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Review Article

LactoDHA™: SAFE VEGETARIAN SOURCE OF OMEGA-3 FATTY ACIDGovind shukla^{1*}, Sharat Kumar² and CJ Sampath Kumar³

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ABSTRACT

Long-chain EPA/DHA omega-3 fatty acid supplementation can be co-preventative and co-therapeutic. Current research suggests increasing accumulated long chain omega-3s for health benefits and as natural medicine in several major diseases. But many believe plant omega-3 sources are nutritionally and therapeutically equivalent to the EPA/DHA omega-3 in fish oil. Although healthy, precursor ALA bio-conversion to EPA is inefficient and production of DHA is nearly absent, limiting the protective value of ALA supplementation from flax-oil, for example. Along with pollutants certain fish acquire high levels of EPA/DHA as predatory species. However, the origin of EPA/DHA in aquatic ecosystems is algae. Certain microalgae produce high levels of EPA or DHA. Now, organically produced DHA-rich microalgae oil is available. Clinical trials with DHA-rich oil indicate comparable efficacies to fish oil for protection from cardiovascular risk factors by lowering plasma triglycerides and oxidative stress. This review discusses about omega-3 fatty acids in nutrition and medicine; possible protective mechanisms of EPA/DHA in major diseases such as coronary heart disease, atherosclerosis, cancer and type 2 diabetes; microalgae EPA and DHA-rich oils and recent clinical results; the importance of DHA during pregnancy and breastfeeding; DHA and human infants.

INTRODUCTION

The foundation of Ayurveda (natural Indian medicine) is diet^{1, 2}. Similarly, Hippocrates stated 'Let food be thy medicine and medicine be thy food'³. To achieve optimum health, contemporary lifestyles must consider food choices, eating habits and strategies for building up omega-3 levels in the body^{4, 5}. For individuals 'at risk' for diet induced diseases everywhere, even dietary restrictions, vitamin supplementation, prescription remedies, alternative medicine, and physical exercise may not be fully protective, preventative, or therapeutic without addressing inherent omega-3 fatty acid deficiencies^{6, 7}. The Indian paradox of diet-induced diseases, whether vegetarian or non-vegetarian, is not isolated from the global dilemmafacing modern society. The paradox is often simultaneous

overeating and undernutrition within the same individual, leading to the initiation of

major diseases. Although various origins are proposed for some cases of obesity in India⁸, urbanization and modernization increase the numbers consuming foods associated with convenience diets⁹.

These include high fat foods, processed foods, snacks, and drinks made available for instantaneous consumption, resulting in a habit of overeating with overly-frequent caloric intake. Too much fatty, oil fried, and processed food often leads to insulin resistance, obesity, type-2 diabetes, high blood pressure, atherosclerosis, and heart disease¹⁰. To the extent that a modern diet departs from traditional dishes with their rich complements of fresh herbs and spices - neglecting

uncooked fruits, vegetables, and leafy greens as functional foods - there may be increased risk of micronutrient undernutrition, particularly in essential omega-3 fatty acids¹². Thus, many processed foods are nonfunctional

and dysfunctional foods and result in diets containing little omega-3 nutritional complement.

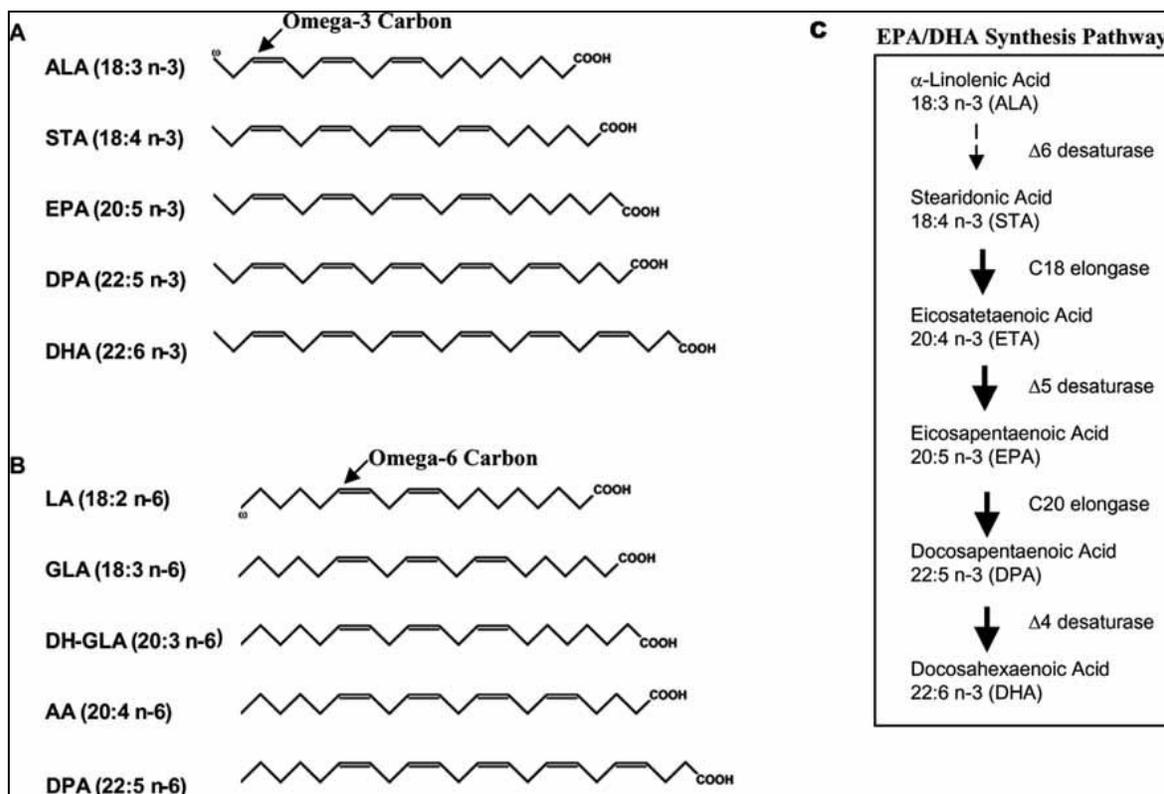


Fig. 1

OMEGA-3 FATTY ACIDS IN NUTRITION AND MEDICINE

Omega-3 fatty acids focused on throughout this review are the bioactive lipids eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Certain fish and microalgae contain high levels of the essential bioactive omega-3 products EPA and/or DHA. Plants also contain various levels of omega-3 fatty acids as 'precursors' mainly in the form of alpha-linolenic acid (ALA). Other dietary long chain fatty acids are the omega-6 fatty acids. Linoleic acid (LA) is the main omega-6 'precursor' in plant/vegetable oils. The omega-6 fatty acid arachidonic acid (AA) is bioactive and found in red meat.

The balance of omega fatty acids is important to consider. The so-called omega-3:omega-6 ratio has become a model for gauging the proper balance of these fats in oils and the diet¹³. Diets with greater than a 1:10 ratio of omega-3 to omega-6 are not recommended, whereas a 1:1 ratio is considered perfect. Very unhealthy ratios of 1:25 and 1:50 are common, especially with regular consumption of 'fast-food', high amounts of fried food, and low intake of fresh whole foods. Thus, 'Eating to live and not living to eat' becomes an important consideration with increases in modern, convenient, non-functional food choices.

Clinical studies are now concluding significant benefits associated with EPA/DHA from fish

oil and DHA-rich oil from microalgae¹⁴⁻²⁵. High doses of oil from both fish and microalgae sources are considered safe, result in increased circulation of both EPA and DHA omega-3s, and both oil sources are protective against cardiovascular risk factors.

Significantly, DHA-rich oil formulations are equally protective compared to fish oil in nearly all human trials conducted. Published clinical studies indicate beneficial effects of DHA-rich oil for cardiovascular risk prevention in healthy men and women, producing significant decreases in plasma triglyceride levels. Results were comparable to fish oil in effects, bioavailability and safety profiles. In direct studies, algae oil cardio-protective effects were similar to fish oil. Furthermore, DHA-rich oil supplementation increased DHA levels in lactating women, in breast milk and in nursing infants²⁶. DHA is particularly important for fetus development, pregnancy outcomes, cognitive development and maintenance, learning and memory, visual function, the immune system, and more²⁷.

SYNTHESIS AND METABOLISM

The vegetarian diet largely depends upon ALA to be synthesized into EPA and DHA in the body, according to the pathway shown in Fig. (1C). Important findings now show that ALA conversion into EPA and DHA is rate limiting^{28, 29}. The first metabolic step in EPA synthesis by the enzyme $\Delta 6$ desaturase is kinetically slow (shown in Fig. (1C) as dashed arrow), so most ALA undergoes oxidative metabolism for energy and is not converted to EPA. That is, omega-3 oil supplementation from plant sources may produce only 7% of the EPA levels compared to the EPA levels gained through fish oil supplementation. DHA accumulation from ALA only occurs at trace levels due to the additional steps required to convert EPA into DHA. Similarly, EPA concentrations increase with dietary EPA, but DHA does not. Also, conversion of omega-6 to omega-3 does not occur. In contrast, dietary DHA supplementation will result in steady state DHA concentrations and modest increases in EPA concentrations through DHA retro-conversion, producing EPA at 5-11% of accumulated DHA levels. With continuous supplementation, EPA/DHA levels plateau, suggesting the body first needs to incorporate omega-3s directly through the diet before

limiting omega-3 fatty acid accumulations by oxidative metabolism. With few ALA rich plant sources for vegetarians, omega-3 supplementation is an important consideration. Beyond flax, walnut and mustard seed oils, ALA supplementation is limited because stearidonic acid (STA) is poorly formed as the second product in the EPA/DHA synthetic pathway³⁰. Bypassing $\Delta 6$ desaturase at the first step with STA supplementation allows for EPA accumulation up to 30% of levels achieved by fish oil regimens. However, plant sources of STA are found most abundantly in the invasive plant species *Echium* and seldom used herb *Borage*. As a staple in the Indian diet, mustard seeds contain just 1-4% ALA omega-3 (~200 mg/tablespoon). Yet mustard oil supplementation does not produce significant effects compared to EPA/DHA omega-3s^{31, 32}. Also, as a food it is not clear what effects pre-roasting or heating of mustard seeds have on ALA stability. As with all unsaturated fats, heating and cooking causes a percentage of the fat to undergo air oxidation. Polyunsaturated fats such as omega-3s and omega-6s are particularly sensitive to oxidation and must be consumed in relatively fresh foods. This suggests conversion of ALA to longer chain omega-3s may be further limited by oxidation of available omega-3 precursors in pre-cooked and/or processed foods. Proper information is particularly important as awareness of the benefits of omega-3 fatty acids increases. Also, because DHA is not readily produced from plant omega-3s, other truly alternative vegetarian sources of DHA are needed. At present, direct EPA/DHA delivery is believed dependent on certain marine fish species, which many fear may not be a pure or sustainable resource. Reputable data suggest wild marine fish populations are already becoming depleted³³. However, microalgae oil products now provide an organic, vegetarian, sustainable alternative source of EPA/DHA. Yet these are still relatively unknown products.

OMEGA-3 EPA/DHA IN PHYSIOLOGY AND GENE REGULATION

Why are the longer chain omega-3 fatty acids different from other fatty acids in terms of their biological effects? Likely, the higher number of double bonds starting at carbon 3 from the end gives these fatty acids

uniqueness and bioactive properties that were harnessed by organisms over evolutionary time. For instance, the brain is enriched in long-chain omega-3 fatty acids, particularly DHA, in phosphatidylcholine and phosphatidylethanol phospholipids concentrated in plasma membranes at the neuronal synapse. Plasma membranes contain various amounts of DHA or EPA in phospholipids. Biophysical studies of omega-3 membrane-protein interactions and functions are emerging areas of research. In addition, long chain omega-3 fatty acids are definite biological regulators. Overall, reduction of chronic inflammation and improvement of lipid metabolism are positive effects that originate through omega-3 effects on gene expression. The bioinformatics of omega-3 gene regulation has been provided here in summary, and because of recent reviews and research articles these data are only briefly covered here³⁴⁻³⁸. Two main groups of genes are affected by EPA/DHA omega-3s. These contain inflammatory genes and energy metabolism genes. First, inflammatory genes are suppressed by EPA/DHA and are as follows: nuclear factor κ B; inhibitory κ Bkinase; inducible nitric oxide synthase; interferon γ ; Interleukin-1b, 2, 6, 8, & 12; E-selectin; intercellular adhesion molecule; vascular cell adhesion molecule; monocyte chemoattractant protein 1; C-reactive protein; von Willebrand factor; matrix metalloproteinase 9; tumor necrosis factor α ; and cyclooxygenase 2. Besides inflammation, endothelial and angiogenesis processes are implicated by these data.

Second, energy metabolism genes are increased by EPA/DHA and are as follows: peroxisome proliferator-activated receptors; sterol responsive element binding proteins; adipocyte fatty acid binding protein; acyl CoA oxidase; uncoupling protein 1; carnitine palmitoyltransferase 1; leptin; pyruvate dehydrogenase kinase 4; glucose transporter 4; Caveolin-1; Caveolin-2; fatty acid transporter protein CD36; stearoyl CoA desaturase 1; ATP binding cassette transporter A1; lipoprotein lipase; liver-X-receptors; and apolipoprotein E. Because lipid metabolism is linked to insulin resistance, treatment of type 2 diabetes by omega-3s is implicated, but likely indirect. Notably, in treated hypertensive type 2 diabetic patients either EPA or DHA

independently reduced oxidative stress but not markers of inflammation³⁹, yet EPA and DHA reduced triglyceride levels in these patients⁴⁰. EPA and DHA may also act as free and/or acyl-CoA conjugated fatty acids, implicating Long-chain Acyl-CoA Synthetases (ACSLs) in their activation⁴¹. Because ACSLs are regulated by peroxisome proliferator-activated receptors⁴², by analyzing specific ACSL isoforms via RT-PCR⁴³, ACSL expression patterns in tissues could provide some insight into omega-3 metabolism. Also, metabolized forms of EPA and DHA bind to the PPAR family of transcription factors⁴⁴, which may differentially regulate ACSL isoforms in different tissues. Therefore, bioactive PPAR ligands derived from EPA/DHA potentially signal through ACSLs to activate genes in a positive feedback loop that could make the effects of EPA/DHA increasingly effectual in tissues as regulators of lipid homeostasis. Genes influencing innate and acquired immunity in type 1 diabetes is also an area of active research⁴⁵. How omega-3s affect these would be interesting to know.

OMEGA-3 FATTY ACIDS FOR COMBATING MAJOR DISEASES

Coronary Heart Disease: In the 1970's researchers noted that Eskimo populations consumed extremely high levels of fat from fish and blubber, but this contradicted hypotheses of coronary heart disease at the time because the indigenous populations had no signs of cardiovascular disease. High levels of EPA/DHA are thought to be the protective complement. Recently, DART and GISSI-P clinical studies of fish oil supplementation revealed 15-45% reduction in mortality in 'at risk' patients for coronary heart disease^{46, 47}. Reductions in sudden death were particularly significant. One small fish oil supplementation study in Norway did not show significant improvements, probably because of habitual incorporation of cold water fish as a regular part of the diet. The latter result can be explained by assuming that omega-3 status was optimum to begin with, suggesting that with full EPA/DHA essentiality in the background this meant coronary heart diseases in Norway were likely due to additional factors such as obesity or genetic factors that played dominant roles in participating subjects. However, most of the risks of coronary heart disease globally are

associated with diet. A high incidence of diet induced coronary heart disease occurs in many countries, including India.

Arrhythmias: The benefits of omega-3s were originally thought to be due to their antithrombotic effects, but recent evidence has indicated that the predominant effect may be antiarrhythmic. Omega-3 supplementation decreased heart rate variability in patients after

myocardial infarction, which correlated with a lower risk of mortality and malignant arrhythmia⁴⁸. In fact, direct addition of EPA/DHA into media with cultured cardiomyocytes prevents or terminates pharmacologically induced or electrically clamped arrhythmias. The modulation of plasma membrane permeability and the stabilization of ion channel functions are suggested to be acute protective properties of EPA/DHA on heart muscle cells.

Atherosclerosis and Inflammation: Omega 3 fatty acids may also influence the atherosclerotic process. Again, in patients with coronary heart disease EPA/DHA supplementation versus placebo for two years resulted in modest improvements in atherosclerosis as assessed by angiography. An important recent study of patients awaiting carotid artery surgery randomized cohorts to fish oil capsules, sunflower oil capsules, or controls up until surgery and then assessed morphology of the plaque⁴⁹. Omega 3 fatty acids incorporated into atherosclerotic plaques in the fish oil group, and these plaques were more likely to have reduced mass with less inflammatory infiltrate and increases in thickness of fibrous caps from protective responses. These features imply a plaque that is less vulnerable to rupture and indicates EPA/DHA may help to establish plaque stability. Additional improvements in overall endothelial function and decreases in pro-inflammatory signals have also been noted. The fundamental cellular processes activated or suppressed by omega-3 supplementation and their potential impact on coronary heart disease are active areas of research.

Vitamin D in the prevention and treatment of coronary heart disease

There is accumulating evidence that the vitamin D hormone calcitriol exerts important physiological effects in cardiomyocytes, vascular smooth muscle cells, and the vascular endothelium. Low levels of the calcitriol precursor 25-hydroxyvitamin D are associated with myocardial infarction, congestive heart failure, and calcific aortic stenosis. Deficient calcitriol concentrations probably contribute to the massive vascular calcification seen in chronic kidney disease. In patients with end-stage renal disease and end-stage heart failure, very low-circulating calcitriol levels or nonuse of active vitamin D or both are independently associated with high mortality rates. (Ref; Zittermann, Armin; Koerfer, Reiner Vitamin D in the prevention and treatment of coronary heart disease)

Cancer: Epidemiologic studies indicate populations that habitually consume high amounts of EPA/DHA fatty acids also have lower incidences of breast, prostate and colon cancers than those that consume less of these fatty acids in their diets. Many of the mechanisms that are thought to slow or prevent the growth of cancers may also slow or prevent the growth of residual metastatic cancer cells as well. Therefore, increasing the consumption of EPA/DHA from food or supplementation can naturally augment cancer therapy. However, clinical research is not complete in humans. The results of animal studies have demonstrated that the consumption of EPA/DHA can slow the growth of cancer xenografts, increase the efficacy of chemotherapy, and reduce the side effects of chemotherapy. Mechanisms that may be involved include the suppression of cyclooxygenase-2 expressions in tumors, decreased AP-1 and ras oncogene levels, and decreased NF- κ B activation and bcl-2 expression. Suppressing these would reduce proliferation and angiogenesis and increase apoptosis.

The Role of Vitamin D in Cancer Prevention

Vitamin D status differs by latitude and race, with residents of the northeastern United States and individuals with more skin pigmentation being at increased risk of deficiency. A PubMed database search yielded 63 observational studies of vitamin D status in relation to cancer risk, including 30 of colon, 13

of breast, 26 of prostate, and 7 of ovarian cancer, and several that assessed the association of vitamin D receptor genotype with cancer risk. The majority of studies found a protective relationship between sufficient vitamin D status and lower risk of cancer. The evidence suggests that efforts to improve vitamin D status, for example by vitamin D supplementation, could reduce cancer incidence and mortality at low cost, with few or no adverse effects.

Type-2 Diabetes: EPA/DHA supplements may indirectly help prevent the development of type 2 diabetes through modulation of lipid metabolism. These effects are likely mediated through transcription factors by decreasing inflammatory NF- κ B activity and increasing pro-metabolic PPAR activities. Weight reduction by restriction of total calories, increasing physical activity, and deriving total intake of fats from healthy sources is always advisable. Nutritional causes seem to be the main culprit in this wide-spread epidemic. Nutritional therapy appears to be the main option for treatment⁵⁰. Again, omega-3s may be significant co-therapeutic treatments for lowering triglyceride levels in pre-diabetic and type 2 diabetic patients⁵¹. However, omega-3 supplements may not directly affect glucose homeostasis, yet these essential fatty acids are protective against lipid oxidative stress in diabetic patients.

The importance of DHA during pregnancy and breast feeding: Microalgae oil can be considered safer than fish and fish oil for pregnant and nursing mothers for purity and better for direct delivery of the right DHA levels for pregnant women.

The DHA intake from an average diet during pregnancy is only 80 mg DHA per day, based on a paper in the *Journal of Nutrition*, 2005 (Denomme et al. 135: 206-211).

A minimum 300 mg DHA daily is suggested based on a 1999 NIH body of experts recommending needed levels to support fetal brain development and visual acuity benefits. Most notably, the last trimester is the most critical period for DHA during pregnancy for the baby's brain growth in the womb. Fish consumption is not enough, partly because DHA is only a minor part of fish and fish oils. DHA (Docosahexaenoic acid, an omega-3 long chain polyunsaturated fatty acid) is found in every cell in our bodies. It is critical for brain,

eye and central nervous system development and functioning.

During pregnancy, developing babies rely on their mothers to get needed DHA. Since DHA is derived from the foods we eat, the content of DHA in a mother's diet determines the amount of DHA passed on to her developing baby. Unfortunately, the majority of pregnant women fail to get the recommended amount of DHA in their diets and DHA is not found in most prenatal vitamins.

Current research suggests adequate levels of DHA may help increase a developing baby's cognitive functioning, reduce the risk of pre-term labor and decrease the risk of postpartum depression. Consider the following:

- ◆ A 2003 study published in the journal *Pediatrics* showed children whose mothers took a DHA supplement during pregnancy scored higher on intelligence tests at four years of age than children of mothers not taking DHA supplements.

- ◆ A 2004 study published in *Child Development* found that babies whose mothers had high blood levels of DHA at delivery had advanced attention spans into their second year of life. During the first six months of life these infants were two months ahead of babies whose mothers had lower DHA levels.

- ◆ Other research studies suggest breastfed babies have IQs of six to 10 points higher than formula-fed babies. Medical and nutritional experts attribute this difference to the DHA infants receive while nursing. (*Obstetrics & Gynecology*, 2003).

- ◆ In a trial of women receiving DHA supplementation during the third trimester, the average length of gestation increased six days (*Obstetrics & Gynecology*, 2003).

- ◆ Research has found low levels of DHA in mother's milk and in the red blood cells of women with postpartum depression. (*Journal of Affective Disorders*, 2002). Some scientists believe increasing levels of maternal DHA may reduce the risk of postpartum depression.

Renowned figures speak out about DHA

Research findings such as these have led pregnancy and child health experts to spread the word about the importance of DHA during pregnancy. Dr. William Sears, one of the nation's leading experts on child health and development and longtime advocate of

DHA, states "DHA is the most important brain-building nutrient at all ages, *especially* during pregnancy and the pre-school years when the child's brain is growing the fastest." Dr. Sears hosts DHADOC.com, a web site providing information on the importance of DHA in infant and maternal nutrition. In light of research findings and what he calls "common sense," Dr. Sears recommends on his web site that pregnant and lactating women supplement their normal diet with 200 milligrams of DHA a day.

In what many consider the modern day pregnancy bible, *What to Expect When You are Expecting*, Heidi Murkoff, *et. al.*, devotes a section to the importance of adequate DHA in the pregnancy diet chapter of her book. She explains that DHA is important during pregnancy, "especially during the last three months, when your baby's brain grows at a rapid pace and lactation (the DHA content of a baby's brain triples during the first three months of life)." Another maternity expert, Rebecca Matthias, president of Mothers Work, Inc., the nation's leading maternity retailer touts the benefits of DHA in her latest book, *51 Secrets of Motherhood*. She celebrates DHA as "the new wonder supplement that actually increases your baby's growth."

DHA is hard to get in your diet

Why do pregnant and lactating women, who so critically need DHA, find it difficult to get the recommended amount of this crucial nutrient in their diets? There are two primary reasons. First, during pregnancy the daily requirements of DHA increase from 220 mg to somewhere between 300 and 1,000 mg. Second, DHA isn't easy to get in your diet.

Despite popular belief DHA is not found in flax seed. Flax seed (and green leafy vegetables) are sources of alpha-linolenic acid which may convert to DHA, but the process is inefficient and according to some experts may not happen at all. Alternatively you might choose to eat some of the "safer" fish choices such as pollock, haddock and cod. But according to Dr. Barbara Levine, associate professor of nutrition in medicine at Weill Medical College of Cornell University, "the purest source of DHA is not the fish itself, but rather what fish consume: the ocean's vegetarian plant algae. Taking DHA supplements produced from marine algae is

therefore a safe way for pregnant women to boost their fatty acid stores," Levine says.

Advantages of vitamin D for women in lactoDHA

Women in northern hemisphere have increased risk of vitamin D deficiency due to lack of available sunlight exposure (ref: journal of clin ped. jan2000edition). The addition of vitamin D in lacto DHA prevents uterocomplications in mothers such as – Pre eclampsia, Gestational diabetes (ref: journal of clinical endocrine2007), Bacterial vaginitis (ref: journal nutr 2009jun139 (6)1157-1161).

The Importance of DHA in Infants

Docosahexaenoic acid, DHA, is an omega-3 fatty acid that is found throughout the body. More specifically, it is an important structural fat in the brain and eyes and a key component of the heart. A growing body of research continues to support the role that DHA plays in maintaining good health throughout life. DHA is important for brain and eye development and function throughout the life cycle, but is particularly important during the first years of life and early childhood, as a child's brain is still steadily growing at age four.

- DHA is the predominant omega-3 fatty acid found in the brain. DHA represents about 97% of all omega-3 fatty acids in the brain and 93% of all the omega-3 fatty acids in the eye (retina). (65-67)

- Uptake of preformed DHA by the brain is significant between ages 2 and 5 and supports the substantial accumulation of DHA by the brain during this critical growth period.⁶⁸

- One-year-old infants who received DHA-supplemented baby food since weaning showed improved vision, equivalent to 1.5 lines on the eye chart, compared to those who received baby food that did not contain DHA.⁶⁹

- The body's production of DHA from alpha-linolenic acid (ALA) is limited. Including DHA in the diet is the most reliable way to ensure that DHA is available to support optimal brain and eye development and function.

- DHA supplementation has been shown to improve blood lipid profiles and restore endothelial function in children with high cholesterol levels.

- Children ages 1-5 years were shown to have low DHA intakes ranging from 30-50 mg/day. This is because the primary dietary sources of

DHA are fatty fish and organ meats which are not popular food choices for young children and DHA-fortified foods geared toward toddlers are limited.

Importance of vitamin D in lactoDHA

There are some associated risks to developmental as well as overall health risks & benefits from optimal vitamin D status during pregnancy & in early years. These include-

- Schizophrenia(ref; schizophrbull.2009 may35(3):582-95)
- Autism (ref; scientific american,april24 2009)

- Lower respiratory tract infections (ref; eur.J clin. Nutr.2009 april 63 (4) 473-77)
- Birth weight (ref;jour. Of early human dev.2009apr85(4) 231-4)
- Diabetes (ref; diabetologia2000 sep;43(9):1093-8)
- Seizures (ref;Am. J dis child 1978 oct;132(10)973-77)
- Heart failure (ref;epub 2007 August)
- Weak bones (ref; lancet 2006 jan7;367(9504):36-43)
- Asthma (ref; J. clin. Immune. 2007nov;120(5);1031-5)

Table 1: Long Chain Polyunsaturated Fatty Acids of Some Non-Toxic Algae versus Fish Oil Supplements

Kingdom	Phyla	Species Name (Common)	DHA	EPA	AA
Plantae	Chlorophyceae	(Green Algae)	0%	25%	0.1%
Plantae	Rhodophyteae	(Red Algae)	0%	20%	4%
Chromista	Heterokontaeae	(Yellow-Green)	0.5%	27%	6%
chromista	Heterokontaeae	Schizochytrium	37.4%	28%	1.0%

PREVENTION AND SAFETY

Current analysis reveals red blood cell levels of EPA/DHA omega-3s in urban Indian populations, and urban populations elsewhere, average between 3% and 4%. Protective, preventative, and therapeutic EPA/DHA levels may require nutritional accumulation to between 6% and 8%. Thus, to achieve nutritional essentiality for omega-3 fatty acids many individuals may need to nearly double their circulating EPA/DHA status. The topic of benefit versus risk of regular fish consumption and fish oil supplementation is still hotly debated. Various and prevalent concerns exist regarding the safety, sustainability, and predatory species sources of EPA and DHA omega-3 fatty acids from fish. Although fish oil from reputable companies is regarded as safe, long term exposure through supplementation is often feared since trace pollutants from ocean ecosystems contaminate both fresh caught and farm-raised fish that feed on or are fed marine organisms⁵¹⁻⁵³. Some types of fish contain relatively high levels of mercury, polychlorinated biphenyls [PCBs], dioxins and other environmental contaminants⁵⁴. In general, older, larger predatory fish contain the highest level of contaminants. Fish can also contain significant levels of methyl mercury, considered one of the more dangerous food contaminants today^{55, 56}. PCBs and methyl mercury are believed to have long half-lives in

the body and can accumulate in people who consume fish on a frequent basis. Recommendations currently suggest limiting intake of fish to twice per week. A study in the journal Diabetes Care links persistent organic pesticide circulation from pollutants in fish to insulin resistance and type- 2 diabetes⁵⁷. The study reports the action of these pesticides may be critical during the early stages of diabetes. Similarly, contaminants in fish oil supplements should not be ignored, especially in light of recent mass market trends towards high level daily consumption. Heavy metals, dioxins and PCBs in fish oil supplements and in cod liver oil supplements are documented to occur at persistent low levels^{58, 59}. More toxicology studies are needed, particularly with respect to fish based supplements. The possible effects of these trace toxins in the human population over years of exposure from omega-3 fish oil supplementation is not known and a cause for cautious concern. Even though clinically tractable effects of the contaminants found in fish require long term exposure at higher levels than found in supplements, pre-clinical effects cannot be determined or ruled out with confidence at this time. Thus, one safe and sustainable solution for supplementing long chain omega-3 may be to derive omega-3s from microalgae sources, which are currently the only well developed EPA and DHA omega-3 fish oil alternatives.

Table 2: Fatty Acid Species and Sterol Content of Microalgae *Schizochytrium* DHA-Rich Oil

Species	Omega	% Total Oil	% Sterols in Oil
Myristate		10.8	
Palmitate		25.2	
Palmitoleate		1.9	
DH-GLA	6	2.4	
Arachidonate	6	1.0	
EPA	3	2.8	
DPA	6	14.4	
DHA	3	37.7	
Total	6	17.8	
Total	3	40.2	
Ratio	3/ 6 = 2.3		
Cholesterol			0.8 (8 mg/g)
Total Sterols			3.1 (31 mg/g)

MICROALGAE AS A SAFE VEGETARIAN SOURCE OF

EPA AND DHA *Schizochytrium* as an Alternative Source of Dietary Omega-3s

Analysis of vegetarian sources of omega-3 fatty acids inevitably leads to microalgae. Overall, algae are a unique branch of life on planet earth. Most marine EPA and DHA do not originate with fish, but accumulate up the marine food chain from sources like microalgae. Certain non-toxic algal phyla contain high levels of EPA compared to DHA, but commercial development of these has not taken place, possibly due to aquaculture limitations for raising photosynthetic organisms, and higher AA levels may be another concern (Table 1). In comparison, the microalgae grown for making DHA-rich oil has low AA and low EPA levels, but very high DHA levels. The discussion that follows focuses on the *Schizochytrium* microalgae strain, which makes high levels of DHA and some EPA⁶⁰ (Table 1).

Schizochytrium is a Thraustochytrid, a member of the kingdom Chromista. *Schizochytrium* is an ancient non-photosynthetic detritus feeding organism that does not assemble into higher ordered structures, as do some photosynthetic green, red, and yellow-green algae. Thraustochytrids form a part of the coastal food chains as a food source for shellfish, which form a significant part of the human diet in coastal regions around the world. Chromista are not related to toxic algae forms, such as some blue-green algae and dinoflagellates, which are in completely separate Kingdoms. *Schizochytrium* DHA-rich oil has no unpleasant flavor, no detectable environmental pollutants, and may be

supplied as oil or in starch powder formulations for cooking, encapsulation, infant milk formula, rice powder, and as additives to cereal and other products (Martek unpublished reports, 2006). This microalgae is rapidly grown in culture where tons of carbon dioxide is removed from the atmosphere for each ton of oil produced, making it environmentally friendly. Martek currently owns the patents to the *Schizochytrium* production strain and the oil extraction process for making DHA-rich oil. Martek acquired these patents by their purchase of OmegaTech. Trademarked as 'Life'sDHA', this product is available on the market in the form of supplements. DHA-rich oil could become available in countries like India with increasing market demand due to its large vegetarian population. India's current unmet needs for omega-3 supplementation may include preventative treatment for conditions such as heart disease, and dyslipidemia in pre-diabetic or type 2 diabetes patient, but omega-3 supplements may not affect, or may only indirectly affect, insulin signaling and glucose homeostasis.

***Schizochytrium* Oil Safety and Efficacy:**

DHA-rich oil produced from *Schizochytrium* has undergone extensive analysis, showing that the individual components of the extracted oil are all present elsewhere in normal food consumed by communities. Thus, DHA rich oil is inherently safe in its fatty acid and sterol components (Table 2) (OmegaTech unpublished reports, 1997). Safety is further supported by the historically safe use of fish oils of similar composition.

In addition, its safety is also based on the small quantities expected to be consumed per

dose, the knowledge of the metabolism of individual lipid components and the lack of published reports of inherent toxicity, thyroid problems, or allergic reactions. There have been no reports in the literature of allergic responses to any members of the kingdom Chromista, including the Thraustochytrids. Allergic responses by humans to microorganisms can sometimes be related to microbial toxins, but only certain types of algae are allergenic. It has been shown that individual ratios of polyunsaturated fatty acids vary according to food source. For DHA-rich *Schizochytrium* oil the key points to note are very high levels of DHA, the low levels of EPA and moderate content of docosapentaenoic acid (DPA n-6) (Table 2)⁶⁰. DPA n-6 is an omega-6 fatty acid that does not have the same bioactivity as AA. It is present in a wide variety of foods and is relatively abundant in eggs and breast milk. The ratio of DPA n-6 to DHA n-3 in human breast milk is reported to range normally from 1:1 to 1:6⁶¹. The ratio of DPA n-6 to DHA n-3 in DHA-rich *Schizochytrium* oil is 1:3, the median range of breast milk. *Schizochytrium* microalgae strains have been developed by conventional techniques and no Genetic Modifications were used or needed. An independent panel of experts in the US has concluded that DHA-rich oil from *Schizochytrium* microalgae can be "Generally Regarded as Safe" as a nutritional food ingredient (OmegaTech unpublished reports, 1997). When DHA-rich *Schizochytrium* oil and fish oil were used in cell viability and proliferation tests with human colon adenocarcinoma Caco-2 cells, tests showed no differences between algal oil and fish oil, indicating safety and potency⁶². One question is whether DHA-rich microalgae oil can function as a universal fish oil alternative? Based on the effects and benefits of fish oil, it will be important to know how well DHA-rich oil compares in clinical efficacy. Early clinical indications strongly support the efficacy of microalgae oil compared to fish oil.

FUTURE DIRECTIONS

Although advances in our knowledge of the protective effects of EPA/DHA have increased, many issues remain. Additional

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biochemical understanding of the individual and/or overlapping roles of EPA and DHA are important scientifically. At the same time, longterm risk factors associated with fish oil supplementation are not known regarding pollutant and heavy metal accumulations in the body. Safe, sustainable alternatives to fish oil EPA/DHA capsules are available, but education and awareness of this option is limited. Double blind, placebo controlled trials of microalgae oils are also needed in more studies, particularly with life threatening heart conditions. Oil formulations of at least two microalgae classes may be considered as *Schizochytrium* mainly provides DHA while other microalgae classes are enriched with EPA. However, DHA retro-conversion in the body is a notable topic for making comparisons to fish oil supplements. Additionally, a standardized blood test for EPA/DHA levels is needed for diagnostic purposes to determine inherent deficiencies in individuals and to assign proper regimens. For general use, EPA/DHA fatty acids should be named vitamin-F, as was the practice with fish oil formulations in the early part of the 1900s. Currently, pharmaceutical and agricultural industries are rapidly increasing their research and development investments in omega-3 fatty acids for production of genetically modified plant organisms and for prescription of highly purified fish oil tablets⁶³. All of these efforts are likely helpful, but controversial. Finally, individuals must carefully choose omega-3 fatty acid sources to suit their needs, keeping in mind that plant sources are healthy, but not fully preventative or therapeutic. Beyond omega-3s, supplementation as a single treatment of any disease is unwise. Always consult a physician and definitely include weight loss and a healthy diet as needed. In general, omega-3s are at minimum essential, but these may even be co-therapeutic. Since many individuals may be deficient in their total accumulated omega-3 status, global health education about these important nutrients and their sources will benefit public health.

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