetable oils. People at CHD risk eat oil-rich fish 1/wk. People who don't eat fish should take 200 mg EPA/DHA. Eat moderate amounts of oil-rich fish (200-300g/wk) or taking FO capsules decrease fetal CHD. Plant n-3 FA reduce VHD risk, ALA intake 2g/d (1%E) is recommended. Some countries and international organization have made dietary recommendation for n-3 fatty acids. A group of scientist in US recommends intake of ALA 2.2g/d and 0.65g/d combination of EPA and DHA. British Nutrition Foundation recommends 2.4 g/d of ALA and 1.2g/d of EPA and DHA. WHO, Canada and Sweden recommend a total n-3 fatty acids (ALA+EPA+DHA) intake of 2.8g/d, 1.2-1.6g/d and 1.7g/d respectively. Some recommendation have been made on the basis of the ratio of n-6 to n-3 fatty acids. WHO recommended a ratio of 5-1:1.

The Effect on Long Polyunsaturated Fatty Acid to Malignant Tumor

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Omega-3 long polyunsaturated fatty acid eicosapentaenoic acid (EPA) and docosapentaenoic (DHA) have effect on inhibiting the growth differentiation and proliferation of cancer cells. There is no effects existing with cancer cells in other fatty acid increase growth rate of cancer cells. Most of studies are summarized in the following four aspects:

1. Population epidemiological survey

It had conducted a series of retrospective survey and intervention trails it found that the higher intake of omega-3 polyunsaturated fatty acid such as docosapentaenoic acid (DHA) and eicosapentaenoic acid (EPA) **can against human cancer and effectively reduce the risks of colon cancer, breast cancer, prostate cancer, leukemia, skin cancer, as well as brain tumor**. If continuously administration of a fish oil riched EPA or DHA can alleviate symptoms and improve cashexia of cancer.

2. Animal trails

The trail of mutagen and inculated with tumor cell in mice, rats and nude mice, after treatment of EPA and DHA to colon cancer, breast cancer, fibro-sarcoma, it found that the dose >2%EPA and DHA can inhibit the growth rate of tumor cells significantly (P<0.05). But there is no effect under the dose lower than 2% EPA or DHA. The study of inducing sarcoma cells (Meth-A cells) with the oleic acid, linoleic acid, gamma-linolenic acid, alpha-linolenic and EPA. These studies suggest that several fatty acids can inhibit the growth and enhancing the survival of tumor-bearing animals.

3. In vitro experiments

The effect of EPA and DHA can inhibit tumor cells growth rate and proliferation. There has a time-dependent and dose-dependent relationship existing in the vitro experiment. The

effects can induce under the condition of a higher concentration of EPA and DHA. The dose over 50 μg/ml or 60 μg DHA can inhibit human breast cancer cells (Bcap-37) growth and proliferation. The DHA in 60μM-90μM can inhibit leukemia cells their effect better than EPA. Fish oil experiment shows that there has a different dose caused different effect existing in the inhibit of tumor cells.

4. Clinical trails

There is an improvement in the cashexia of cancer patient it shows beneficial control metastasis of carcinoma and strengthening anti carcinoma in the case using DHA and EPA in their diet. These data shows that consumption rich DHA and EPA diet which can assistant nutrients during the period of treatment in the patients of colon cancer, breast cancer, pancreatic cancer, and prostate cancer. The maximum tolerated dose of fish oil is 0.3 mg/kgbw per day. Among these the fish oil content 62% EPA.

Changes in Phospholipid-Fatty Acid Patterns of Mucosa in Colorectal Adenoma Carcinoma Sequence

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During the last 10 years, the incidence of colon cancer has remarkably increased in Korea. Few studies have focused on phospholipid-fatty acids in mucosa of colorectal adenoma and cancer. The purpose of this study was to investigate phospholipid-fatty acid patterns in diseased mucosa of the patients with colorectal cancer (n=8), adenomatous polyps (n=29) known as potential precursor lesions of carcinoma, and in normal mucosa of controls (n=5 1). Fatty acids were analyzed by TLC and GC systems.

There were considerable differences in fatty acid profiles between the normal mucosa and the diseased mucosa of both adenoma and cancer. In adenoma polyps and cancer, the significantly higher levels of C18:1, C24:1, C18:2n-6, C20:4n-6, C18:3n-3, C22:6n-3 and the lower levels of C14:0, C16:0, C18:0, C24:0, C20:5n-3, C22:5n-3 were observed in their compositions(%). It was interesting to observe that some desaturation indices, such as the ratios of C18:1/C18:0 (desaturation to oleic acid) and C22:6n-3/C22:5n-3 (desaturation to DHA), were significantly higher both in colorectal adenomatous polyps and in cancer tissues. Especially, the ratio of C20:4n-6/C20:3n-6 (desaturation to arachidonic acid) was significantly higher in cancer than in adenoma ($p<0.01$) and also in adenoma than in normal mucosa ($p<0.01$). This study suggests that the profiles of individual fatty acids and some of their ratios in phospholipids of colorectal mucosa were abnormal already at the stage of adenomatous polyposis. These changes may probably occur in adenoma phase prior to cancer formation and can be influenced by the differences in phospholipid metabolism and eicosanoid balance which has been suggested to participate in colorectal caricinogenesis.

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Something's Fishy About Fish Oils

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This presentation reviews the benefits of fish oils as a source of EPA while at the same time addressing some of the precautions that need to be weighed. **Since linolenic acid is a precursor of EPA, and readily available in the food supply, plant based alternatives to fish oils are offered.**

In the 1970's a number of studies conducted on Greenland Eskimos led to the opinion that an increase in marine based *n-3* fatty acids was associated with a lowered risk of coronary artery disease. This apparent correlation was strengthened in 1985 by three articles printed in the same issue of the prestigious New England Journal of Medicine.[2]

Since then other studies have indicated that fish oils may have numerous benefits including reduction of plasma triacylglycerols, platelet function, blood viscosity, and inflammatory processes. Possible positive effects may also be seen in rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease, depression, and it is even suggested they may have a preventative effect for breast and colon cancer[4] and chronic obstructive pulmonary disease.[5]

However, I believe that some cautions are warranted and should be addressed. First, an increased consumption of fish will increase dietary cholesterol and very likely thereby increase plasma LDL in most people.[6] Other potential hazards include a worsening of blood sugar levels in diabetics[7], increased clotting time,[8] and increase in body weight. Fish oil tablets are expensive. Sea food is a large reservoir for infectious diseases.[9] And toxins in polluted waters concentrate in the fatty tissues of fish.

Most studies looking at the beneficial aspects of *n-3* fatty acids have based their studies on the 20 carbon long chain eicosapentaenoic acid (EPA). However EPA is not an essential fatty acid as the body can synthesize it from linolenic acid, and abundance of which can be found in vegetable oils especially flaxseed, walnuts, and canola oil. These foods have the potential for providing all the benefits of fish oils, without their potential harmful side effects.

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Essential Fatty Acids and Brain Development

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Essential fatty acids (EFA) are structural components of all tissues and are indispensable for cell membrane synthesis; the brain, retina and other neural tissues are particularly rich in long-chain polyunsaturated fatty acids (LCPUFA). Human infants require n-6 and n-3 LCPUFA for growth and neural development. Animals fed diets deficient in the essential fatty acids have low neural LCPUFA and behavioral changes that imply adverse effects on brain function. These fatty acids serve as specific precursors for eicosanoids that regulate numerous cell and organ functions. Some reports showed that the n-6 and n-3 fatty acids influence eicosanoid metabolism, gene expression, and intercellular cell-to-cell communication. Linoleic acid (LA) is the major n-6 fatty acid, and alpha-linolenic acid (ALA) is the major n-3 fatty acid. In the body, LA is metabolized to arachidonic acid (AA), and ALA is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). DHA is found in unusually high concentrations in the brain and is selectively accumulated during fetal and infant brain growth. Studies with nonhuman primates and human newborns indicate that DHA is essential for the normal functional development of the retina and brain, particularly in premature infants. Results from animal and recent human studies support the essential nature of n-3 and n-6 EFA for human subjects, particularly in early life. The most significant effects relate to neural development and maturation of sensory systems. The PUFA composition of cell membranes is, to a great extent, dependent on dietary intake. Even preterm infants are able to form AA and DHA, but that synthesis is extremely low. Attention Deficit/Hyperactivity Disorder (ADHD) is the most common behavioral disorder in children. Nutrient deficiencies are common in ADHD. **Supplementation with n-3 and n-6 essential fatty acids, and minerals**, the B vitamins, flavonoids, and the essential phospholipid phosphatidylserine (PS) **can ameliorate ADHD symptoms**. Comprehensive clinical studies have also shown that dietary supplementation with marine oil or single-cell oils, sources of LCPUFA, results in increased blood levels of DHA and AA, as well as an associated improvement in visual function in formula-fed premature infants to match that of human milk-fed infant. A number

of findings have indicated that the administration of a diet deficient in essential fatty acids during development causes hypomyeIination in the rat brain. **Therefore, appropriate amounts of dietary n-6 and n-3 fatty acids need to be considered in making dietary recommendations.**

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