PUFA NEWSLETTER

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EDITORIAL

Good News on PUFAs

Dear Fats of Life readers,

As we approach 2016, what can we say noteworthy about the past year? Perhaps the main observation stands: research on PUFAs just keeps expanding year over year and 2015 was no exception. Progress in clinical, nutritional and biochemical research on PUFA provides increasing appreciation of the importance of essential fatty acids in virtually all areas of health. That is good news because it indicates that we are still learning more about their roles and relevance. And good news because there are plenty of opportunities to tell society about the salutatory aspects of different PUFAs. With knowledge gathered to date there may also be possibilities to determine where improvements in nutritional and supplemental intake of fatty acids may be made, both in prevention and in treatment of disease.

In contrast to this progress, however, 2015 has also been a period in which major media outlets have displayed that they have a preference for highlighting a few visible scientific "stories" that focus on the absence or lack of benefits of specific nutrients or foods. This curious superficiality and lack of acknowledgement for the bulk of scientific studies that mark progressive scientific advancement is not constructive, is confusing to everyone, and wastes a lot of valuable time. So 2015 was a year of taking note that we should all keep trying to place new findings in perspective, and make a stronger effort in pointing out quality research.

And there are plenty of good studies to point out, too many. At Fats of Life we attempt to add a little to the knowledge base by reporting on a selection of what is going on in PUFA research in different parts of the world. Hopefully we are able to provide examples of representative research work that would otherwise go unnoticed to most of us. Covered by the Worth Noting references, many additional interesting publications that signal progress are also indicated.

This December issue of the PUFA Newsletter brings you two very interesting gifts. The first is a Guest Article by Anne Marie Minihane who summarizes the stage of our understanding about genetic variability and our response to omega-3 LCPUFA. And in an Invited Opinion ,the Global Organization for EPA and DHA Omega-3s (which owns Fats of Life) presents its summarized views on the role of EPA/DHA in heart health.

In this new issue a few studies on the topics of cardiovascular health, maternal and infant health, the central nervous system, immune health, and clinical conditions are given as regular summaries. We highlight a study on aortic calcification and its relation to omega-3 intake. A study on chia oil intake during pregnancy and nursing has revealed interesting insight that the body of nursing women is endowed with an impressively adaptable mechanism to provide DHA in breast milk to an infant. Another study shows that children with treatment-resistant epilepsy may find benefit from fish oil consumption, which may perhaps someday help close a major treatment gap in this area.

Two new and relatively large population-based studies have examined the associations of biomarkers of the acute phase response and inflammation with the intake and blood levels of various PUFA. Finally, two complementary studies have taken a close look at the potential for sustaining DHA levels in children that are recovering from acute malnutrition through reformulating the fatty acid composition Ready-to-Use Therapeutic Foods that are already employed in over 50 countries and set to reach more children in the next few years.

Best wishes for 2016!

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CARDIOVASCULAR HEALTH

Higher Total Omega-3 Intake is Associated with Less Vascular Calcification in Older Women

THIS ARTICLE AT A GLANCE

- *Calcification of the abdominal aorta is a predictor of cardiovascular disease (CVD), independent of other CVD risk factors.*
- *This study addressed whether vascular calcification development in adults followed over an 18-year period displays a relation to dietary intake of omega-3 polyunsaturated fatty acids.*
- *The study reports that higher alpha-linolenic acid and total omega-3 fatty acid intake is associated with reduced severity of aortic calcification in women. This association was not found in men.*
- *The results may help identify additional patient groups that are at risk for CVD.*

Arterial calcification is a process that involves the accumulation of calcium phosphate crystals within the walls of the arteries. Ectopic calcification can occur in different extra-skeletal tissues and frequently associates with aging. Within the cardiovascular system, calcification leads to a reduction in vascular function because stiffened arteries become poor capacity and conduit vessels that cannot adapt to flow pulsatilty and organ blood flow demands. Arterial calcification is classified into two general types: calcification of the medial smooth muscle layer (Mönckeberg's sclerosis), and calcification of the intima of an artery. The second type has received significant research attention because the process of intimal calcification can also occur in the development of atherosclerosis, and hence is a relevant process given the high prevalence of this metabolic vascular pathology. A clear differentiation of both forms of calcification in humans is frequently impossible as it requires histopathology. In some arterial sites both forms are believed to occur, such as in the abdominal aorta, whereas in coronary arteries biomineralization of the intima predominates. Calcification can already occur early during atherosclerotic plaque development, and accelerates during further progressive lesion development, but not all atherosclerotic plaques calcify.

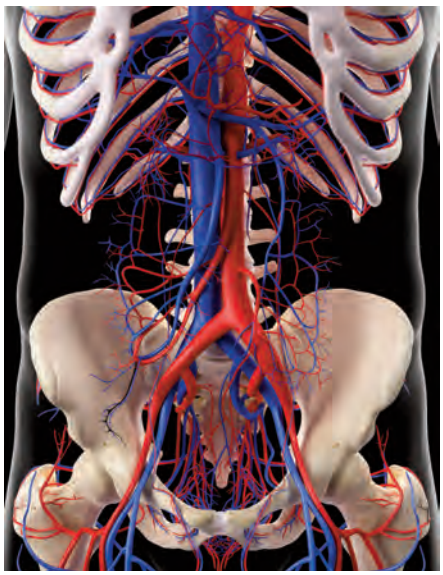
Interestingly, research in the last few years has generated a shift in the understanding of the vascular calcification process from a passive mineralization with deposition of calcium phosphate crystals within tissues to a dysregulated but active formation of true bone-like structures. This new insight views calcification within the arterial vasculature as a result of the improper activation of cells and hormones that normally regulate skeletal bone formation. This comprises the differentiation of vascular smooth muscle cells to acquire a mineralizing chondrocyte-like function. These osteoblast-like cells produce calcium-containing vesicles and a matrix of bone collagen and noncollagenous proteins that can then mineralize if the balance of pro-mineralizing factors outweighs inhibitory factors. Also, monocyte-derived cells with osteoclast-like activity are present in atherosclerotic calcified structures.

> *Calcification of the abdominal aorta is associated with a marked increased risk of cardiovascular mortality, coronary heart disease and stroke, and constitutes a risk factor for CVD independent of other risk factors.*

In contrast to medial calcification, intimal biomineralization and deregulated bone biology has been shown to be closely associated with chronic inflammation, which is also important to the atherosclerotic process. Previous interpretations that calcification of atherosclerotic plaques confers a stabilizing effect on atherosclerotic plaques that become less susceptible to rupturing and subsequent thrombus formation have in recent years become increasingly challenged by new views. This evolving topic of research demonstrates that calcification may also be involved in promoting further plaque remodelling, and is intimately involved in destabilization of the fibrous cap that covers advanced atherosclerotic plaques through the seeding of microcalcified structures from matrix vesicles. Mineralization of the arterial vasculature is also considered an intermediate step in the progression to either weakening of the arterial wall (potentially leading to aneurism) or to vascular stenosis with more extensive calcification and arterial obstruction.

Calcification of arterial vessels other than coronary or cerebral vessels was suggested to have predictive relevance for CVD death 30 years ago. Calcification of the abdominal

aorta is associated with a marked increased risk of cardiovascular mortality, coronary heart disease and stroke, and constitutes a risk factor for CVD independent of other risk factors. Aortic calcification is positively associated with age, with older people having more severe calcification of the abdominal aorta. Other factors associated to calcification of the aorta are hypertension, smoking, the presence of circulating markers of inflammation (CRP) and bone metabolism (osteoprotegerin, osteopontin), and disturbed lipoprotein and lipid metabolism. Aortic calcification is a common feature in patients with diabetes, chronic kidney disease/renal failure, and osteoporosis.



Given the involvement of inflammation in the misdirected biomineralization process of the intima, and the etiological role of vascular inflammation in the atherosclerotic process, it may be possible to modify or redirect the calcification process and associated arterial complications. Results from animal studies have suggested that vascular calcification can be reduced by increased omega-3 LCPUFA intake. The potential relationship between omega-3 status or omega-3 PUFA dietary intake and aortic calcification in humans has never been assessed. A recent report on the results of a large and long-lasting prospective cohort study has now investigated if dietary alpha-linolenic acid (ALA), EPA plus DHA, or total omega-3 PUFA were associated with the development of abdominal aortic calcification. The study focused on arterial calcification in older people, where the calcification load is highest.

Older men and women selected from a long-term prospective cohort study, and for whom radiographic images of the abdominal aorta were available, were investigated with respect to their daily intake of omega-3 PUFA and the extent of arterial calcification.

The study results were reported by Shang and colleagues from the Faculty of Medicine, University of Melbourne, Australia, and several other institutes in Melbourne and Delhi, India.

The study examined 312 selected study participants from the >41000 adults that enrolled at baseline between 1990 and 1994, at age 45-64 years old, for whom readable lateral thoraco-lumbar radiographs were available at follow-up and whose dietary intake characteristics had been determined at study onset. Dietary intake was again assessed at follow-up when the selected study subjects were on average 18 ± 1 years older. Dietary intake was measured using food-frequency questionnaires designed specifically for the Melbourne Collaborative Cohort Study, the prospective cohort study from which the 312 selected participants were selected. Of note, only subjects with a calcium intake greater than 1300 mg/d or below 500 mg/d were recruited. Information on dietary habits including use of milk, sugar, supplements, and type of oils and other fats was collected. The intake of omega-3 PUFA (total, ALA, EPA, DHA) was calculated using an Australian food and compositional database.

The degree of vascular calcification of the descending aorta was measured at follow-up at the level of the thoraco-lumbar region of the spine. Two complementary non-invasive imaging techniques for measuring mineralized tissue (bone) density were used: thoraco-lumbar lateral X-ray radiography and dual-energy X-ray absorptiometric imaging (DXA). A semi-quantitative composite and summed score of calcified deposits in the abdominal aortic vascular wall was made by independent and trained evaluators to grade the severity of arterial calcification at the level of the first through fourth lumbar vertebrae.

Males and females were found to have significant differences in energy-adjusted daily intake of omega-3 PUFA, and baseline characteristics were therefore evaluated by gender according to tertiles of total omega-3 PUFA intake. Men in the highest tertile intake of total omega-3 PUFA had significantly higher energy intake, as well as higher intake of fiber ALA, EPA plus DHA, fruit vegetables, fish, meat and nuts. Women in the highest tertile of total omega-3 intake had significantly higher energy intake, as well as higher ALA, EPA plus DHA, and vegetable intake. Women in the lowest tertile had significantly lower fish and meat intake. Calcium intake was significantly lower in the second tertile in both men and women, with no difference between higher calcium intake in lowest and highest tertiles of total omega-3 PUFA intake. No other dietary or anthropometric differences were measurable.

No significant difference in the extent of calcification of the abdominal aorta was found between men with low, intermediate or high total omega-3 PUFA daily intake. However, a significantly higher proportion of women with the highest omega-3 PUFA daily intake had no calcification. Accordingly, fewer women had high or moderate calcification scores when measured by radiography. When measured by DXA, a significantly lower proportion of women in the lowest tertile of total omega-3 intake were free of aortic calcification.

In older women there was a statistically significant inverse relation between energy-adjusted ALA intake and calcification after adjustment for a number of potential confounders (age, smoking, physical activity, BMI, systolic and diastolic blood pressure, plasma cholesterol, total energy and calcium intakes). An inverse association was also observed for total omega-3 PUFA intake and aortic calcification in women that was also apparent in regression analyses adjusted for age only. The strength of the associations varied somewhat depending on the imaging technique used, and was stronger after adjustment for a larger number of potential confounders. For EPA plus DHA intake no significant association was found (0.42 g/d median intake in tertile 3). There were no associations between total omega-3 PUFA, ALA intake, or EPA plus DHA (median intake in highest tertile, 0.35 g/d) in men. Changes in tertile of energy-adjusted ALA, EPA/DHA, or total omega-3 PUFA intake over the eighteen-year study period were not associated with the level of abdominal aortic calcification. The results of this study indicate that baseline intake of ALA and total omega-3 PUFA in older women is associated with lower aortic calcification severity over an eighteen-year period.

The study found a significant inverse association between abdominal aortic calcification and dietary intake of alpha-linolenic acid and total omega-3 PUFA in older women. This association was not observed in men.

Men have a higher prevalence of arterial calcification than women, but calcification of the abdominal aorta is the most common arterial site for calcification in women. A potentially detrimental relation between abdominal aortic calcification and bone loss has been reported in older women, but appears absent in men. This study evaluated men and women with median intakes of EPA plus DHA that were relatively low, and accounted for a small proportion of total omega-3 LCPUFA. It is possible that at higher daily EPA/DHA intake, an association with lower calcification severity may also become apparent. Men in this study were not meeting the recommended daily intake level for ALA (in Australia). No relationship between low or high calcium intake and calcification of the abdominal aorta was found in this study.

The results of this study indicate for the first time that biomineralization of the abdominal aorta as an independent risk factor for cardiovascular disease is inversely associated with dietary intake of omega-3 PUFA and ALA, albeit only in older women. Whether in older men arterial calcification may be modifiable or is related to dietary omega-3 PUFA intake at higher intake levels will require further research. As a potentially modifiable outcome by diet, addressing calcification of the abdominal aorta may furthermore have important implications for bone health in older women.

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FOL

MATERNAL AND INFANT HEALTH

Enhanced Production of Breast Milk DHA during Chia Oil Intake

THIS ARTICLE AT A GLANCE

• A randomized controlled intervention trial has addressed the effect of chia oil intake during pregnancy and nursing on breast milk fatty acid composition in healthy women.

• In addition to increased levels of alpha-linolenic acid, a marked elevation in docosahexaenoic acid (DHA) in breast milk is observed during the first three months in nursing women taking chia oil.

• The study indicates that chia oil consumption can promote additional accumulation of DHA into breast milk, a potentially useful application to support dietary omega-3 LCPUFA needs by infants in conditions where maternal DHA consumption is low or absent.

• Further studies are necessary to confirm that this mechanism is functional in nursing women beyond the group of women studied here.

Breast-feeding is very important for the developing child for many reasons, one major reason being that breast milk provides many if not all of the nutrients that a growing infant needs, including long-chain polyunsaturated fatty acids (LCPUFA) that contribute to the development of the nervous system. The level of docosahexaenoic acid (DHA) in breast milk is determined by the DHA status of the mother. The fatty acid composition of blood cells and breast milk is a reflection of the dietary fatty acid intake during pregnancy and nursing. To attain a sufficient level of DHA in breast milk that supports adequate biomagnification (active accretion of DHA by the infant from breast milk), it has been estimated that a mother needs to carry approximately 6% of fatty acids in red blood cell membranes as DHA throughout pregnancy. However, significant variability in the transfer of DHA from the mother to the fetus during pregnancy is also recognized. Breast milk DHA is believed to be largely transported from a mother's depot tissues to the mammary glands that produce breast milk.

Alpha-linolenic acid (ALA) is the precursor for the biosynthesis of the longer chain omega-3 PUFAs in mammals. The efficiency (or fractional conversion) whereby dietary ALA is transformed in adults into DHA is considered very low, although in women it is somewhat higher than in men. A diet low in DHA is inadequate to satisfy the physiological requirements for DHA of some tissues in the body, particularly during specific periods in life when requirements are high such as during fetal and infant growth. Provision of ALA to a mother's diet will lead to the formation of EPA, as measurable in red blood cell membranes, but not to significant increases in DHA. EPA is not present in human breast milk at substantial levels, and the requirement for it in infant development is less clear than for DHA. Hence, it is widely considered that during pregnancy ALA may not contribute substantially to breast milk DHA levels, and direct intake of DHA by pregnant and nursing mothers is widely recommended to increase DHA availability during nursing. However, it is possible that under conditions of very low or no DHA intake, augmented ALA to DHA conversion may support DHA production, provided sufficient ALA is ingested. An interesting question that is not completely answered is to what extent can ALA compensate for a lack of DHA in a pregnant or nursing mother's diet?

Chia oil is a plant oil extracted from the seeds of the plant *Salvia hispanica*, native to Central America, and now cultivated in many parts of the Americas. Among plant oils, chia oil has a very high level of ALA (60-65% of fatty acids as triglyceride). To address the issue whether chia oil consumption during pregnancy and after delivery can support adequate breast milk DHA levels, Valenzuela and colleagues carried out a controlled intervention study in pregnant women. The study was performed at the Department of Nutrition, the Lipid Center at the Institute of Nutrition and Food Technology, and the Obstetrics and Gynecology department at the Clinical Hospital, all at the University of Chile, Santiago, Chile.

Forty pregnant women were randomly assigned to either a control group (n=21) or to a second group that received 16 ml chia oil per day (4 teaspoons of oil containing 10.1 g of ALA). The control group (n=19) took the same volume of a sunflower/soy-

This study explored the effect of chia oil intake on fatty acid levels in breast milk in nursing women with a very low omega-3 LCPUFA intake.

bean oil (80:20 v/v). The women were instructed to replace the oil they usually used at home with the test oil, mainly in salads eaten at lunch or dinner. The oils were taken from the sixth month of pregnancy onwards, until the sixth month of nursing. The chia oil was a cold-pressed chia seed oil. A dietary record was maintained to follow the daily consumption of test oil.

All women were of Hispanic origin, between 22 and 35 years old, and had had prior successful nursing (1 to 4 children). Women with an illness that could affect their pregnancy, or those taking fatty acid supplements, were excluded from participating in this study. The women that were studied were mostly of medium socio-economic status, mean age 29 years, and a pre-pregnancy body mass index that was normal (borderline to overweight). At study onset, the pregnant women were clinically evaluated, with measurement of weight and height. Dietary intake was evaluated using a food-frequency questionnaire combined with a photographic display of Chilean foods, at study initiation (six months of pregnancy), one week after delivery, and six months after delivery. A dedicated software program was used to calculate the energy and nutrient intake, using a food composition database. The fatty acid composition of red blood cell membrane phospholipids was measured by gas-liquid chromatography, from frozen erythrocyte samples collected at the study onset, immediately after delivery, and six months after delivery. The fatty acid content of the phospholipid fraction in breast milk was also determined. Breast milk samples (5 ml) were collected by the mothers themselves during breast feeding, each month during the six months after delivery.

There were no significant differences in anthropometric measures between women in the chia and control groups, no differences in gestational length, or gender, birthweight and height of their babies. Beyond changes in PUFA intake, there were no significant changes in the energy and composition of the diet ingested by mothers during pregnancy, at delivery, and while nursing, except for a small decrease in total energy and carbohydrate intake in the control group at six months of nursing compared to six months of pregnancy. Total omega-6 LCPUFA and linoleic acid intake, as well as the omega-6 to omega-3 intake ratio, were significantly lower in mothers in the chia oil group at delivery and at six months of nursing. As intended, total omega-3 LCPUFA and ALA intake were significantly increased during chia oil consumption, by a factor six to eight-fold, compared to mothers in the control group. These changes in fatty acid intake at delivery and six months of nursing were mirrored by the fatty acid composition of red blood cell membranes. Levels of EPA in red blood cell membranes, but not of DHA, were significantly increased (2.7 fold at delivery and 2.5 fold at six months nursing). Basal dietary DHA intake in these women was very low and ranged from 0.02 ± 0.03 to 0.1 ± 0.1 g/ day throughout the study period.

A comparison of the fatty acid content of breast milk produced each month after delivery showed no changes in saturated fatty acids, mono-unsaturated fatty acids, or total PUFA. Throughout the 6-month nursing period total omega-6 PUFA levels in maternal milk were significantly lower, and total omega-3 PUFA levels were approximately twice as high in the chia oil group compared to controls. The omega-6 to omega-3 PUFA ratio in breast milk was about three-fold lower in the chia group during the six month nursing period. Breast milk linoleic acid was lower in the chia oil group, but arachidonic acid levels were similar in the groups. ALA levels were higher throughout the nursing period, and the already low EPA levels remained similar between the chia oil and control group. Remarkably, during the first three months after delivery DHA was significantly elevated in the breast milk of the mothers that took chia oil. DHA levels were four times higher than in controls during the first two months post-partum, decreasing to three-fold higher in the third month. DHA levels in maternal milk returned to the levels of the control group four months after birth, even with chia oil supplementation still ongoing.

Daily intake of 15 grams of chia oil during the third trimester of pregnancy and the first six months after delivery stimulated the appearance of DHA in breast milk during the three months following delivery.

In summary, chia oil supplementation during pregnancy and nursing to healthy mothers led to a marked increase in the DHA content of breast milk during the first three months of nursing. During the course of the study, chia oil supplementation changed the relative dietary intake of linoleic acid to ALA from a ratio of 15 to nearly 1. The biotransformation of ALA derived from chia oil intake to downstream omega-3 LCPUFA was reflected in the composition of the red blood cell membrane phospholipids, with an increase in EPA concentration. Although a marked increase of DHA was found in breast milk, there was no increase in red blood cell DHA levels. DHA formation from the dietary intake of another ALA-rich plant seed oil, sacha inchi oil, has been shown to increase the levels of DHA in plasma. DHA levels in red blood cells during chia oil consump-

tion were held to a similar basal level observed in the control group, suggesting a dynamic regulation of DHA content in maternal milk permitting an elevated level during just the first three months of a nursing infant. This observation suggests that regulation of DHA formation from ALA is taking place during pregnancy in a way that is even more intricate than previously anticipated.

This study was carried out in mothers of Hispanic origin in Chile, and interesting new studies await to establish whether chia oil consumption may support increased DHA levels in the maternal milk in mothers of different regional and/or ethnic origin. The concommitant amount of dietary linoleic acid is an important factor to take into account, since lower dietary intake of linoleic acid resulting from a change in dietary oil use, as observed in the present study, will allow more efficient conversion of ALA to longer chain downstream metabolites, including DHA. This study has exposed chia oil as a potentially nutritionally valuable seed oil during pregnancy and nursing. Understanding how chia oil consumption augments maternal milk DHA levels may provide further insight into how the body regulates the delivery of adequate amounts of DHA to an infant.

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BRAIN AND CNS

Achieving Seizure Freedom with Fish Oil in Children with Treatment-Resistant Epilepsy

THIS ARTICLE AT A GLANCE

- *This study addressed the question whether daily intake of fish oil during three months had an effect on treatment-resistant epilepsy in children.*
- *More than half of the children had no epileptic attacks during the intervention period.*
- *The results support a limited number of studies in humans showing that omega-3 LCPUFA may have beneficial effects in epilepsy.*
- *Future studies with optimized protocols will be required to confirm the results.*

Epilepsy is a chronic neurological disorder that can be idiopathic, *i.e.* occur without a clearly identifiable cause, or can be secondary to an injury of the brain such as a brain lesion following trauma, or cerebral tumours. Epilepsy involves disturbed electrical signaling in the brain, which depending on the site and magnitude, can range from short-lasting absence seizures to generalized seizures with loss of consciousness. In the US it is estimated that approximately 7 out of 1000 people have epilepsy, and 17 out of every 1000 have had some period of a seizure disorder in their lifetimes. Both the frequency and the severity of epileptic attacks are of importance when considering an individualized approach to epilepsy in daily life, or when evaluating potential treatment approaches. The severity of epilepsy involves for example to what extent an attack generalizes, the time from onset to loss of consciousness, the time of recovery from an attack, and the associated injuries from falls, and the intellectual decline.

The most commonly reported efficacy measures in clinical intervention trials in adult epilepsy are the median percent reduction in seizure frequency from baseline, and the percentage of patients that experience a ≥50% reduction in seizure frequency from baseline (≥50% responder rate). The frequency of epileptic attacks varies substantially between individuals. Intractable epilepsy, also termed refractory epilepsy, treatment-resistant epilepsy, or drug-resistant epilepsy is when seizure frequency and severity cannot be adequately controlled with existing anticonvulsant medication. Formally, drug-resistant epilepsy is the failure of adequate trials of two tolerated, appropriately chosen and commonly used antiepileptic drug regimens (whether as monotherapies or in combination) to achieve sustained seizure freedom. Seizure freedom is defined as remission from seizures following an intervention, after a period without seizures has elapsed equal to three times the longest pre-intervention inter-seizure interval over the previous year. Patients with intractable epilepsy need to be very careful in specific situations, for example in traffic or near water, to reduce the risk of serious accidents.

Some 20% of children with epilepsy develop intractable or treatment-resistant epilepsy. This study addressed the question whether these children benefit from a daily dose of fish oil.

Childhood epilepsy has its own characteristics, such as a significant risk to develop co-morbid anxiety and depression, long-term social problems, learning disorders, and a risk of permanent cognitive disturbance. Epilepsy presenting in childhood requires careful evaluation to discern whether a child may forgo medication, would be a candidate for resection epilepsy surgery, or would benefit from treatment with an anti-epileptic drug. Up to 20% of children that develop new onset epilepsy may develop a period of intractable epilepsy, which can last several years. Satisfactory seizure control is furthermore not achieved in a high percentage of children with epilepsy. In addition, children with treatment-resistant epilepsy are at risk of intellectual disability, psychiatric comorbidity, and sudden unexpected death (SUDEP). The early diagnosis and a decision on the best treatment for developing pharmaco-resistant epilepsy is therefore very important in childhood epilepsy.

Lack of access to epilepsy treatment, also termed the "treatment gap," is a particularly serious problem in many low and middle-income countries due to the limited availability of anti-epileptic drugs, lack of primary health-care providers or neurology specialists that can diagnose and treat epilepsy, and social stigma. Although a remission rate of up to 80% of childhood-onset epilepsy has been reported, a similarly high percentage of children that have

epilepsy also have to deal with psychological, cognitive and behavioral problems. Good management of epilepsy in children is thus increasingly recognized as necessary before and after the transition into adulthood to limit the development of social and psychiatric complications. There is also a significant need for the identification of existing and new anti-epileptic drugs that have minimum adverse cognitive and behavioral effects.

Studies in animals have indicated that omega-3 long-chain polyunsaturated fatty acids (LCPUFA) have anti-convulsant activity, which is mediated by the ability to raise the electrical threshold of experimentally-induced seizure activity. A randomized placebo-controlled double-blind cross-over study in US American adults with partial onset epilepsy, showed that ~1 gram daily omega-3 LCPUFA for 10 weeks reduced seizure frequency by 34% in individuals that continued their anti-epileptic medications. A negative correlation between serum DHA level and seizure duration as well as seizure severity has also been reported in Egyptian children with treatment-resistant epilepsy.

A recent intervention study assessed whether it was possible to modify the frequency or intensity of epileptic seizures in children with treatment-resistant epilepsy by dietary supplementation with fish oil. The study was performed by Reda and colleagues from the Department of Nutrition and the Department of Pediatrics, at the Faculty of Medicine, High Institution of Public Health, Alexandria University, Egypt. The children (n=70) had a median number of four recurrent seizures a month at baseline (the month preceding the start of the intervention). They were randomly assigned to two study groups: a fish oil group that received 3 milliliters of fish oil containing 18% EPA and 12% DHA every day during 3 months. The control group received instead the same volume of corn oil. No information was provided on whether the oils were encapsulated. At the start of the study and at monthly study visits, an assessment of the frequency and severity of epileptic seizures was made.

Seizure frequency (number of seizures per month) was determined through the use of a diary that the parents filled out every day. The variation in seizure frequency between the individual children at baseline (the month preceding study initiation) was large; it ranged from 1 seizure per month in approximately 35% of children in both groups, to more than 300 attacks per month in three children (8.6%) in the fish oil group, and one child (2.9%) in the control group. The median seizure frequency was 4.0 attacks per month, with no difference between the two groups. Seizure severity was scored following monthly interviews with the children's parents, according to the National Hospital Seizure Severity Scale (NHS3) that employs standardized scoring of seven seizure-related factors (scale ranging from 0 to 27). The

seizure severity score before intervention was 13 and 12 in the treatment and control groups at baseline, respectively (no significant difference). The mean age of the children receiving fish oil (n=35) was 6.9 + 2.5 years, and that of the children in the control group (n=35) was 6.6 + 2.4 years. No further evaluation of baseline characteristics of the study population or assessment of random differences between the two study groups was made. The ongoing medication of the children was not described in detail.

In the first month after starting the daily intake of the fish oil, a ~50% reduction in the median number of seizures was noted. After two and three months this reduction was statistically significant, with the median seizure frequency having decreased to zero. Freedom from seizures was achieved in 60% of the children after two months. After three months, children with the most refractory seizures were two children with 30 seizures per month, whereas 20 of the 35 children in the fish oil group were seizure-free. In the control group no significant effects were noted; the median seizure frequency remained at 4 seizures a month. With respect to seizure severity, no statistically significant changes were noted in both groups. Of the children in the fish oil group that still had seizures at month 3, none had high seizure severity scores (scores of 17-24), whereas in the control group 21% were still having severe seizures. In summary, the results of this study indicate that a 3-month daily intake of fish oil as an add-on to existing medication significantly reduced seizure frequency in children with intractable epilepsy, with a large proportion of children becoming seizure-free during the study period.

Placebo effects have recently been noted to be particularly prominent in studies addressing pharmaco-resistance in

The study indicates that seizure freedom was achieved within two months in 60% of the children who were not responsive to other anti-epileptic drugs.

epilepsy patients. The phenomenon involves increasing responder rates in more recent studies, in both the placebo and active medication groups of recent clinical trials of new anti-epileptic drugs. Correction for the response rate to placebo is therefore considered critical in epilepsy research. However, in the present study no effect of the control intervention was measurable at all. It also has to be considered that the influence of patient and physician expectations on trial outcomes in epilepsy might be much less trivial than expected. For example, participants may have greater incentives to report improvements, to obtain better access to an otherwise not available drug treatment. The present study was single blinded, with only the children not knowing what supplement they received. Also no proper description of the source or formulation of the oils was provided in the study description, although it is expected that the oils were provided in identically looking and smelling gelatin capsules. Replicate studies are warranted to confirm these results by adequate blinding of study staff as well as the children and their parents, and employing indistinguishable test oils.

Through extrapolation from placebo-adjusted clinical efficacy in adults, the efficacy of several of the most commonly used anti-epileptic drugs in children has been estimated to range from 11% to 31% (reduction in seizure frequency) and ≥50% responder rates ranging from 3% to 26%. The results of the present study are therefore remarkable. The present study was relatively short, and a longer treatment is desirable for studies assessing seizure freedom as an efficacy endpoint (at least 24 weeks). In summary, this study reports favorable effects of 3 g fish oil a day as a therapeutic approach in children with intractable epilepsy, to significantly reduce the frequency of epileptic attacks. These results are of particular relevance to countries where the treatment gap is largest. The results that seizure freedom may be attained by fish oil supplementation in children with treatment-resistant epilepsy are important to confirm in the future. In Egypt the average daily intake of omega-3 LCPUFA is very low (below 100 mg/day), and it is possible that EPA/DHA provision via fish oil supplementation provided significant benefits because baseline omega-3 status was low. Future studies of childhood epilepsy may also benefit from examining background fatty acid status and dietary intake.

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IMMUNE FUNCTION

Modulation of the Acute Phase Response by Polyunsaturated Fatty Acids in Older People

THIS ARTICLE AT A GLANCE

• Two recently published studies report on the relations between dietary intake and levels of poly-unsaturated fatty acids (PUFA) in blood and the acute phase response C-reactive protein (CRP) in older people.

• In one study, the omega-3 index was negatively associated with the levels of CRP and several other circulating markers of inflammation.

• In a second study, higher intake of omega-6 PUFA, and total PUFA, but not omega-3 PUFA, were found to be negatively associated with CRP, particularly in older women.

• The results reflect current challenges in understanding the roles of PUFA families in inflammation and the acute phase response.

The acute phase response is a systemic reaction of the body to control inflammation that follows a local tissue injury. An acute-phase response involves fever, leukocytosis, release of cortisol, activation of complement and clotting cascades, changes in metal homeostasis, and a marked increase in the concentration of certain plasma proteins of hepatic origin. The latter hepatic acute phase response is initiated via the release of interleukin-6 (IL-6) from monocytes and macrophages that are activated during the course of an inflammatory response. IL-6 activates the secretion of a range of proteins by the liver into the circulation, each having specific roles in host defense, microbial clearance, and organ functioning and healing in the face of trauma and infection. Additional pro-inflammatory factors, such as the cytokines IL-1 and tumor necrosis factor-α, that are secreted into the circulation by an inflamed tissue also take part in fine-tuning the hepatic acute phase response. Kupffer cells, macrophages located in the liver, sense infection in blood and form additional cytokines that stimulate acute protein formation and secretion. Whereas the local inflammatory response involves its own mechanisms of onset and its return to homeostasis, the acute phase response is a systemic reaction that initially helps control infections and limit trauma, while at later stages it promotes the resolution of system-wide aspects of inflammation such as fever, normalizes hormonal adaptations, and the release of mediators that promote tissue healing.

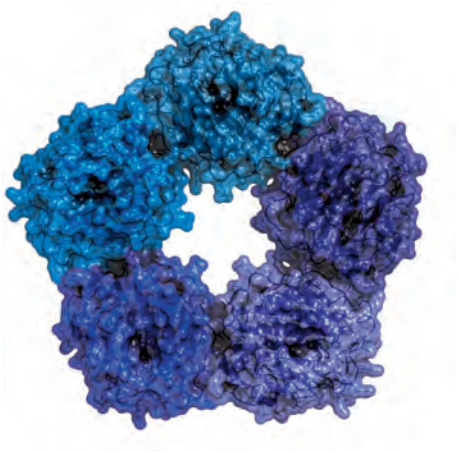
Although the term "acute" implies a fast response, the appearance of acute phase proteins in the circulation can take several hours, and in the case of a chronic inflammatory disease, elevations in the concentrations of acute phase proteins can be found in blood over long periods of time. Disturbances in the acute phase response are involved in pathophysiological conditions such as the systemic inflammatory response syndrome. Since individual acute phase proteins play important roles in both supporting and controlling inflammatory reactions, their levels in blood constitute diagnostic and prognostic biomarkers in patients, for infection and inflammation. One of the acute phase proteins, C-reactive protein (CRP), has found widespread use as one such biomarker of inflammation. It is a "distal" marker though, *i.e.* an acute phase protein that is induced by pro-inflammatory cytokines.

C-reactive protein (CRP) is often measured as a distal marker of inflammation. It is an acute-phase protein secreted by the liver and has an important role in host protection.

The levels of CRP acute phase protein in the circulation can increase up to 10,000 fold during injury, involving both IL-6 and IL-1 for optimal hepatic synthesis and secretion. Originally recognized for its ability to bind to the phosphocholine part of teichoic acid of the pneumococcal C-polysaccharide cell wall ("C-reactivity"), CRP plays an important role in the opsonization of microbes. Complement activation targets CRP-opsonized microbes for phagocytic removal. CRP also plays a role in regulating local inflammatory responses, modulates angiogenesis and platelet formation, and can promote the non-phlogistic clearance of apoptotic cells by phagocytosis, an important event in turning off inflammation. An increase in CRP level is not specific for some particular disease, but correlates well with a range of chronic inflammatory disorders in humans. CRP-mediated complement activation is believed to be involved in the pathogenesis of a number of chronic inflammatory diseases, such as atherosclerosis, cardiomyopathy, Alzheimer's disease, and rheumatoid arthritis. The measurement of CRP levels has

thus found widespread predictive utility for its association with chronic disease risk.

CRP circulates as a soluble pentameric structure in blood that can recognize infectious microbes or oxidatively-damaged cells. The active form of CRP is a monomer that is formed when CRP binds to its target structures (microbial and oxidized lipid epitopes). Current research is increasingly focussed on how pharmacological intervention can mitigate the involvement of CRP in chronic inflammatory pathologies, either through reducing elevated levels seen in chronic inflammatory disease, interfering with the transition from CRP pentamers to monomers, or by inhibition of CRP-mediated complement activation. Successful intervention to readjust a disturbed acute phase response may also need to look beyond one acute phase protein alone, as the acute phase response involves upregulation of specific proteins (positive acute phase reactants) and downregulation of others (negative acute phase reactants) during inflammatory reactions. However, results from recent genetic studies are not supportive in assigning a causal involvement of elevated CRP levels in cardiovascular disease, casting doubt on a direct role of the acute phase response in the etiology of chronic inflammatory disorders.



Omega-3 LCPUFA have long been recognized to be involved in regulating inflammatory reactions, and both enhanced intake as well as higher blood levels are associated with lower levels of positive acute phase reactants, and decreased tissue inflammation. Omega-6 LCPUFA can also serve anti-inflammatory roles in the context of inflammation, since arachidonic acid is the substrate for the formation of several anti-inflammatory mediators that regulate the extent and duration of inflammatory reactions. For example, a negative association between plasma concentrations of arachidonic acid and IL-6, and a positive association with anti-inflammatory transforming growth factor-β,have been reported. On the other hand, intake of omega-6 PUFA, in particular linoleic acid, in significant excess compared to levels that cover the essential requirement, may contribute to chronic inflammatory disorders typical of a Westernized lifestyle, particularly with a low dietary intake of omega-3 PUFA. On the background of this awareness, two recent prospective studies carried out in relatively large population-based cohorts assessed the association of dietary and blood fatty acids, with the levels of CRP, and other markers of systemic inflammation.

The first study aimed to determine the association of dietary intake of total PUFA, omega-6 PUFA and omega-3 PUFA, with CRP levels in adults aged 55 years or older. Data were collected over two periods during the 1990s in participants of the Rotterdam Study by Muka and colleagues at the Department of Epidemiology, Erasmus University Medical Center, in Rotterdam, The Netherlands. The Rotterdam Study is a prospective cohort study that has been ongoing since 1989 in the city of Rotterdam and has provided epidemiological insight in multiple disease endpoints. A large group of people (nearly 3000 individuals) was followed over a 6-8 year period. The study examined the influence of a large number of potential confounders and used separate analysis by gender. At baseline (1990-1993), study participants had been extensively interviewed at home by trained personnel about their lifestyle and health status. A clinical examination was subsequently performed at the study center. Dietary intake of different foods was assessed via a validated food-frequency questionnaire at baseline, allowing the recording of monthly food item intake during a preceding one-year period. Total energy intake and fatty acid intake was calculated from the dietary intake data using a food composition table. CRP was measured in serum samples obtained during the first and a third examination cycle that was organized approximately seven years later (1997-1999). Of all participants that had been evaluated in the first study period, a CRP measurement was available together with a dietary intake assessment for 2911 persons.

A large prospective cohort study in older people found that total PUFA intake and total omega-6 PUFA intake was negatively associated with CRP, suggesting an anti-inflammatory role for omega-6 PUFA.

The mean age of the study participants was ~67 years at baseline. Total PUFA intake was 18.1 g/d in men, and 13.6 g/d in women. Omega-6 PUFA and omega-3 PUFA intake was 14.7 g/d and 1.2 g/d in men, and 11.1 and 0.98 g/d in women, respectively. The women had a comparatively higher level of education than the men. Nearly 47% of the men and 30% of the women had some prevalent chronic dis-

ease. Dietary intake of fish was relatively low: 15 g/d in men and 14 g/d in women. They had a relatively high intake of butter, margarine and hard frying fats (38.2 g/d in men and 26.46 g/d in women). At baseline, intake of vegetable oil, butter, margarine and hard frying fats, and whole grains correlated best with omega-6 PUFA intake. Butter, margarines and hard frying fats, fish, and red and processed meat intakes correlated best with omega-3 PUFA intake. The reason why meat as well as butter, margarine and frying fats correlated with omega-3 intake is because of the use of alpha-linolenic acid (ALA)-containing vegetable oils in the cooking/frying of meats, and this fatty acid being present in widely used margarine and sauces.

Mean CRP levels in the first study period were 0.187 mg/dl in men, and 0.176 mg/dl in women. These levels had risen to 0.235 and 0.241 mg/dl, respectively, in the third study period (1997-1999). Analyses examined dietary fatty acid intake as categorical variables organized in quartiles of energy-adjusted PUFA intake against CRP levels. The median CRP level was significantly lower as total PUFA intake or omega-6 PUFA intake increased. These associations remained statistically significant after adjustment for a range of covariates (such as whether people had some form of chronic disease, were complying or not with the recommended healthy diet standards, took anti-inflammatory drugs, their physical activity, educational level and household income). In contrast, there was no consistent association between omega-3 PUFA intake or the omega-3 to omega-6 PUFA intake ratio and CRP levels.

A further analysis addressed whether the relationship between PUFA intake and CRP levels was different between women and men. That proved to be the case; in women a clear trend in higher total PUFA intake or higher omega-6 intake was associated with lower CRP levels. The ratio of omega-3 to omega-6 intake (but not omega-3 PUFA intake alone) showed a positive association with CRP levels in women. There were no significant associations between CRP levels and any of the studied fatty acid intake variables in men. In this older Dutch population, CRP levels in women were negatively associated with the amount of PUFA in their diet, in particular omega-6 PUFA intake. The associations between intake of individual fatty acids of omega-6 and omega-3 families with CRP levels were not determined. The results suggest that higher dietary PUFA intake, in particular omega-6 PUFA, is associated with a less intense acute phase response, and more notably in older women.

In the second study, Fontes and colleagues investigated the relationship between red blood cell fatty acid composition and CRP in older people. The study was carried out by members of research groups in the Schools of Medicine, and Public Health at Boston University MA, and the Sanford School of Medicine, University of South Dakota, coordinated by the National Heart Lung and Blood Institute and Boston University's Framingham Heart Study, Framingham, MA. Participants from two cohorts of the Framingham Heart Study, the Framingham Offspring Cohort (recruited in 1970) and the Framingham Omni Cohort (participants with a racially-diverse background, recruited in 1994), were evaluated in their eighth and third examination cycles, respectively. The average age of the participants determined at clinical examination was 66 years. Of 2724 subjects in the combined cohorts the fatty acid composition of red blood cells (RBC), levels of a range of plasma and urine inflammatory biomarkers, and measurement of several clinical covariates was available. RBC fatty acid measurements were made after methylation of RBCs followed by gas chromatographic detection. The omega-3 index was calculated as the percentage of EPA plus DHA of total fatty acids. In addition to CRP and IL-6, eight biomarkers of inflammation were measured.

Prevalent cardiovascular disease was relatively low in the participants (6%), but nearly half received blood-pressure lowering or lipid-lowering drugs, and took aspirin at least three times a week. About one in ten persons used fish oil supplements. The subjects had a lipid profile within the normal range, but were slightly overweight on average. All measured inflammatory markers, including CRP and IL-6, were negatively correlated with the omega-3 index after adjustment for age and sex. After further adjustment for a range of potential confounders, all correlations remained statistically significant, except for osteoprotegerin and MCP-1. The associations for IL-6, 8-epi-$PGF_2\alpha$ (a marker for non-enzymatic oxidation and rearrangement of AA), and lipoprotein phospholipase A_2, were strongest.

The results of a large cross-sectional study in older American people indicated that CRP and a range of circulating markers of inflammation are negatively associated with the omega-3 index (content of EPA plus DHA as % of total fatty acids in red blood cell membranes).

The two studies had a comparable size and age range of participants. The results show CRP levels associated with total PUFA and omega-6 PUFA intake, and omega-3 PUFA EPA

and DHA levels in RBC membranes. The study by Muka and colleagues offers appreciation for a potential association of omega-6 PUFA intake with the acute phase response in older people. A much weaker support for an association with omega-3 PUFA intake is noted. The study by Fontes and colleagues shows EPA+DHA present in red blood cell membranes, as a marker of omega-3 LCPUFA, associated with a number of inflammatory biomarkers, suggesting perhaps that EPA and DHA can influence inflammation through multiple pathways. None of the associations should, however, be interpreted as causal relations.

In the study by Muka and colleagues a correlation between a higher intake of omega-6 PUFA and lower CRP levels may suggest that omega-6 PUFA reduce the acute phase response, for example by modifying the mechanisms by which CRP is formed and released by the liver. Alternatively, there may be effects on enhanced CRP turn-over, or anti-inflammatory actions associated with omega-6 PUFA intake, which provide a lower stimulus for hepatic CRP release. At present it is not possible to discriminate between such possibilities, but the observation that higher PUFA intake and particularly that of omega-6 PUFA intake are associated with a lower acute phase response is an important observation. The authors indicate that in the studied population cohort omega-3 intake is dominated by ALA. A conclusion that marine fish-derived omega-3 would not have an influence over CRP levels may be premature, since this population may have had a low omega-3 status and a low fish intake.

The results of the Muka study are not completely in line with associations identified in a third prospective study (12 year follow-up) carried out in a large cohort of middle-age French individuals published by Julia *et al* in the *British Journal of Nutrition* in 2013. In that study, a dietary pattern reflecting a high omega-6 to omega-3 fatty acid intake ratio was positively associated with elevated CRP. That dietary pattern corresponded to a diet poor in fatty fish, seafood, and margarines, and rich in other animal products (meats and eggs). In a further analysis, total omega-3 PUFA, omega-3 LCPUFA, and omega-6 PUFA intake were each individually negatively associated with CRP levels, but the associations were modified by the dietary intake of vitamin E, suggesting that additional dietary factors may modify the associations. The studies of Muka and Fontes provide interesting data of associations between total and omega-6 FUFA intake, and RBC omega-3 PUFA with lower CRP levels. These data however are in contrast with randomized controlled trials that have shown for example that omega-3 LCPUFAs do not affect CRP levels. Results obtained from population studies need to be considered in light of possible effects of unmeasured confounders such as demonstrated by Julia *et al*.

The results of the two studies together suggest that older people may receive benefit from higher intakes of total PUFA, omega-3 PUFA as well as omega-6 PUFA that may contribute to maintain inflammatory and acute phase responses under control. However, further detailed studies are necessary to understand which fatty acids and associated factors are most important.

Future studies will likely increasingly focus on the specific contributions that individual PUFA species make to immune regulation, and further determine if PUFA status is helpful in making sense of how the biological system is modulated by its dietary habits. A recent example of further comprehension is the recognition that docosapentaenoic acid (n-3) abundancy in RBC membranes also displays an inverse association with CRP levels. Whether older people have a need for both a higher omega-6 and omega-3 intake to control inflammation will be an important topic to determine in the future. A better understanding of a biomarker's biological functions, such as for CRP in the context of the acute phase response and in chronic inflammatory pathologies in older people, will also be helpful for interpreting what changes in its levels mean.

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CLINICAL CONDITIONS

Sustaining DHA Accretion during Recovery from Acute Malnutrition with Improvements in Ready-To-Use Therapeutic Foods

THIS ARTICLE AT A GLANCE

• Two recent studies have explored the possibility of adjusting the fatty acid composition of Ready-to-Use Therapeutic Foods (RUTF) that are used to rehabilitate malnourished children, with the aim of better supporting omega-3 LCPUFA status.

• DHA levels drop during the recovery of malnourished children treated with a standard RUTF – this is likely to be caused by suppression by excess omega-6 linoleic acid of increased accretion of DHA for the growth of the central nervous system.

• Two ways to sustain DHA accretion are shown: direct supplementation with fish oil alongside a standard RUTF, and adjustment of RUTF composition with the inclusion of a low-linoleic acid/high-oleic acid peanut oil, together with a vegetable oil high in linolenic acid.

Ready-to-Use-Therapeutic-Foods (RUTF) are lipid- and carbohydrate-rich paste-like foods that have been developed for the purpose of providing a nutrient-complete diet to malnourished children, and to assist in their rehabilitation. The use of RUTFs has seen significant implementation on a global scale in the last 15 years. RUTFs are used in at least 50 countries, and are now produced by several organizations. RUTFs are composed of several of the following ingredients in emulsified form; one or more vegetable oils, such as peanut, palm and soybean oil, purified phospholipid (lecithin), carbohydrates such as powdered sugar and corn syrup, milk powder, a vitamin premix, and some emulsifying agent. RUTF is mostly packaged in light-impermeable sachets and has a low water content to minimize microbial growth, allowing preservation for several months in tropical conditions. The use of RUTF is primarily intended for outpatient care of severely malnourished children who have unimpaired appetite and are otherwise medically stable.

Acute malnutrition in infants and children remains a grave problem worldwide, causing more than half a million preventable deaths each year (~35% of deaths in children under five years globally). The most severe form of acute malnutrition in children is defined as having a weight-for-height Z-score of <3 according to WHO growth standards, or less than 70% of the median National Center for Health Statistics (NHCS)/WHO reference values (both are measures of visible wasting), or the presence of nutritional edema (kwashiorkor; which can represent up to 50% of malnutrition cases). The availability of RUTF is one of the essential interventions known to improve the health outcomes of children globally. The United Nations post-2015 development agenda foresees further implementation of RUTF usage to increase the coverage of malnourished children globally, of which currently only around 15% are being reached.

Photo by I. Trehan

Children do not only need diets containing the necessary nutrients and energy to prevent malnutrition, or help recover from it – they also need those nutrients that support optimal functional development of tissues. This is of particular relevance to the central nervous system, as apparent normal body growth can be accomplished but optimal neurological development may lag or is irreversibly compromised when nutrients that support brain development are not present in an infant's or child's diet. Sufficiency in the dietary intake of essential fatty acids is an important aspect for normal brain development. In RUTFs that are being used to date, the composition has no rational

Ready-to-Use-Therapeutic-Foods are lipid-rich pastes that provide a nutrient-complete diet to malnourished children and assist in their rehabilitation. Their use has seen significant implementation on a global scale in the last 15 years.

design with respect to essential fatty acid composition and content, and broad ranges of omega-6 and omega-3 fatty acids can be found. Ample evidence gathered in the last decades has indicated that children have a substantial need for omega-3 LCPUFA, with DHA in particular known to be important for brain development. Omega-3 LCPUFA are obtained from breast milk and from foods that contain preformed EPA/DHA. Besides being lipid-rich, RUTFs developed to date have no precise guidelines to ensure sufficiency in omega-3 LCPUFA provision.

Since the lipid portion of current RUTFs is provided nearly entirely by vegetable oils (groundnut and seed oils), the level of the omega-6 PUFA precursor linoleic acid (LA) is in substantial excess to estimated nutritional needs (around 2% of energy in adults). RUTFs used currently favor a dominant intake of omega-6 PUFA and very little, if any, omega-3 PUFA. Alpha-linolenic acid (ALA) is present in low levels in some of the vegetable oils used for RUTF manufacturing. Depending on the dose, this ALA may support some endogenous EPA biosynthesis. However, the further conversion to DHA in humans has been demonstrated as extremely low and most probably insufficient to satisfy accretion rates in children. High LA levels will likely inhibit any ALA conversion, since both fatty acids share the same elongation and desaturation pathways. In addition, given the high overall PUFA content in current RUTF (mainly linoleic acid (LA) up to ~10 weight percent of RUTF paste, and 25% of the fatty acid content), omega-3 LCPUFA biosynthesis from precursor ALA may be effectively shut down when combined precursor PUFA content (LA plus ALA) in the diet exceeds a certain energy percentage (for example around 3%). In other words, there is a worthy opportunity to adjust the fatty acid content of RUTFs to provide the correct amounts of specific fatty acids and/or facilitate precursor fatty acid conversion in children recovering from malnutrition.

It is possible that by markedly lowering the content of LA in RUTFs, improvements in omega-3 status may be achieved. In addition, improvements in omega-3 status could possibly be attained by increasing ALA content, and likely by the direct inclusion of preformed omega-3 LCPUFA. How changes in RUTF composition affect fatty acid status in malnourished children that might help improve their clinical and neurological outcome is unknown. Exactly what the best strategies would be to achieve this is the topic of some very interesting new research. Two recent studies have taken a step in this direction by investigating the changes in fatty acid status of malnourished children in Malawi and Kenya following the ingestion of RUTF with purposefully adjusted fatty acid compositions.

In the first study, Hsieh and colleagues made use of a custom-made "high-oleic" RUTF (HO-RUTF) made with peanut oil from a high-oleic acid peanut cultivar. This research was carried out by researchers from the College of Medicine, University of Blantyre, Malawi, and colleagues at the Department of Pediatrics, Washington University, St. Louis, MO, Cornell University, Ithaca, NY and College of William and Mary, Williamsburg, VA, in the US. High-oleic peanut oils contain significantly higher levels of oleic acid (up to 80%) and 5-7 fold lower levels of LA than found in common peanut cultivar groundnut oils. Furthermore, replacing part of the palm oil and peanut oil used in standard RUTF, omitting any soybean oil, and using ALA-rich linseed (flax) oil (up to 8%) led to a HO-RUTF with markedly increased levels of ALA and reduced levels of LA. Compared to currently used standard RUTFs, the total PUFA content was similar, but the LA content was reduced from 8.9 g/100 g (21.3% of fatty acids) to 4.4 g/100 g (13.1 % of fatty acids). ALA content was increased from 0.17 g/100 g to 4.4 g/100 g (0.4 and 13.1 %, respectively). Achieving a 1:1 ratio of LA to ALA in the new HO-RUTF, this offered the possibility to compare the effect of a more balanced essential fatty acid intake on fatty acid status in malnourished children with that of a standard RUTF that contained 53 times more LA than ALA.

The effectiveness of the HO-RUTF was determined in a double-blind placebo controlled randomized intervention study in 141 Malawian children with acute normal (uncomplicated by other diseases) malnutrition. Some 44% of the children had edematous malnutrition. The children (75% girls, age range of six months to five years), were assigned at random to either the RUTF group (n=70) or the HO-RUTF group (n=71). RUTF and HO-RUTF were given daily to children, with help from their caretakers who were well instructed by nurses. All study personnel and care-takers were blinded to the two types of RUTF. Besides fatty acid content, both RUTF and HO-RUTF had a similar nutrient content, with 175 kcal/kg provided to each child every day. The principal objective of the trial was to assess the effect of the therapeutic foods on the level of EPA and DHA in plasma phospholipids, measured at baseline and after four weeks. Families were provided with enough RUTF for two week periods, and the children visited a study clinic every two weeks. RUTF was provided for as long as children needed it until recovery, up to three months after the start of the trial. At study visits, the investigators measured the recovery from malnutrition (mid-arm circumference >12.4 cm without edema), the child's health and growth status, and eating habits.

In both groups about half of the children recovered from malnutrition. Overall no differences in clinical outcome were

noted: rates of recovery, death, and the number of children who remained malnourished were similar. The children who had received HO-RUTF had a significantly better weight-for-height at the time they recovered from malnutrition, although still marginally below average weight-for-height. The levels of EPA, docosapentaenoic acid n-3 (DPA n-3), and DPA n-6 did not change in children on standard RUTF. However, their DHA levels had decreased from 3.2 to 2.4%, indicating that the standard RUTF received during the four-week period did not support circulating DHA levels. In contrast, in the children on HO-RUTF, the levels of EPA and DPA n-3 increased, and DHA levels remained at the level observed at study onset. Apparently, HO-RUTF supported the endogenous formation of omega-3 LCPUFA, and protected the levels of DHA from decreasing during recovery from malnutrition. Compared to children receiving RUTF, HO-RUTF also induced a lowering of arachidonic acid (AA) levels, indicating that less LA was transformed to DHA or that AA was displaced from plasma phospholipids by the increased levels of omega-3 LCPUFA.

The two studies provide complementary new insight into the potential for further improvements in the fatty acid composition of therapeutic foods to support the demands for DHA of growing children when recovering from malnutrition.

In the second study, a decrease in DHA levels in children recovering from malnutrition was also observed. This study was carried out by Jones and colleagues at the KEMRI-Wellcome Trust Research Programme in Kilifi, Kenya, together with colleagues at the Centre for Global Health Research, Imperial College, London, and several other institutes in the UK and Ireland. This study focused on children with severe acute malnutrition (uncomplicated by other diseases) who were treated in an on-site outpatient therapeutic feeding program of a rural Kenyan referral hospital. Children (age 6-60 months, approximately 50% girls) were randomly assigned to three groups. Twenty children per group received either a daily dose of standard RUTF, a flax oil-RUTF, or a flax oil-RUTF plus fish oil (1 ml daily containing both EPA and DHA) during four months. There were no significant differences between the three groups in a range of variables at baseline. The objective was to determine how an RUTF enriched with ALA (by addition of linseed/flax oil), or with both ALA and EPA/DHA (from fish oil) would affect the PUFA status of children with severe malnutrition, in comparison to a standard RUTF. The flax oil-RUTF contained 3.3 energy % (en%) ALA compared to a standard RUTF with 0.7 en% ALA, and nearly similar levels of LA (7.9% vs 8.2 en%). The children and study personnel were blinded to the two RUTF preparations, with reportedly indistinguishable organoleptic properties. RUTF doses were determined according to national guidelines and given until recovery, followed by supplemental use of the RUTFs as 50% of dietary intake alongside food eaten at home until the end of the four months. The treatment with fish oil together with flax oil-RUTF was provided as an open-label administration, with the oil squeezed directly from a ruptured gelatin capsule into the children's mouths.

No difference in growth rates between the children in the three groups was noted over the treatment period. Recovery was accompanied by increases in insulin growth factor-1 and hemoglobin levels in all groups. DHA levels in RBC membranes significantly decreased after four weeks of outpatient support with standard RUTF, from marginally low baseline levels of 5.2% to ~4%. In the children receiving flax oil-RUTF alone the DHA level similarly decreased. In children also receiving fish oil, DHA levels in RBC membranes increased over time to a level around 6-8%. Only supplementation with fish oil increased EPA levels. Flax oil-RUTF did not change the overall omega-6 to omega-3 ratio compared to baseline, whereas this ratio increased with standard RUTF and decreased with the fish oil supplementation to flax oil RUTF.

Improvements in fatty acid status of recovering malnourished children can be achieved by use of newly formulated Ready-to-Use Therapeutic Foods employing vegetable oils with low LA content. Alternatively, when possible, direct supplementation with fish oil alongside a standard RUTF can help sustain DHA accretion.

In summary, in this group of children with severe malnutrition the addition of flax oil to a standard RUTF did not protect DHA levels from decreasing during rehabilitation. Only the addition of fish oil increased EPA and DHA in RBCs. The improvement in omega-3 status in the children receiving fish oil was not associated with marked changes in the levels of circulating markers of inflammation and T-cell function.

Both studies also addressed organoleptic acceptability and oxidative stability of the RUTFs. Scoring of taste/likeability and assessment of the consumed quantities of a fixed weight

of RUTF led to the conclusion that children liked the new RUTFs equally well to standard RUTF pastes, and there was no difference in intake reported. Peroxidation of PUFA content is considered important to remain within certain limits. The study by Jones measured peroxide levels in the standard and flax oil-RUTF products after one year storage and found 29.7 and 17.9 meq/kg of paste, respectively. Although both were above limits recommended for newly manufactured batches (<10 meq/kg UNICEF), there was no effect on palatability as tested.

The view that the addition to RUTFs of an ALA-rich oil alone can contribute to maintaining DHA levels in malnourished children is unsupported, consistent with more than 20 studies yielding similar results in normally nourished adults. The replacement of groundnut oils with a high-oleic groundnut or seed oil alone may bring about favorable changes in omega-3 LCPUFA status. The value of "high-oleic" oils in this context lies in their low LA content. Concomitant to lowering LA content, the extent to which a significant increase in ALA is needed for support of DHA levels by novel RUTF preparations remains to be addressed. Availability of high-oleic groundnut oils obtained from cultivars that can be grown locally where they are needed will provide a valuable new RUTF ingredient. High-oleic peanut oils can offer additional favorable properties such as enhanced resistance to oxidation.

Interestingly, the fact that a high-oleic/high-ALA RUTF supports DHA levels is somewhat surprising as total PUFA levels remain elevated, which would be expected to down-regulate conversion of ALA to omega-3 LCPUFA. This observation, at least made here in children, suggests that additional components in the high-oleic oil stimulate increased fractional conversion rates of ALA to DHA. Indications have recently been obtained in animal studies that other dietary fatty acids, such as short-chain saturated fatty acids, can promote the conversion of ALA to omega-3 LCPUFA. As shown in the study by Jones, the more direct approach to administer preformed DHA, such as present in a fish oil or an omega-3 concentrate, in addition to a standard RUTF, can also serve to provide sufficient DHA during the recovery of malnourished children. Whether the more direct approach or a reformulation of RUTFs will be practical to implement will likely depend on local conditions. Cost and lability of DHA may be limiting factors. In any case, reducing LA to levels more similar to natural foods is supported by the Hsieh study.

The studies highlighted here are complementary and signal the progress in RUTF development. RUTFs can be improved to attain a better fatty acid status to, in principle, support neurological development in children enrolled in therapeutic feeding programs. Such improved RUTF formulations are palatable, and may find applicability under conditions where fish oil capsules cannot be taken as a complementary dietary intervention, or a rehabilitation diet containing fish cannot be provided. For readers further interested in this topic, an informative commentary on both studies has been published by several specialists.

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GUEST ARTICLE

The Impact of Common Gene Variants on EPA and DHA Status and Responsiveness to Increased Intakes

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Setting the Scene

A large body of human prospective epidemiology, as well as rodent feeding studies, have demonstrated the beneficial impact of the 'marine' long chain n-3 PUFA (LC n-3 PUFA), EPA (20:5n-3) and DHA (22:6n-3), on fetal development, and cardiovascular and cognitive health. Randomised controlled trials (RCTs) have been less congruent, with this apparent lack of consistency likely attributable to a whole host of determinants of response, including habitual diet (including EPA and DHA intake and associated tissue status), medication use, the length of intervention, the total EPA+DHA dose and the EPA: DHA ratio, and the age, sex, BMI and health/disease status of the individual. In addition, it is likely that a large proportion of the inter-individual variability in response both within and between populations is attributable to common variants in genes involved in (i) modulating EPA and DHA status, including those central to EPA and DHA synthesis, transport, cell uptake and partitioning, and (ii) modulating their cellular and physiological targets, including transcription factor status (1).

Although there is wide recognition that response to increased EPA+DHA intake is highly variable, currently generic dietary EPA and DHA recommendations are provided by international and national learned organisations such as the UK Scientific Advisory Committee in Nutrition (SACN), the International Society for the Study of Fatty Acids and Lipids (ISSFAL) and the American Heart Association (AHA). Typically a minimum dietary intake of 500 mg per day of EPA+DHA (achieved through the consumption of 1-2 portions of oily fish per week) is recommended for the general population, increasing to 1 g or 2-4 g per day for the secondary prevention of cardiovascular diseases (CVD) or as a TG lowering therapy, respectively. In the future, with the emergence of a fuller understanding of the aetiology of response, more stratified dietary guidelines may emerge, with recommended total EPA+DHA dose and EPA/DHA composition potentially based on a number of phenotypic and genotypic variables. Such a more targeted approach towards increased intake in responsive groups would also address the issue of marine n-3 PUFA sustainability. Current marine stocks provide only 40% of what is needed for all individuals globally to meet the conservative recommendation of > 500mg per day (2).

Research providing an understanding of the genetic basis of the response to EPA and DHA intake and status is very much "work in progress." Here, rather than be exhaustive, this invited opinion will focus on fatty acid desaturases *(FADS), APOE,* and *PPAR* genotypes, along with blood pressure and vascular function, as exemplars of variants in genes involved in EPA and DHA, synthesis, transport and cell uptake, transcription factor activation and a physiological target respectively, which may modulate EPA/DHA status and response to increased intakes.

The Concise Temporal Guide to Genetics and Genotype and Its Assessment

The origin of the science of genetics dates back to the 1850s-1860s, with Darwin and Wallace's concept of *Natural Selection* and Gregor Mendel's peas and the *Laws of Inheritance.* In 1953 Watson, Crick and Franklin described DNA as the molecule that carries genetic information and the unit of inheritance. But it was only in 2004 that the full sequence of the human genome (DNA) was described (3) and came the recognition that it contains 3 billion nucleotides (the structural unit consisting of a base, sugar and phosphate group) and far less genes (~22,000) than was originally thought. The genome sequence was considered by many to be the "panacea" in clinical medicine and public health, which would within a few years lead to considerable refinement in the prediction of risk of disease and the effective and wide-scale personalisation of preventive and therapeutic strategies. However, this was certainly an overly-optimistic speculation.

The latest output from the *1000 Genome Consortium* published in October 2015 (4) indicates that there are typically 88 million variants in a human genome. The identification of which variant predicts the progression from health to disease and response to environmental exposure, including altered food intake, will take a while to fully establish.

Genetic variation comes in many "guises," ranging from gross structural alterations (affecting more than 1000 bases/nucleotides in the DNA sequence) such as copy number variation, deletions, insertions and inversions, to single nucleotide polymorphisms (SNPs), where as the name sug-

gests, a single base in a nucleotide is changed. Structural variants are relatively rare and often of high and largely unmodifiable penetrance (impact on health end-point). In contrast SNPs, which constitute > 90% of all genetic variability, are common and therefore most relevant for public health and will be the form of genetic variation considered here. Depending on the location of a SNP in the genome, it may have no functional impact or may affect gene expression (and therefore levels of proteins produced), or protein amino acid content, structure, stability and function.

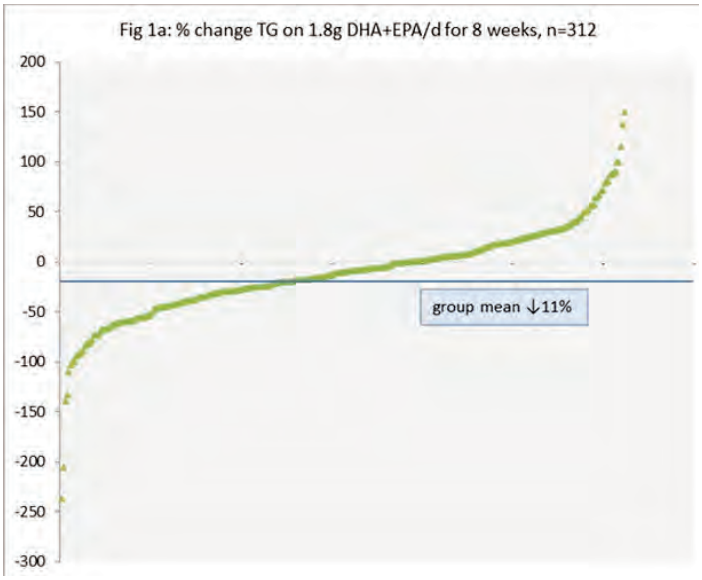
Approaches used to examine genotype*diet*phenotype associations can be targeted and hypothesis driven, with a focus on candidate genes with a known role in the metabolic process of interest. Publically available SNP databases such as the site curated by the National Centre for Biotechnology Information, US (www.ncbi.nlm.nih.gov/snp) are used to select SNPs of interest. In contrast non-targeted approaches, such as genome wide association studies (GWAS), which first appeared in 2005, may be used to identify novel loci associated with a phenotype of interest. In GWAS, the majority of the genome is assessed and genetic variation is compared between different sub-groups (such as those without or without disease, or responders and non-responders to intervention) to identify genotypes associated with a trait.

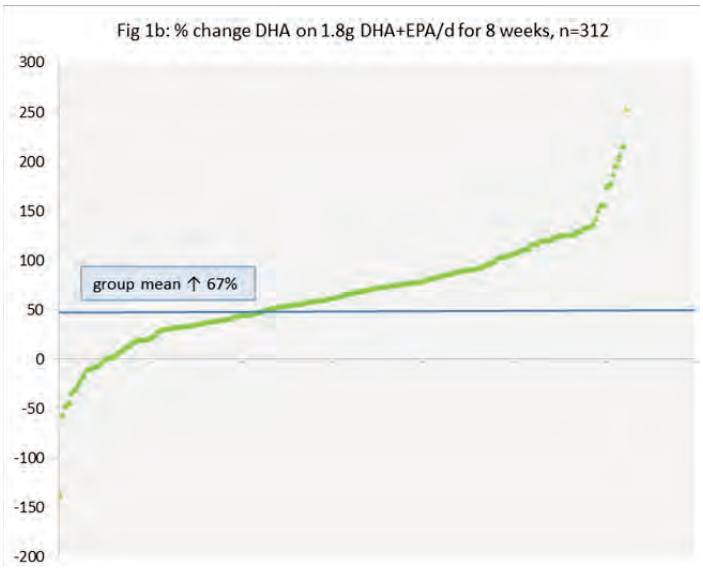
How Variable is the Response to EPA and DHA Supplementation?

In scientific publications data is almost exclusively presented as group means with some measure of variability in the form of e.g., SD or SEM, which disguises the large inter-individual variability in response. In the FINGEN study a total of n=312 healthy UK adults underwent a dose-response cross-over study consuming a control oil of fish oil providing 0.7 and 1.8g EPA+DHA per day in random order (5). A number of cardiovascular disease biomarkers were assessed including the plasma DHA and TG response. According to the records of returned capsules, compliance to treatment was 95% overall. In the group as a whole, a mean (SD) 11 (45) % and 67 (51) % decrease and increase in TG and DHA were evident following the high dose intervention for eight weeks, which represented ranges of -237% to 150% for TG and -137% to 253% for DHA (Figure 1) (5). Overall 118/312 participants had an increase in TG following intervention.

Fatty Acid Desaturase *(FADS)* Genotype, EPA/DHA Status and Health Endpoints

Endogenous production of LC PUFAs from shorter-chain precursors (AA from LA, and EPA and DHA from αLNA) is mediated by the delta 5 (D5DS) and delta 6 desaturase (D6DS) enzymes, which are encoded by the *FADS1* and *FADS2* genes. In general, the efficiency rate for n-3 PUFAs is relatively low in humans, with an estimated conversion of αLNA to EPA of 0.2-6 % and <0.1% for DHA (6). It may be predicted that gene variants associated with greater D6DS and D5DS and activities would be associated with a higher tissue EPA and DHA status. This may be of particular relevance to individuals with a low consumption of fish who rely on plant derived αLNA to improve tissue EPA and DHA status.





In a 2006 publication, which included an analysis of 18 SNPs of *FADS1* and *FADS2,* the minor (less frequent) alleles were associated with higher levels of αLNA and LA and lower levels of DGLA, AA, EPA, and n-3 DPA, with no significant impact evident on DHA or n-6 DPA (7). These initial findings were broadly consistent with the output from the *CHARGE* consortium, which performed GWAS in five population-based cohorts comprising almost 9000 individuals of European ancestry (8).

Associations between *FADS1-FADS2* SNPs and haplotypes (grouping of SNPs) and blood lipids have also been observed using GWAS approaches (9). Furthermore, associations between variation in these gene loci and the incidence of diseases with a chronic inflammatory component have been reported (10), although Baylin et al. (11) failed to detect FADS SNP associations with incidence of myocardial infarction, despite genotype-dependent variation in tissue PUFA levels. In a fascinating recent GWAS analysis to investigate genetic signatures of diet and climate adaptation in Greenland Inuits (who have a high LC n-3 PUFA intake), *FADS* was the strongest loci associated with height, weight, growth hormone regulation and membrane fatty acid composition.

In summary, individuals with a *FADS* genetic profile associated with low EPA and DHA synthesis may be particularly at risk from a lower tissue status and benefit from higher dietary intakes (12).

APOE Genotype, EPA/DHA Status Lipids and Cognition

Apolipoprotein E is an important lipid transporter in the circulation and in particular in the brain and acts as a high affinity ligand for cell uptake (13). Two common SNPs lead to three apoE isoforms, namely E2, E3 and E4. Individuals who carry an *APOE4* allele, which is about 25% of Caucasians, are at significantly higher risk of dementia.

Brain tissue is particularly enriched in DHA with concentrations 2-10 fold higher than in systemic tissues (14). The higher rate of cognitive decline in *APOE4* carriers is likely to be in part due to a lower brain DHA status, defective uptake of DHA across the blood brain barrier (BBB) and increased DHA oxidation (15, 16). Lower enrichment in plasma DHA has been reported in *APOE3/E4* individuals relative to the wild-type *APOE3/E3* genotype (17) and the cognitive benefits of LC n-3 PUFA have been shown to be significantly less in *APOE4* carriers relative to non-carriers (18).

In addition to influencing DHA metabolism and cognition, *APOE* genotype is known to influence the plasma lipid response to EPA+ DHA supplementation. A greater TG lowering effect has been observed in *APOE4* carriers and in particular in males (5, 19), which may be due to a greater up-

regulation of lipoprotein lipase (LPL) (20), a key enzyme hydrolysing TG-rich lipoproteins (TRL) in the circulation.

Therefore, *APOE4* carriers represent a large 'at-risk' population subgroup who may particularly benefit from higher DHA intakes, although supra-physiological doses should be avoided in hyperlipidaemic individuals given that modest LDL-cholesterol raising effects have been observed (21).

PPAR Genotype and the Plasma Lipid Response to EPA and DHA

PPAR-α regulates multiple genes involved in TRL and HDL homeostasis such as LPL, apoA5, and apoA1 and in lipogenesis and fatty acid oxidation. It is the major transcription factor modulating the impact of EPA and DHA on plasma lipids. A number of publications have examined the impact of the *PPAR-α Leu162Val* variant, although findings have not been fully consistent (1). In the first of these analyses, based on the Framingham cohort, *Val* allele carriers had lower plasma TG and apoC3 concentrations relative to non-carriers, when consuming a high-PUFA diet, with n-6 PUFAs and n-3 PUFAs having comparable effects (22). No specific analysis of LC n-3 PUFAs was conducted. Rudkowska *et al.* indicated that the effect of *PPAR-α Leu162Val* genotype on the TG response to EPA+DHA supplementation in humans may be due to a differential impact on LPL activity (23). The authors went on to show in transfection studies that *Leu162Leu* homozygotes were more responsive to LC n-3 PUFAs with regard to LPL transcription (23).

PPAR-γ regulates adipogenesis and lipid (TG) uptake and storage in adipose tissue. *In vitro* studies have shown that the Ala12 isoform of PPAR-γ2 is associated with a reduced

ability to induce transcription and adipogenesis (24). In the *KANWU* Study carriers of the *Ala12* allele had significantly greater reductions in serum TG levels in response to LC n-3 PUFA supplementation relative to the PPAR-γ2 *Pro12Pro* group (25). In a more recent publication, Jensen and co-workers reported that in addition to sex, this variant modulated the impact of *FADS* genotype on behaviour in children (26), highlighting the complexity of penetrance and interpretation of individual genotypes in isolation.

Although not fully elucidated, given the central role PPARs play in modulating the effects of fatty acids on lipid metabolism, insulin sensitivity, vascular function, and inflammation, a research focus on this loci in the identification of the aetiology of the responsiveness of these physiological targets to EPA/DHA supplementation is merited.

Genotype and the Variable Blood Pressure and Vascular Response to EPA and DHA Supplementation

At a population level, it is thought that the hypotensive actions of EPA and DHA and their effect on vascular function and stiffness is only evident at high intakes, which could only be realistically achieved through high dose supplementation. However, it is likely that lower doses are effective in responsive individuals. In endothelial cells, endothelial nitric oxide synthase (eNOS) produces NO, a potent vasodilator. The eNOS *Asp298Asp* genotype has been associated with vascular function and cardiovascular risk (27) along with the vasodilatory response to EPA+DHA intake (28). Recent GWAS have identified a SNP in the c-Src tyrosine kinase (CSK) gene to be associated with blood pressure with an increase of systolic blood pressure of 0.6mmHg per allele (29). In the MARINA study CSK genotype emerged as a significant modulator of the systolic, diastolic and mean arterial blood pressure responses to EPA+DHA supplementation (30).

Closing Remarks

Although at a population level, dose-response relationships between EPA and DHA intakes and CVD clinical endpoints and biomarkers of CVD risk have been relatively quantitatively described, there is still a distinct lack of information on factors that determine individual responses. While progress is slower than originally expected, the published literature is providing insight into what factors may be important, with *FADS* and *APOE* genotypes emerging as important genetic modulators. Further identification of genetic determinants requires investigation in cohort studies where EPA and DHA intakes and status are accurately characterised, or in human RCTs that are sufficiently powered to study genotype*diet*phenotype associations. In the past small sample sizes are likely to be a major source of apparent lack of consistency between studies, and erroneous findings from such studies stunt progress in the field.

Given the recognised biopotency of LC n-3 PUFAs along with evidence of considerable heterogeneity in response, current moves toward a more stratified approach to preventive and therapeutic medicine, and diminishing worldwide marine stocks, investigations in this area are of wide public health relevance. However, we must not get too "gene-centric" in our thinking and research focus, and recognise that effective future personalisation must consider other major factors that are likely to influence response to EPA and DHA such as sex, habitual intake and disease status.

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FOL

INVITED OPINION

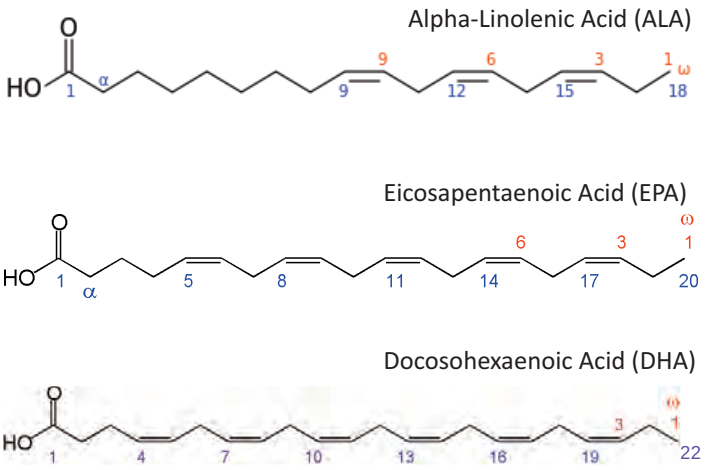
Heart Health Benefits of EPA and DHA

GOED/Omega-3 Science Advisory Council White Paper

Decades of research have uncovered many health benefits of long-chain omega-3 fatty acids, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA are both essential building blocks for tissue structures and important biological mediators in health and disease, which is why the Dietary Guidelines for Americans, as well as health advocacy groups from around the globe, recommend eating foods rich in EPA and DHA as part of an overall healthy eating pattern. Yet, there are mixed viewpoints about the cardiovascular benefits of EPA and DHA when taken in supplement form. This document reviews the scientific evidence about EPA and DHA and its association with cardiovascular health and disease risk reduction.

Omega-3 Basics

There are three primary omega-3 fatty acids consumed in the diet: alpha linolenic acid (ALA), EPA and DHA. All are polyunsaturated fatty acids with varying lengths of the carbon backbone. ALA has 18 carbon atoms with three double



bonds; EPA has 20 carbon atoms and five double bonds; and DHA has 22 carbon atoms and six double bonds. ALA is found in plant foods such as soybeans, black walnuts, flaxseeds, and chia, whereas EPA and DHA are marine-based and found in seafood (especially fatty fish). Sources of EPA and DHA for supplements include sardines, anchovies, certain types of algae and krill.

While the body can elongate ALA into its longer-chain omega-3 fatty acid counterparts, the process is inefficient with less than 5 percent converting to EPA and less than 1 percent to DHA. The best way to get EPA and DHA is by consuming it directly, through foods and supplements.

Why EPA and DHA are Important

The roles and functions of EPA and DHA are distinct but complementary. As precursors to several families of biologically active molecules (resolvins, protectins, maresins, prostaglandins, leukotrienes, etc.), EPA and DHA both help to control inflammatory responses and regulate blood coagulation. They both play a role in determining what genes are turned on and off. DHA, by virtue of its greater presence in cell membranes than EPA, plays an important structural role in cells, helping make cell walls more flexible. This in turn allows the proteins in the membrane to control the inflow and outflow of important cell components, making the cell operate most efficiently. DHA is found in virtually all cells, but it is particularly concentrated in cells of the brain, heart and retina.

EPA and DHA and Heart Health

What Is Known

Scores of research studies support the view that EPA and DHA omega-3s are cardioprotective nutrients. Higher intakes or blood levels of these important fatty acids are associated with reduced risk of mortality from coronary heart disease (CHD) and sudden cardiac death.[1] The USDA's National Evidence Library notes that the evidence supporting EPA and DHA for heart health is "moderate" indicating that some of the evidence is conflicting and more research needs to be done.[1] Importantly, recent meta-analyses of randomized-controlled studies found that dosages greater than 1 gram (1,000 mg) EPA+DHA per day offer significant cardioprotection,[2] reducing risk for CHD by 20 percent or greater.[3] Studies with even lower intakes find that overall risk for cardiac death is reduced,[4] and a recent modeling exercise looking at prospective studies found that intake of EPA+DHA at *any dose level* was associated with reduced risk for any type of CHD event.[5]

EPA and DHA, especially at relatively high doses (i.e. >3 g/day), have been shown to lower triglycerides and raise HDL-cholesterol (the "good" cholesterol).[6] The same study found that in patients with extremely elevated triglyceride levels, fish oil supplementation may also slightly raise LDL-cholesterol, yet total cholesterol remained virtually unchanged. Other studies have also found that EPA+DHA improve blood vessel function,[7] reduce inflammation,[8] and lower blood pressure.[9]

What is Unclear and Needs More Research

Several major early studies using fish or fish oils to reduce CHD events were favorable. These included DART,[10] JELIS,[11] and two GISSI[12,13] studies. It was upon the basis of two studies (DART and GISSI-Prevenzione) that most recommendations in the late 1990s and early 2000s for the use of EPA+DHA in secondary prevention were founded.

However, at least six trials published between 2010 and 2014 did not find a benefit for EPA+DHA in patients with known CHD or with risk factors for heart disease.[14-19] These "failed" studies obviously beg the question of whether, in today's world of modern medical care, omega-3 fatty acid supplementation still reduces risk for CHD. It's a reasonable question, because even though the safety of EPA and DHA supplementation is certain, consumers – and their healthcare providers – may express concern about the cost and/or inconvenience of supplementation when the perceived benefits are not as clear. These are valid concerns.

But before any conclusions can be drawn about fish oils and EPA/DHA supplements failing to prevent heart disease, we need to interpret findings of recent clinical studies in context. That is to say that if we are going to accept the findings of these studies, then we must keep in mind the conditions under which they were done. When we do so, we find that fish oils "did not work" in the following setting where patients:

- were given a **< 1 g/day** for about **2-3 years** (i.e., a low dose for a short period of time);
- were in their **early 60s**;
- were typically on **one to five other heart/diabetes medicines** and had been **treated in the hospital immediately after their heart attack to restore blood flow to the heart muscle (which greatly reduces the damage)**; and
- whose **background omega-3 intake (from diet) was close to a cardioprotective dose already (i.e., around 250 mg/d)**.

In addition, most of these studies included too few of the specific kinds of heart attacks most likely to benefit from omega-3 to draw any valid statistical conclusions. These concerns have been raised by several experts in the field.[20-24] Under *these* conditions, it is not surprising at all that the addition of a relatively small amount of EPA and DHA had no detectable effect.

The important question is not whether a course of low dose of omega-3s given over the short term with other CHD drugs to older people with disease helps, but whether taking either higher doses or taking lower doses for decades (not a couple of years) will be helpful. Potential positive effects of long-term intakes of 1 g or more of EPA+DHA beginning in young adulthood (or even *in utero*!) was not addressed in these studies and remains an open question, and it is this question that must be answered before one can conclude that "fish oils don't work." The strong findings of epidemiological studies supporting a cardioprotective effect of higher intakes of EPA+DHA and of higher blood levels of these important fatty acids argues that, in the long-term, EPA and DHA are cardioprotective nutrients, and calls to abandon their use are premature.

In this light, future studies are needed, but they **must be properly powered** (i.e., include enough clinical events to allow for proper statistical analysis), must **measure dietary intake and blood levels** of all omega-3 fatty acids **before and during** the study, use doses of omega-3 fatty acids **significantly higher than those provided in background diets**, focus on **patient populations with low EPA+DHA** levels, treat for **longer periods of time**, advise taking omega-3s with meals, and consider the effects of these agents in the great majority of patients who are not actually taking the drugs that they were prescribed (which is far more common in the real world than in clinical trials). The strong evidence-base from prospective cohort studies and the ever-deepening understanding of the cellular effects of long-chain omega-3 fatty acids together support the need for these nutrients in reducing cardiovascular risk. Short-term findings from randomized controlled trials need to be interpreted in the light of all the evidence.

Safety of Omega-3 Supplementation

When it comes to recommending omega-3 supplements to patients or consumers, it is important to also consider both safety and costs associated. The good news is that omega-3 supplementation of up to 3 grams per day is generally recognized as safe (GRAS) by the Food and Drug Administration.[25] Omega-3 fish oil supplementation greater than 3 grams per day is possible but should be done only under the care of a physician.

A recent report from Norway concluded that there was no evidence for safety concerns at daily intakes of up to 6.9 g of EPA+DHA per day; a dose that is higher than the currently approved dose for omega-3 prescription drugs (4 g/day, which supplies up to 3.6 g of omega-3 fatty acids). This applies to risk for "excessive bleeding" as well, which is a common misconception among consumers and physicians alike. Fish oils do not increase risk for clinically-significant bleeding.[26]

Recommended Amounts of Omega-3s

The Dietary Guidelines for Americans,[27] American Heart Association (AHA),[28] the World Health Organization (WHO),[29] the International Society for the Study of Fatty Acids and Lipids (ISSFAL)[30] and other public health groups around the globe recommend dietary patterns that would supply adequate amounts of EPA and DHA, averaging between 250 and 500 mg per day. This can be accomplished by eating two 4-oz servings every week of fatty fish such as salmon, mackerel, trout, halibut, or albacore tuna; or by taking an omega-3 supplement with EPA and DHA (which typically contains 250 to 500 mg per capsule). The Global Organization for EPA and DHA Omega-3s (GOED) recommends a minimum intake of 500 mg per day for effective cardiovascular protection.

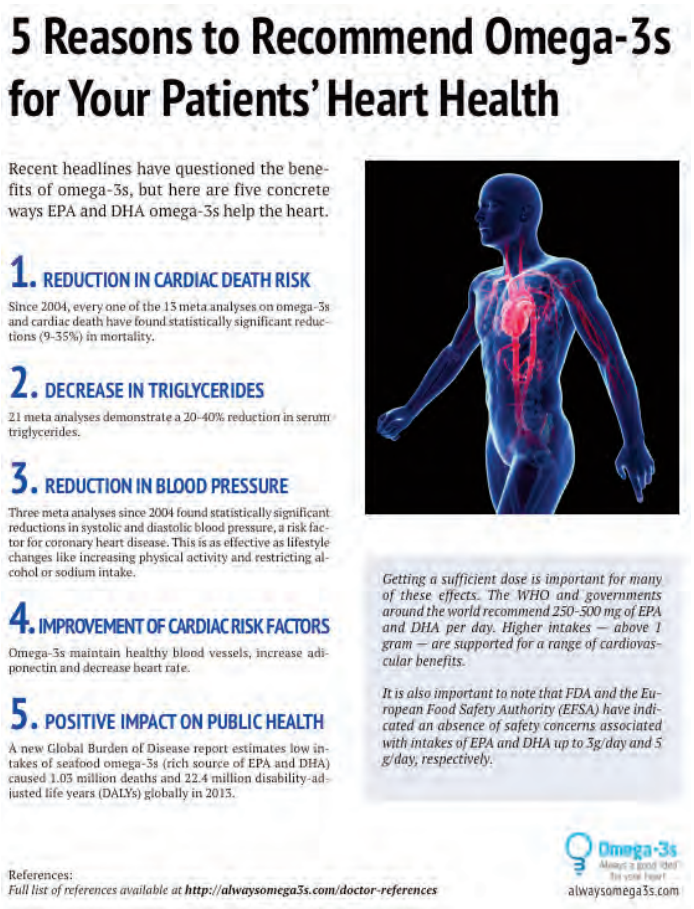
According to the American Heart Association, people with coronary heart disease should increase their omega-3 intake to about 1 gram per day. Individuals with high triglycerides may require intakes of EPA+DHA in greater amounts and should be done under the care of a phycisian.[28]

Summary

People should be eating fatty fish at least twice a week to get adequate amounts of EPA and DHA to support a healthy heart – as underscored by position pieces from the AHA,[28] the Academy of Nutrition and Dietetics,[31] and the U.S. government.[27] Yet, the typical American diet is far from ideal, with people consuming only about 3.5 ounces of fish per week – a fraction of which is omega-3-rich fatty fish, and a far cry from the 8 ounce weekly recommendation.[32] Until we can close the gap, health professionals should continue to advocate for increased fish consumption. For those individuals who will not or cannot comply, recommending a high-quality omega-3 supplement to support a healthy lifestyle, including a healthy heart, is reasonable.

It's important to note that omega-3s, as with most supplements, **are not meant to be a proxy for pharmaceutical interventions – they are not drugs**. They are not meant to treat disease (except in the case of severe hypertriglyceridemia), but rather to supplement nutrient gaps in the diet and contribute towards maintaining health and wellbeing. While the science behind omega-3s and cardiovascular disease is not yet conclusive, the weight of the evidence coupled with the low risk and potential high reward associated with supplementation indicates that recommending omega-3s is a smart strategy.

An infographic to provide information for health practitioners on the benefits of EPA and DHA in cardiovascular health has also been developed by GOED.



The flyer is designed to be distributed to patients and colleagues and can be found here.

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