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EDITORIAL

Understanding the Fundamentals to Appreciate the News on Omega-3s

Dear Fats of Life readers,

Understanding what polyunsaturated fatty acids do in the body is a real challenge. These fatty acids are biologically active, but they are not drugs that one can easily follow after intake. They will be stored in cell membranes, and sometimes released again and used for a range of purposes. Both the absolute amounts that we ingest and the relative concentrations of individual fatty acid classes determine how cellular activities are modulated. Their individual roles furthermore vary by life-stage – long-chain PUFA are conditionally essential for good health. But discerning what each fatty acid type does is very difficult, since fatty acids regulate each other's fate and activities, and can be transformed to other bioactive fatty acid types. In the view of this complicated landscape, research is making steady progress in delineating the rules of our body that are obeyed in health, and deranged in disease.

This August issue of the PUFA Newsletter brings you fresh insight into the booming research field of omega-3s and other polyunsaturated fatty acids. To properly understand their function we need to keep reminding ourselves about the fundamental learnings. Particularly important for understanding biologically active nutrients, studies need to be performed as well as possible based on the learnings of prior research, to be able to report increasingly meaningful results and uncover the true activities fatty acids display in our tissues.

In order to address the basics, for this issue two experienced scientists provide us with top-level views of two important areas. Bill Lands in an Invited Opinion explains in a succinct way the essence of the role of omega-3 LCPUFA intake in view of our diet. The crucial understanding relates to how the absolute intake of omega-3 LCPUFA modulates the narrow therapeutic window of linoleic acid, an abundant dietary fatty acid that contributes fundamentally to a range of low-grade chronic inflammatory disorders typical for current Westernized lifestyles.

In a Guest Article, Jacques Delarue reviews the important role of omega-3 LCPUFA in regulating insulin sensitivity, a pivotal mechanism that is central to energy metabolism in our body. A careful reading will provide you with a good overview of the main arguments to understand in which way omega-3 LCPUFA can prevent type II diabetes, a disorder that is displaying a marked increase in incidence around the world.

In our summaries, we highlight recent studies on cardiovascular health, maternal and infant health, the central nervous system, and the visual system. The role of omega-3 LCPUFA in the cardiovascular system is highlighted often since much research is continuously being published in this area. In this issue we highlight recent studies addressing the effects of omega-3 LCPUFA supplementation on cardio-respiratory adaptations in endurance athletes. A second summary looks at the relationship between omega-3 status and outcomes of reperfusion therapy after acute myocardial infarction.

In the topic on Maternal and Infant Health we highlight a study that has taken a close look at the potentially key developmental roles for individual PUFA in the development of body composition of male and female newborns. We also highlight two studies that have addressed the potential importance of omega-3 LCPUFA dietary requirements for preventing and treating the behavioral and emotional problems associated with ADHD in school-age children.

Remarkable understanding has come out of the ongoing efforts from the OPTIMA network at Oxford University. Elderly people with mild cognitive decline, which will ultimately progress to develop Alzheimer's Disease, are reported to benefit from supplemental combined intake of folic acid, vitamin B-6 and vitamin B-12. The recent

studies indicate that this preventive action towards brain atrophy may be governed by the omega-3 status of the body, with associations of markedly decreased risk for brain loss progression with the highest omega-3 levels in blood. From a mechanistic point of view, the essential fatty acids and B-vitamins have strong inter-relationships, and this study provides a smart basis for further research in preventing, and possibly treating, Alzheimer's Disease.

Dry eye disease is a common ailment with multiple origins that lead to major discomfort in daily life. Three recent studies have investigated the effectiveness of omega-3 LCUFA supplementation to make dry eye disease more bearable in different settings, with marked levels of success in improving symptoms and restoring some of the underlying defects.

Finally, the analysis of the plasma samples of a double blind randomized controlled intervention study of patients with advanced chronic kidney disease with omega-3 LCPUFA has shown that the body is capable of converting the EPA and DHA to biologically active anti-inflammatory derivatives. This is an important study since this response is noted under carefully controlled and unbiased conditions and indicates that people with chronic kidney disease remain capable of responding with a protective endogenous response that may potentially contribute to counteracting some of the fundamental deranged cardiovascular adaptations characteristic for this condition.

As a word of closure, the PUFA Newsletter aims to regularly provide you with some valuable examples of research progress, recognizing that most of the literature goes uncovered. By highlighting examples and placing the articles in perspective through related notable publications in the "Worth Noting" reference list, over time we will be able to provide a good overview of much of the overall progress being made.

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CARDIOVASCULAR HEALTH

The Influence of Omega-3 LCPUFA Supplementation on Cardio-Respiratory Function during Exercise in Endurance Athletes

THIS ARTICLE AT A GLANCE

- *How specific nutrients modulate adaptations to exercise and endurance training is a topic of growing interest.*
- *Two recent small studies have made a careful analysis of potential cardio-respiratory changes in response to omega-3 LCPUFA supplementation in highly-trained athletes at rest, during exercise, and during recovery.*
- *The results suggest that specific improvements such as lowered heart rate, increased oxygen uptake, and faster heart rate recovery can be achieved.*

Introduction

Training of exercise endurance involves adaptations in the efficiency by which the cardiovascular, respiratory and skeletal muscle organ systems support the delivery of energy substrates and oxygen (O_2) to muscles during periods of increased physical demand. The human body is well developed to adapt to endurance exercise, and has a tremendous capacity to adjust with major increases in ventilation and cardiac output capacities to achieve its physiological needs. The maximum transport rate for O_2 to reach skeletal muscle from the lungs is limited by two principal factors: the maximum rate of O_2 uptake from the lungs (VO_{2max}), and the resistance of the diffusion pathway from capillaries to muscle tissue. The major ways in which the human body addresses these limitations is by making adaptations in cardiac output and hematocrit to facilitate increased oxygen transport from the lungs to the circulation and carry the oxygen to the muscles. While reactive hyperemia facilitates increased muscle tissue perfusion during exercise, the principal adaptive features to overcome an increased diffusion limitation in trained muscles is an angiogenic response that increases skeletal muscle capillarity, an increase in mitochondrial density, and an intensification of the parallel activation of ATP usage and ATP supply.

Endurance exercise training activates several changes to achieve a higher cardiac output: enlargement of the ventricular cavities, increased myocardial vascularity, an increased blood volume that increases central venous pressure, and a decrease in resting heart rate. Resting bradycardia permits a markedly increased dynamic range in cardiac output from rest to exercise. Along with a tremendous increase in pulmonary ventilation during exercise, highly trained people can meet oxygen consumption demands of ~ 6 liters/min, compared to an average untrained person with an oxygen demand of ~ 3 l/min. Along with these physiological adaptations, several metabolic adaptations improve the utilization of energy substrates to optimize power-generating capabilities in muscle, and increase the lactate threshold. Neuronal and endocrine signaling regulate the adaptations to increased training, and are likely sensitive to different types and forms of exercise training, *e.g.* endurance, interval, and muscle power training. How specific dietary components influence the physiological and metabolic adaptations to exercise in relation to increased performance in athletes, and in people that exercise in general, is a subject of growing interest.

The role for specific nutrients in the adaptation of cardio-respiratory function in exercising people and in athletes is of growing interest.

Omega-3 LCPUFA improve flow-mediated vascular dilation, an important response of conduit arteries that permits increased blood flow to an active vascular bed. Omega-3 LCPUFA also have measurable effects on the heart, resulting in a lower resting heart rate and small decreases in resting blood pressure. Since these effects may also be beneficial in improving exercise performance in athletes, understanding the extent of the influence of omega-3 LCPUFA intake and status in exercise adaptation is of interest. However, very few studies have examined the effects of EPA and DHA supplementation on exercise physiology in athletes. Two recent studies have addressed in more detail whether supplementation with omega-3

LCPUFA influences cardio-respiratory function in highly-trained athletes and elite endurance cyclists.

First Study Looks at Low-Dose Supplementation

A recent study has addressed the effect of low-dose fish oil supplementation on the heart rate response to intense exercise in physically fit men. The research was carried out by Macartney and colleagues at the Centre for Human and Applied Physiology at the School of Medicine, Medical and Exercise Science, University of Wollongong, Australia. Heart rate, heart rate variability and heart rate recovery during rest, intense exercise, and recovery were measured following eight weeks of supplementation in physically fit and healthy males. The study was a double blind randomized intervention study with men (18-40 years old) who regularly trained intensely with at least four sessions of 60 minutes of moderate-to-vigorous exercise sessions per week. Participants were allocated in a random fashion to 2 g of tuna oil every day providing 140 mg EPA and 560 mg DHA daily, or soya bean oil, with 13 persons in both groups completing the trial and the post-supplementation exercise testing protocol. Men consuming more than two fish meals per week, already taking omega-3 LCPUFA supplements, or who could not be confirmed to regularly exercise at the desired level, were excluded from the study. The oils were provided in unmarked and indistinguishable capsules (two capsules a day). Compliance was confirmed by capsule counts, and by incorporation of omega-3 LCPUFA into erythrocyte membrane lipids.

Men that regularly exercised intensely took 700 mg EPA/DHA every day for eight weeks. Their cardio-respiratory functioning was tested in detail in sub-maximal, maximal exercise, and during recovery from exercise.

Measurements of cardiovascular function were taken before and after the eight-week supplementation period. Resting heart rate was measured at home using a heart rate monitor upon going to bed, and during sleep (mean and minimum heart rate), as well as in the laboratory 20 minutes before the exercise test. Blood pressure was monitored at home in the morning and evening using an automated blood pressure monitor over a three-day period. In order to assess the effects of dietary supplementation with omega-3 LCPUFA on cardiovascular function during exercise, participants performed a cycling exercise composed of three parts: a 10 minute sub-maximal cycling at 125W (steady-state sub-maximal cycling), six 30 second cycling sprints with 150 seconds recovery between each sprint (repeat-bout sprints), and after five minutes of active recovery (slow cycling), a five-minute work capacity trial of uninterrupted maximal cycling effort. This was followed by a 10-minute recovery in the supine position under controlled laboratory conditions. During the entire exercise cardiac frequency, heart rate variability, aerobic work, and oxygen consumption were measured.

Results

There were no significant differences between the control and treatment groups in age, height, mass, blood pressure, resting heart rate, heart rate during the steady-state exercise, maximal oxygen consumption (mean of ~51 ml/kg x min), or the amount of work exerted during peak exercise activity. There were no differences in self-reported physical activity during the week prior to study commencement (average of ~450 minutes), and consumption of fish (1.4-1.8 100 g portions a week) in the two study groups. After eight weeks of supplementation with the DHA-rich tuna oil, the treatment group had a significantly higher level of DHA in erythrocyte membrane lipids, as well as an increased omega-3 index (percentage EPA plus DHA of total fatty acids in erythrocyte membrane lipids). There was no increase in EPA content in the treatment group, and no difference in linoleic acid content was found between the two groups. In control subjects there were no significant changes in membrane fatty acid composition.

Fish oil supplementation did not change blood pressure, heart rate, or heart rate variability under resting conditions. There was no difference in resting heart rate variability between the two groups, obtained from a beat-to-beat analysis of heart rate, although a trend for a lower vagal (parasympathetic) autonomic tone under resting conditions in men taking the fish oil supplement was noted. During the steady-state sub-maximal cycling period, the fish oil group had a significantly reduced heart rate (23 beats per minute lower during the second five minute cycling period) compared to the control group. During peak cardio-respiratory efforts, whether during sprints or the work capacity trial, no differences in any of the measured cardio-respiratory parameters were found between the two groups. However, during the recovery phase, the time to half

During sub-maximal exercise the men that had taken EPA/DHA for eight weeks had a significantly lower heart rate. During recovery from maximal exercise, the time to half recovery (the restoration of the elevated heart rate towards a baseline value) was significantly shorter.

recovery (the restoration of the elevated heart rate towards a baseline value) was significantly shorter in the fish oil group (eight seconds at the end of supplementation period compared to baseline) compared to control (no change with respect to baseline). There was no difference in the heart rate variability between supplemented and control subjects, suggesting that EPA and DHA supplementation did not affect autonomic cardiac tone during recovery from peak exercise.

Elite Cyclists Focus of Second Study

In a second recently published study on highly trained endurance cyclists, similar findings were reported. Zebrowska and colleagues at the Department of Physiological and Medical Sciences at the Academy of Physical Education, Katowice, Poland, examined the effect of omega-3 LCPUFA supplementation on cardiovascular and respiratory parameters in a small group of elite cyclists. The average age of the cyclists was 23 years, and they had a maximal oxygen consumption of 69.8 ml/kg x min, and a mean training volume of 655 km/month. The study was a cross-over design with the 13 cyclists randomly assigned to receive a three-week supplementation with either placebo (lactose) or an omega-3 LCPUFA supplement. After a two-week break, the study subjects switched to the opposite treatment. The daily 1.3 g dose contained 660 mg EPA and 440 mg DHA in gelatin capsules. Three weeks prior to initiation of the cross-over intervention trial, all participants were placed on the same isocaloric and nutrient-balanced diet, which was continued throughout the study. Before and after each three-week intervention period, all subjects were asked to perform a cycling exercise on an ergometer bicycle while being monitored continuously for respiratory and cardiovascular function. Participants performed three-minute periods of increasing intensity (40 W increases) up to maximal exercise intensity.

Results

The study showed some notable changes in cardiovascular and respiratory parameters after the omega-3 LCPUFA supplement. These included statistically significant changes after supplementation (compared to both baseline before intervention, as well as to controls) (**Table 1**) in aortic pulse pressure (increase), brachial artery diameter (a small decrease), flow-mediated dilation of the brachial artery (increase), maximal oxygen consumption at peak exercise (VO_{2max}; increase), and maximal heart rate (decrease). Blood pressure measured at brachial level did not change. The increase in aortic pulse pressure under resting conditions may likely reflect an increased ventricular inotropy (cardiac output was not studied). Increased serum free fatty acids and glucose levels were found after omega-3 supplementation. No significant changes were identified in the control group. After omega-3 supplementation, both the baseline and the exercise-induced nitric oxide levels (measured from total NO/nitrite/nitrate in serum) were significantly higher than in subjects at the end of the placebo intervention period. Exercise-stimulated nitric oxide formation at maximal exercise intensity was correlated with maximal oxygen consumption. No information was provided on the masking of study subjects or study organizers to the interventions. Baseline omega-3 status, and the change in omega-3 status as a result of supplementation, were not reported.

Table 1: Changes in cardio-respiratory function in endurance-trained elite cyclists after three weeks of omega-3 LCPUFA supplementation

Function	Control cyclists	Omega-3 LCPUFA supplemented cyclists
Maximal oxygen uptake VO_{2max} (ml x kg-1 x min-1)	71.0 ± 4.0	74.8 ± 5.6 *
Heart rate$_{max}$ (beats min-1)	196.0 ± 3.0	186.0 ± 5.0 *
Aortic pulse pressure at rest (mm Hg)	26.2 ± 7.1	30.2 ± 9.1 *
Brachial artery diameter at rest (mm)	4.2 ± 0.2	4.1 ± 0.1 *
Flow-mediated dilation at rest (%)	10.0 ± 4.6	15.2 ± 7.6 *

*Values indicated are means +/- S.D. of 13 elite cyclists. *P <0.05. Zebrowska et al 2015*

Elite cyclists taking a daily dose of 700 mg EPA/DHA for three weeks had markedly improved maximum oxygen uptake and a lower heart rate during maximal exercise.

Discussion

These two studies provide preliminary indications that in highly trained athletes, supplementation with relatively low doses of omega-3 LCPUFA for a period of three to eight weeks can provide some benefits in cardio-respiratory function. This refers specifically to a lower heart rate during submaximal power training, faster heart rate recovery after maximal intensity training, and improved maximal oxygen

uptake. Surprisingly, even relatively short supplementation periods appear to provide measurable benefits to cardiovascular and respiratory function in these highly trained endurance athletes.

The authors of the first study point out that omega-3 LCPUFA, in particular DHA, may have an intrinsic action on the beat rate of the heart, independent from sympathetic or parasympathetic influences, possibly within the pacemaker region of the heart. Improved endothelial function may also be associated with the effects of omega-3 LCPUFA on cardiovascular and respiratory function, as suggested by the second study. Since the studies were both small, further studies are warranted to obtain confirmation of the observed effects of omega-3 LCPUFA supplementation on adaptations in cardio-respiratory function during periods of intense exercise by well-trained athletes. Whether optimizing the dietary intake of omega-3 LCPUFA in endurance athletes translates to improved performance remains to be established.

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Cardio-Protective Effects of Omega-3 LCPUFA in Percutaneous Coronary Intervention Reperfusion Therapy after ST-Segment Elevation Acute Myocardial Infarction

THIS ARTICLE AT A GLANCE

- *Acute myocardial infarction (AMI) remains one of the most common causes of death globally.*
- *The study reports the results of an observational study of Japanese patients who had a STEMI-type AMI and that received percutaneous coronary intervention (PCI) reperfusion therapy.*
- *The results indicate that people who had EPA + DHA levels in serum over 155 mg/ml had a markedly better outcome of PCI than people with low omega-3 LCPUFA levels; ischemia-reperfusion injury was decreased as measured by fewer reperfusion ventricular arrhythmias, lower ST-segment re-elevation, and less myocardial tissue injury.*

Introduction

A growing body of animal and clinical research continues to document the cardioprotective effects of the omega-3 long-chain polyunsaturated fatty acids (LCPUFA) and their mechanisms of action, supporting the view that their intake can contribute to a reduced risk of cardiovascular mortality. A reduced incidence in sudden cardiac death associated with arrhythmias of the ventricles following a heart infarct is one important contribution that higher dietary intake and endogenous levels of EPA and DHA make to this risk reduction. Loss of viable myocardial tissue resulting from an ischemic event in the coronary circulation is considered an important contributor to deranged electrical conduction that precipitates fatal arrhythmia. The length of the ischemic insult due to a coronary artery obstruction is a critical factor that defines myocardial damage in acute myocardial infarction (AMI). In current clinical practice, however, impaired myocardial reperfusion is also a significant determinant of clinical outcome in AMI patients, since in many countries the larger part of people with a heart infarct are treated with reperfusion therapy. Reperfusion therapy through timely removal, disruption, or dissolution of a blood clot obstructing a coronary artery by percutaneous coronary intervention (PCI) or thrombolysis is live-saving. It limits the size of an infarct, preserves contractile function of the left ventricle, and reduces the development of heart failure. However, PCI and thrombolytic therapy also impart their own risks, which are related to so-called ischemia/reperfusion (I/R) tissue injury.

Age-standardized mortality rates from ischemic heart disease have decreased in several developed countries in recent years. However, due to a global aging population, myocardial infarction remains one of the most common causes of death globally.

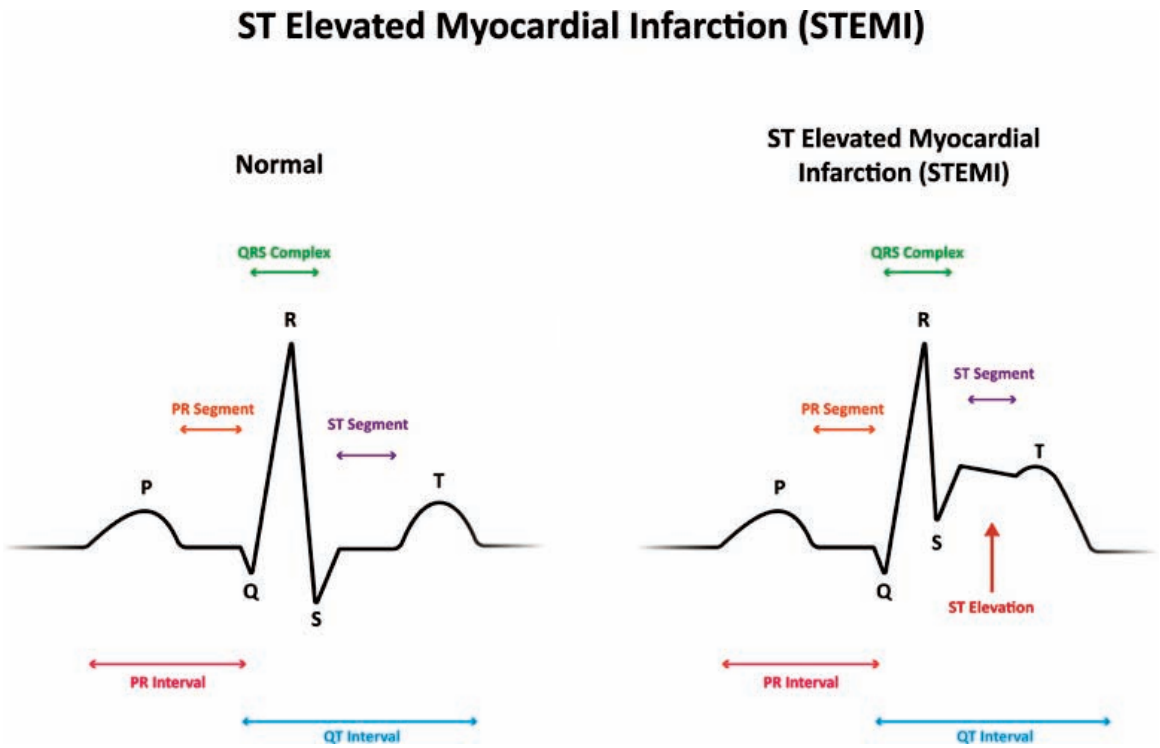
The reperfusion of a temporally-occluded part of any organ can contribute to a significant extent to loss of viable organ tissue. Activation of oxygen- and nitrogen radical-mediated tissue damage occurs upon reoxygenation, as well as aberrant activation of neutrophil-dominated inflammation. In PCI, a widely employed surgical procedure to re-open one or more blocked coronary arteries, reperfusion of the affected ischemic tissue will occur, causing a certain degree of ischemia-reperfusion injury depending on the time of ischemia until PCI or thrombolysis, and on the endogenous tissue-protective and anti-inflammatory local mechanisms that are operative. The success of reperfusion therapy is an important determinant for clinical outcome. Pharmacological and mechanical approaches to protect myocardial tissue from reperfusion injury have been developed. Progress in PCI techniques and improved thrombolytic approaches, as well as an improved mechanistic understanding of myocardial I/R injury, and of secondary microvascular obstruction, and microvascular damage with intramyocardial hemorrhage, after PCI, will likely bring about further improvements. Still, even with much understanding and many intervention and treatment options, ischemic heart disease remains the most common cause of death in many countries in the world.

Narrowing Down Patient Variables

A role for omega-3 LCPUFA in protecting the myocardium has not been formally demonstrated within the context of reperfusion therapy in ischemic heart disease in humans. DHA is known to be rapidly incorporated and retained in myocardial membrane lipids, and may play a regulatory function in myocardial electrophysiology. A recent observational study has addressed the potential association of serum omega-3 LCPUFA levels with I/R-injury and clinical effects of myocardial damage in patients with ST-segment elevation acute myocardial infarction (STEMI) that underwent PCI. The research was by Dr. Kantaro Arakawa at the Division of Cardiology at Fujisawa

Hospital in Kanagawa, Japan, together with colleagues from Yokohama City University Hospital Medical Center and

In order to appreciate if the blood levels of omega-3 LCPUFA might carry any predictive value to the extent of myocardial



School of Medicine. Data from 221 patients with STEMI were analyzed to identify which patient variables correlated with ST-segment re-elevation and reperfusion arrhythmias as outcome of myocardial reperfusion injury.

These patients had shown ST–segment re-elevation on electrocardiograms recorded during pre-hospital cardiopulmonary resuscitation, stenosis of ≥90% of lesion diameter, a TIMI flow grade of 0 or 1 (a standardized measure of coronary blood flow), and an increase in serum creatinine kinase that surpassed twice the maximal normal level. Emergency PCI had been performed within six hours after chest pain onset. The patients included 160 men and 51 women, with an average age of 67 years. Blood samples were analyzed for fatty acid composition of serum after transmethylation of all serum lipids. Infarct size was estimated using the serial QRS scoring system prior to PCI, one hour after PCI, and at hospital discharge.

This study focused on the relationship between blood levels of EPA and DHA and the clinical outcome of myocardial reperfusion therapy by percutaneous coronary intervention in patients with an ST-segment elevation type acute myocardial infarction.

I/R injury, the patients were divided into two equally sized groups on the basis of the sum of EPA and DHA before angiography (median 155.0 microgram/ml), one (group L) had EPA ± DHA levels < 155 microgram/ml (n=106), and the second group (H) had levels ≥ 155 microgram/ml (n=105). Besides the significantly different blood levels of EPA and DHA, the baseline clinical characteristics of these two groups did not show significant differences for a range of variables related to other disease incidence, drug use, clinical chemistry, blood pressure and heart rate, and an estimation of the myocardial area at risk. Some differences were noted: a lower percentage of smokers in group H (29% vs 52%), and a higher use of calcium channel blockers in group H (33% vs 19%). Group H also showed a small but significant increase in the level of arachidonic acid, but the ratio of EPA to AA was more than two-fold higher in group H compared to group L.

Results

The researchers did not find any correlation between the time of blood sampling after symptom onset and EPA+DHA levels in blood, indicating that acute myocardial infraction did not alter blood omega-3 LCPUFA levels. In patients of group L the increase in QRS score after PCI was significantly higher than in group H, indicating that infarct size was considerably higher (**Table 1**). It remained high upon hospital discharge, suggesting that myocardial scarring remained significantly greater in patients with a lower blood omega-3

Myocardial reperfusion therapy by PCI in patients with an ST-segment elevation type acute myocardial infarction resulted in significantly smaller infarct sizes, lower ST-segment re-elevation and ventricular arrhythmias in patients that had higher serum EPA+DHA levels.

LCPUFA levels. Immediately after PCI-initiated reperfusion, both ST-segment re-elevation and reperfusion ventricular arrhythmias were significantly lower in group H patients (**Table 1**). These findings indicate that I/R injury is substantially reduced in patients with higher EPA+DHA levels. Serum EPA+DHA levels correlated well with I/R injury when analyzed by univariate and multivariate regression analysis. Having pre-myocardial infarction angina, as well as the length of ischemia prior to PCI, also correlated significantly with I/R injury. Based on the results, the authors calculated a highest predictive level (a combination of optimal sensitivity (true positive rate) and specificity (true negative rate)) of serum EPA+DHA level in calculating ST-segment re-elevation of 157 microgram/ml. For reperfusion arrhythmias, 135 microgram/ml had the highest predictive value.

Table 1: Protective effects of EPA + DHA in percutaneous coronary intervention-induced reperfusion injury in people with an ST-segment elevation myocardial infarct

	Serum EPA + DHA level	
Reperfusion injury	**Below 155 microgram / ml**	**Above 155 microgram /ml**
ST-segment re-elevation (*% of patients*)	44	22 *
Ventricular arrhythmias (*% of patients*)	25	11 **
QRS score after PCI	5.9 ± 3.8	4.2 ± 3.1 **

Ventricular arrhythmias is composed of accelerated idioventricular rhythm, ventricular tachycardia and ventricular fibrillation. Arakawa et al 2015

***P<0.001, *P<0.005*

In summary, this study provides interesting new indications that in people with an acute myocardial infarction (STEMI type) and that are treated with PCI reperfusion therapy within six hours of symptoms onset, lower levels of EPA+DHA associate with a significantly increased risk of I/R injury, namely ST-segment re-elevation, reperfusion arrhythmia, and the extent of myocardial damage. The authors discuss that acute administration of omega-3 LCPUFA before or after PCI might be an adjunctive therapy to offer cardioprotection against I/R injury. This approach could be more effective in patients with a first AMI. This view is based on the fact that other studies in patients with a recurrent ventricular fibrillation have not consistently shown a measurable benefit from omega-3 LCPUFA supplementation, which the authors ascribe to concomitant therapies. The limitation of the current study is that it is an observational study, and other parameters were associated with blood levels of omega-3 LCPUFA (smoking, calcium blockers), or with a change in reperfusion injury incidence (presence of existing angina, length of ischemia). The study is of significant interest since a logical link can be postulated between the known actions of omega-3 LCPUFA and the observed protection. Confirmation of this proposed role awaits a controlled intervention trial in patients with angina that are at risk for STEMI and undergoing PCI as a preferred reperfusion treatment.

Protective Actions during Myocardial Infarction

The authors indicate that the mechanisms whereby omega-3 LCPUFA may reduce myocardial reperfusion injury remain to be established. A recently published study led by Ganesh Halade and colleagues at the Division of Cardiovascular Disease, Department of Medicine at the University of Alabama, Birmingham, AL, USA, has addressed how such an omega-3 LPUFA-mediated protective action may be mediated. Together with colleagues at the Center for Experimental Therapeutics and Reperfusion Injury at Brigham & Women's Hospital, Boston, MA and the Chicago College of Pharmacy at Midwestern University, IL, the investigators examined the molecular and cellular events during ischemia-induced inflammatory damage following myocardial infarction. In a murine model of myocardial ischemia-induced infarction by permanent ligation of the left descending coronary artery, left ventricular dysfunction was induced after one to five days, as a model for ventricular remodeling and heart failure. Functional read-outs for left ventricular function were the fractional shortening (ratio between the diameter of the left ventricle when relaxed and the diameter when it is contracted), end-diastolic dimension (diameter across the left ventricle at the end of diastole), end-systolic dimension, infarcted wall thickness, and heart

rate. The research addressed if the DHA-derived lipid mediator resolvin D1 (RvD1) affected ischemic ventricular remodeling and function.

Results

After one and five days, fractional shortening of the ventricle was markedly reduced, and ventricle diameter at systole and diastole significantly increased. The thickness of the infarcted myocardial lesion was markedly reduced. The sub-cutaneous administration of small doses of RvD1 (3 microgram/kg/day), either as the free acid or incorporated within multi-lamellar liposomes, significantly reduced the remodeling of the ventricle. RvD1 increased neutrophilic infiltration at day 1, but ablated the presence of neutrophils in the ventricle at day 5. RvD1 upregulated the expression of 5-lipoxygenase (a biosynthetic enzyme involved in wound healing and synthesis of lipid mediators), the receptor for RvD1 and lipoxin A_4, an arachidonic acid-derived lipid mediator with anti-inflammatory activity. A small decrease in the number of macrophages was also induced by RvD1, and importantly the phenotype of myocardial macrophages changed from a predominant M1 classical macrophage phenotype to an M2 macrophage phenotype that play a role in resolving inflammation. Collagen deposition in the ventricle at day 5 was significantly reduced by RvD1. The functional effects of myocardial ischemia were not limited to a local response. A marked edema of the lungs appeared after one day, which was abrogated by RvD1. Following myocardial ischemia the spleen reacted rapidly with a marked reduction in weight. The involvement of the spleen, with the mobilization of pro-inflammatory leukocytes, has recently been recognized to be activated after acute myocardial infarct in humans. Also in the spleen macrophages expressed predominantly the M2 macrophage phenotype.

Discussion

These studies provide new insight into the potentially protective roles of omega-3 LCPUFA in the myocardium after an AMI. The studies are important since a better understanding of their role in protection from ischemia, ischemia-reperfusion injury, and incomplete reperfusion therapy, will likely help in refining interventions after acute myocardial insults. The mouse study did not address the involvement of a DHA-derived lipid mediator in reperfusion injury, but rather in a setting of prolonged ischemia-induced ventricular injury. It highlights the possibly important role of splenic activation following a myocardial infarct, and the omega-3 LCPUFA-derived lipid mediators that are formed there may play a role in activating splenic retention of immune cells that mediate ventricular damage. Further studies that directly address the relationship between blood levels or dietary intake of omega-3 LCPUFA and outcome of myocardial I/R injury and reperfusion approaches are now of significant interest and clinical value.

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Visual Sonics – In vivo imaging system : http://www.visualsonics.com/ FOL

Evidence for Sexual Dimorphism in the Neonatal Fat Mass Response to Maternal Long-Chain PUFA Intake

THIS ARTICLE AT A GLANCE

• *Omega-3 long-chain polyunsaturated fatty acid (LCPUFA) intake during pregnancy has been associated with a small but significant increase in the average length of a healthy pregnancy, as well as the body weight of a newborn.*

• *In this study the investigators address the sex-specific effects of maternal LCPUFA intake during pregnancy on body compositon of healthy full-term babies.*

• *The results suggest that the ratio of omega-6 to omega-3 LCPUFA intake favors development of adipose tissue in newborn girls, and that higher DHA intake is associated with increased ponderal index of males at birth, a measure of larger body size unrelated to fat mass.*

Introduction

Omega-3 LCPUFA intake during pregnancy has been associated with a small but significant increase in the average length of a healthy pregnancy. On average, gestational length is 2-3 days longer in mothers with above average omega-3 dietary intake, for example after intake of 600 mg/d DHA during the second half of pregnancy. Omega-3 LCPUFA intake can also increase the body weight of newborns. The studies that have identified the effect of omega-3 LCPUFA on gestational length and neonatal body weight have not studied the changes in body weight as a direct effect of omega-3 LCPUFA status but as a secondary outcome that has been largely regarded as a consequence of longer gestation. Body composition associated with this weight gain has not been studied.

Determination of the specific effects of maternal long-chain PUFA intake on body composition of neonates has not been a primary objective in past studies that have identified increased gestational length in mothers with higher omega-3 LCPUFA intake.

Objective

A recent study has investigated the relationship between maternal LCPUFA intake and fat mass in healthy full-term babies. The study was performed by Pereira-da-Silva and colleagues from the Department of Pediatrics, at the Hospital Dona Estefania, Centro Hospitalar de Lisboa Central, in Lisbon, Portugal, and other research centers in Lisbon, St. John Providence Children's Hospital, Detroit, MI and Rutgers University Newark, NJ, in the USA. The objective of the study was to examine the effects of maternal LCPUFA intake on the body composition of neonates at birth of full-term appropriate-for-gestational age neonates born to mothers without known potential factors that affect intrauterine growth. Specifically the study employed an accurate and reliable technique to measure adipose fat mass in newborns using air-displacement plethysmography.

Methods

Neonatal anthropomorphic measurements were taken within the first 72 hours after birth. These included weight, length, and midarm circumference. From weight and length measurements, indices were calculated to estimate adiposity, weight/length, body mass index (weight per squared length), and the ponderal index (weight per cubed length, *i.e.* expressed as 100 x g/cm^3) as a measure of leanness. Body composition was determined by air-displacement plethysmography, and a model that permitted calculating fat mass and fat-free mass with a precision of 0.1 g. Maternal diet was assessed by a food-frequency questionnaire validated for pregnant women (Portuguese context), and consumption of individual nutrients calculated based on food

composition databases and average portion patterns. The omega-6:omega-3 LCPUFA ratio intake was calculated from the sums of ingested long-chain PUFA. Inclusion criteria were a pregnancy resulting in a singleton birth, and full-term gestation (between 37 and 41 weeks), and appropriate for-gestational-age birth weight. Excluded from the study were pregnant mother-child dyads in the case of ≥5 pregnancies, multiple pregnancies, and small-for-gestational-age, or where mothers had adverse health conditions known to affect intrauterine growth as well as alcohol, tobacco and illicit drugs use. Data were collected on 100 mother-neonate dyads, considered a "convenience" cross-sectional sample from a previously reported study design.

Results

In multivariable regression analysis there was a significant interaction between the estimated omega-6:omega-3 ratio intake and the percentage fat mass of male and female neonates. Further stratification by sex was employed to investigate the effects of maternal LCPUFA intake, pre-pregnancy body mass index, and gestational weight gain, on percentage fat mass and anthropomorphic indices. In female neonates a positive association was found between the n-6:n-3 LCPUFA ratio intake and fat mass and percentage fat mass (corresponding to a 21 g increase in fat mass for each unit increase in n-6:n-3 LCPUFA intake ratio). In boys, a positive association was not found for fat mass and n-6:n-3 LCPUFA, but here the ponderal index was associated with maternal DHA intake, pre-pregnancy body mass index, and with gestational weight gain. After adjustment for body mass index and gestational weight gain of the mother, the ponderal index of male neonates was estimated to be 0.165 g x cm-3 higher when their mothers had an intake ≥200 mg DHA/day, compared to those with mothers with a lower daily DHA intake.

This study in 100 Portuguese women has identified significant associations between LCPUFA intake during pregnancy, and neonatal body size and fat mass, that were different for male neonates and female neonates at term birth.

Discussion

This study provides new indications that at term birth a higher maternal omega-6:omega-3 LCPUFA intake ratio is associated with female offspring having higher fat mass, and fat as percentage of body composition, whereas in male neonates a higher ponderal index is associated with DHA intake above a recommended daily intake of 200 mg/day. Employing measurements within 72 hours after birth, the differences in anthropometry can be ascribed to differences in the prenatal period, in contrast to other studies where effects of post-natal feeding have made it difficult to assign independent effects of variations in LCPUFA supply on intrauterine developing fat depots. A limitation of this study is the reliance on a food frequency questionnaire, sensitive to recall bias, although the authors mention that the quality of the estimated fatty acid intake has been deemed acceptable according to the EURopean micronutrient RECommendations Aligned scoring system. The Portuguese women analyzed in this study had overall relatively high average daily intakes of EPA (115 mg) and DHA (266 mg), and a n-6:n-3 ratio of 7.0. However, almost 40% of women had a daily LCPUFA intake below 200 mg.

Female neonates are known to have greater fat mass and percent fat mass at birth than males. The results of this study suggest that a relative higher maternal intake of omega-6 compared to omega-3 LCPUFA predisposes to higher adiposity at term birth. With respect to male neonates, it has been suggested that ponderal index as an indicator of body size correlates better with lean mass rather than adiposity, and the authors indicate the possibility that increased ponderal index associated with maternal DHA intake may reflect a higher bone mass than adiposity. Sex differences in the response to omega-3 PUFA intake have also been reported with respect to cognitive performance during childhood with more marked effects for female children. Recognition that increased DHA intake and the relative levels of dietary omega-6 to omega-3 LCPUFA may have differential effects on body composition in male and female neonates is an important observation. The adequate development of fat stores in women throughout fetal development and childhood as a physiological fat storage depot may reflect the location for storage of DHA needed for future delivery of DHA to their respective children. Confirmation of the associations iden-

The results of this study suggest that a higher omega-6 to omega-3 ratio intake during pregnancy favors higher adiposity in female neonates, whereas higher DHA intake is associated with increased ponderal index of males at birth, a measure of larger body size unrelated to fat mass.

tified here is required to substantiate the observation that maternal LCPUFA intake may have important early effects on the physiology of men and women.

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Advances in Understanding the Relationship between Child Problem Behavior and Maternal Long-Chain PUFA Intake

THIS ARTICLE AT A GLANCE

• An important area in omega-3 research addresses the possibility to prevent or alleviate ADHD in childhood by omega-3 supplementaton with omega-3 LCPUFA of mothers during pregnancy or the children with ADHD themselves.

• A higher n-3:n-6 ratio during mid-pregnancy was found to be associated with fewer emotional, but not behavioral, problems in six-year old children.

• In a randomized double-blind placebo-controlled intervention trial with 8-14 year old boys with ADHD it was found that attention levels improved in boys that received omega-3 LCPUFA supplementation for four months.

Introduction

Understanding to what extent and how maternal omega-3 long-chain polyunsaturated fatty acid (omega-3 LCPUFA) intake during pregnancy and lactation determines the growth and development of the fetus and newborns has fascinated nutrition researchers from the time these fatty acids were recognized to be essential for human health. This consideration has also expanded into understanding if maternal omega-3 LCPUFA availability determines the emotional well-being and behavior of children at later ages. This interest stems from the fact that omega-3 LCPUFA contribute to a significant extent to membrane lipid structure and function in the central nervous system. Docosahexaenoic acid (DHA) in particular is one of the major PUFA within membrane lipids in brain tissue, imparting functional properties to neuronal cells, glial cells and the blood-brain barrier, allowing control over regulation of immune privilege, cellular viability, neuroplasticity and neurotransmission. The availability of LCPUFA for fetal and infant brain development depends nearly exclusively on maternal fatty acid provision via placental transfer *in utero* and breast milk after birth. Recommendations for adequate intake of PUFA during pregnancy and the post-natal period are hence updated periodically. DHA and arachidonic acid (AA) are now routinely added to infant formula.

Early perinatal nutritional inadequacies that determine behavioral and emotional well-being at later ages (toddler, school-age and adult), and that are modifiable by relatively simple dietary changes of the mother or via infant formulae, are of significant relevance to individuals, parents, and to society at large. Furthermore, the possibility to correct any long-term emotional and cognitive effects of maternal nutritional deficits in omega-3 LCPUFA by supplementation is also an important therapeutic option to assess. Several studies in humans have pointed out a direct relationship between neurocognitive deficits in childhood or adult life and low blood levels of omega-3 LCPUFA and/or increased ratio of omega-6 to omega-3 LCPUFA during pregnancy. However, most of the evidence linking perinatal nutritional inadequacy and later life cognitive and behavioral deficits originates from animal studies. A number of studies have indicated that emotional and cognitive deficits in school age and adult life can be attenuated by increased omega-3 LCPUFA intake via the diet or supplementation with omega-3 LCPUFA.

Behavioral disruption in children is often associated with attention deficit/hyperactivity disorder or ADHD, a disorder with varying signs and symptoms that encompass several of the following: children with ADHD may be clumsy and accident-prone, show erratic and disruptive behavior (fits and tantrums), compulsively touch everything and everyone, are in constant motion, disturb other children and may be aggressive and argumentative, are unable to concentrate, fail to finish activities or tasks, are easily frustrated, are easily distracted and often unable to follow instructions, lack social skills, have a normal or high IQ but may do badly at school, have poor hand and eye coordination, and are uncooperative, defiant and disobedient. ADHD has an estimated prevalence of 7.5% among children worldwide according to a recent systematic analysis. It has been suggested that the prevalence of ADHD is increasing in developed countries but this has not been substantiated. Renewed research is

The prevalence of ADHD, a disorder of childhood characterized by emotional and behavioral disruption, may be of the order of 7.5% worldwide. Significant interest exists in understanding the importance of insufficient availability of essential fatty acids in its etiology, and evaluating the possibility to address central nervous system imbalances by augmenting omega-3 LCPUFA intake.

focusing on determining the effectiveness of dietary adequacy of omega-3 LCPUFA in children affected by ADHD. The results of studies carried out to address the long-term effects of maternal nutritional inadequacy or nutritional intervention studies in ADHD are not unambiguous. The variability in study design and target groups may have contributed to this. Foremost, many cohort studies have been relatively small and underpowered to have properly detected any effects. Additionally, scoring of the variable displays of emotional and behavioral problems is difficult, and most studies rely on measurements from one setting, for example at home or at school, but not in both places. A recent open intervention trial has reported that omega-3 LCPUFA supplementation may be beneficial in children with ADHD that were also being treated with methylphenidate. Also recent meta-analyses of interventional studies have concluded that omega-3 LCPUFA supplementation in ADHD may be modestly efficacious.

Study Examines Effects in Six-Year Olds

In a recently published study, Steenweg-de Graaf and colleagues addressed associations between maternal LCPUFA status during pregnancy and emotional and behavioral problems in six-year old children. The study is part of a larger initiative called the Generation R Study, which is following the development of 10,000 children in the city of Rotterdam, The Netherlands. This study was performed by researchers from the Generation R Study Group and various departments at the Erasmus Medical Center in Rotterdam, The Netherlands, and the Dr. von Hauner Children's Hospital in Munich, Germany. The population-based study involved 6916 mother-child dyads, and measured several parameters related to child behavior and emotions at six years of age scored by both parents and teachers. At least one measure of child behavior at age six was available for 5307 children.

Venous samples drawn at mid-pregnancy (median week 20.5) were analyzed for arachidonic acid (AA), EPA and DHA, and n-3:n-6 ratio, present in plasma phospholipids. The Child-Behavior Checklist (CBCL) questionnaire was filled out by parents to measure the degree of children's problem behavior in the preceding two months, and was compared to the known distribution in the population to assign a cut-off definition for problematic behavior. In this study the so-called CBCL 11/2-5 questionnaire was used since a substantial part of the children had not yet turned six. The CBCL makes possible the calculation of a value for emotional and behavioral problems by rating a large series of problem items. This type of scoring makes possible the ranking of various problems, typical of disrupted children's emotions and behavior in ADHD.

A second test was a version of the CBCL called TRF 6-18, for behavioral and emotional problems scored by the teachers of the studied children. Of importance, recovering information in different contexts, such as home and school, provides complementary information, which may be lost in studies that focus on one context alone. Parent and teacher scores were examined individually and combined. In a subset of children with higher scores on the CBLC scale (>85th percentile) a diagnosis of problem behavior was obtained with a parent-administered interview (DISC-YM or Diagnostic Interview Schedule, Young Children). This highly structured computerized test generates diagnoses on anxiety disorders, mood (emotional) disorders, and behavioral disorders.

When child and maternal characteristics were stratified by maternal n-3:-6 ratio in mid-pregnancy, it was found that a number of variables differed significantly. For example, children of mothers with the lowest quintile n-3:n-6 ratio had a lower birth weight, spent half the time in daycare, and had a higher dietary intake of omega-6 PUFA at 14 months (compared to all other quintiles combined). Mothers in this lowest quintile were of significantly different non-European ethnic background, had received fewer years of higher education, had a lower average family income, presented with a higher level of general psy-

The probability for having emotional problems in six-year old children whose mothers had a higher maternal DHA blood level, or a higher ratio of n-3 to n-6 fatty acids, was significantly lower when symptoms were scored by their parents. When scored by teachers alone, this association was not found.

chiatric symptoms during pregnancy, were more likely to have smoked and less likely to have consumed alcohol during pregnancy. When children-mother dyads of the 208 children that were found to have emotional and behavioral problems were compared to the other mother-child pairs, a number of differences were observed. Firstly, the children in this group contained a lower number of girls, and were less likely to have attended daycare. Of their mothers, fewer were of European ethnicity, fewer had completed higher education, they had a lower average income, they presented with a marked increase in psychiatric symptoms in mid-pregnancy, they were more likely to have smoked during pregnancy, less likely to drink alcohol, and fewer mothers were married.

Results

Parent report of child emotional problems was significantly lower in children whose mothers had a higher maternal DHA level (odds ratio OR 0.82). This was also found when the reporting by parents and teachers was combined (OR 0.79). The n-3:n-6 ratio was significantly inversely associated with parent and combined parent-teacher reporting of emotional problems (OR 0.83 and 0.77, respectively). Teachers reporting alone did not reveal any significant differences in emotional problems. Use of the parent-scored DISC-YM testing method did not reveal any significant differences in emotional problems in the sub-group of children enriched for having emotional and behavioral problems.

With respect to behavioral problems, parent-report did not identify any significant associations between maternal PUFA status during pregnancy and their children's behavior. However, teacher's report showed a significantly increased probability for having behavioral problems associated with increased maternal blood phospholipid levels of AA (OR 1.10), DHA (OR 1.23), as well as the n-3:n-6 ratio (OR 1.23). The combined teacher-parent report also identified a positive relationship between AA and behavioral problems. Again, no significant association between maternal PUFA status and behavioral problems were identified by the DISC-YM testing method.

When the maternal n-3:n-6 ratio was stratified by quintiles, marked differences in the probability to suffer emotional, but not behavioral, problems in the offspring were identified (using parent report of child problem behavior). Compared to the lowest quintile, the three highest quintiles displayed odds ratios of 0.58, 0.39 and 0.35 (after correction for a number of covariates such as gestational age at blood sampling, sex, and age of child at assessment). After correction for additional covariates (such as family income, educational level, European origin, psychiatric symptoms in mid-pregnancy, smoking and alcohol consumption during pregnancy, age at enrollment, parity, marital status, and child care attendance), the odds ratios for the two highest quintiles remained significantly different from the lowest quintile (0.62 and 0.61).

In summary, the results of this cross-sectional study in Dutch six-year old children from a multi-ethnic city suggest that a higher n-3:n-6 ratio during mid-pregnancy of their mothers is predictive for having fewer emotional problems. In particular DHA may contribute to this association. This does not apply to behavioral problems, where no association with LCPUFA status was identified. In contrast, a small increase in behavioral problems by teacher's reporting was found to be associated with an increased maternal AA and DHA level, as well as with an increased n-3:n-6 ratio.

The limitations and virtues of this study are both related to the comprehensive nature of the reporting, where different symptom scoring approaches, and scoring ADHD symptoms in different physical environments, do not appear to provide the same information. This study hence provides important guidance for future studies, as it points out that limiting reporting to a single approach and environment is unlikely to provide a good view of ADHD in children in this age group. A positive association between a higher n-3:n-6 ratio and behavioral problems, and with seemingly incompatible increased DHA and AA levels at the same time, suggests that other omega-3 PUFA or other omega-6 LCPUFA such as linoleic acid, might also contribute to these observations. Future research is needed to better understand in detail the associations between behavioral and emotional problems and individual fatty acids and fatty acid classes. Non-recognized covariates may also be important. Importantly, the population size in this study permits identifying meaningful associations.

A Controlled Trial with ADHD Boys

A second recently published study reports a randomized, double-blind, and placebo-controlled trial that addressed the effect of omega-3 LCPUFA supplementation during 16 weeks in 8-14 yr-old boys with clinically-diagnosed ADHD on ADHD symptoms and cognitive control. The study was performed by Bos and colleagues from the Department of Psychiatry at the Rudolf Magnus Brain Center, University Medical Center, in Utrecht, The Netherlands, the Wilhelmina Children's Hospital in Utrecht, Dr. von Hauner Children's Hospital in Munich, Germany and Unilever Research & Development in The Netherlands. Forty boys with ADHD were allocated in a masked and random 2x2 facto-

rial design to one of two groups. The first group received supplementation with EPA and DHA as part of a margarine spread containing 650 mg EPA and 650 mg DHA per 10 g serving or a control margarine without EPA and DHA. Two further groups comprised boys (n=39; reference) without ADHD and matched in age, handedness, and body mass, allocated to EPA and DHA or a placebo margarine. Total intake and compliance was calculated from left-over margarine on a monthly basis, as well as daily recordings of margarine consumption by both parents and participants. Parents were masked to the test products. The boys otherwise continued their regular diets, fish consumption was limited to once a week, and no other use of omega-3 supplements was permitted. Boys with ADHD were allowed to continue any ongoing treatment with methylphenidate as a psychostimulatory medication.

Before and after the intervention study, samples from buccal cheek tissue were collected, and the PUFA composition of phospholipids determined. Urine was collected in order to measure homovanillic acid excretion (as a measure for dopamine formation and breakdown). The severity of ADHD symptoms was assessed with two tests. The CBCL was used as the primary outcome, and was tested before and after the trial. The Strengths and Weaknesses of ADHD symptoms and Normal behavior scale (SWAN) test were used to collect results over the course of the trial. A teacher reporting test was used but did not receive sufficient feedback, and hence this study was limited to parental reporting. A Go/NoGo test was used as a cognitive control task before and after the trial to measure brain activation by functional magnetic resonance neuroimaging (fMRI), allowing the measurement of any changes in cognitive task performance and brain activation.

In a placebo-controlled, randomized, and masked intervention trial, supplementation of 8-14 yr old boys with ADHD with EPA and DHA during 16 weeks led to a measurable reduction in symptoms of inattention, according to scoring by their parents. No improvements in rule breaking or aggressive behavior, or changes in cognitive control, were found.

Results

Supplementation with EPA and DHA for 16 weeks led to a significant improvement in parent-rated symptoms of inattention in the boys with ADHD (CBCL score changed from 9.1 to 7.7). In the boys in the reference group, with much lower baseline scores for attention problems (CBCL ~2.6) than boys with ADHD, it was noted that EPA and DHA supplementation might stabilize the development of behavioral problems during the course of the trial. These changes were accompanied by significant increases in cheek phospholipid DHA levels in boys that had received EPA and DHA supplementation, whereas they decreased in the reference groups over this period. A negative correlation was identified in boys with ADHD between their cheek DHA levels and attention problems, both before supplementation and after supplementation. No significant effect of EPA and DHA intake was found in rule-breaking behavior, aggressive behavior, or overall ADHD score. The absence of any effect of EPA and DHA supplementation on cognitive control measured by task performance and fMRI, or on dopamine turn-over calculated from homovanillic acid to creatinine ratios in urine, did not support an effect on cognitive control in boys with ADHD.

Discussion

The two new studies provide important new indications about the potential relationship between essential fatty acid intake and tissue status, and the incidence of emotional and behavioral problems in school age children. Importantly, the studies highlight a suitable way forward in addressing these topics, since they have addressed some methodological limitations of previous studies. Firstly, cross-sectional studies need to be large enough to enable measuring outcomes with sufficient power. Secondly, it is important that information on emotional and behavioral problems is scored in the different environments where children spend their time and may express themselves differently, for example at home versus school. The study nicely reflects this, with parent reporting giving different outcomes from teacher reporting.

Whereas this makes assessment of interventional strategies and cohort studies in ADHD more difficult to conduct, it will likely be needed to obtain better insight into the complicated symptoms of this disorder. This limitation is already apparent, as teachers' feedback was insufficient to evaluate results for all children in the second study. A randomized controlled trial can, however, provide information on direct causality and shows that four-month supplementation may help boys with ADHD recover attention level. An indication for a preventive activity on attention problems may also be apparent in normally developing boys, suggesting that basal intake of EPA and DHA could be very important in 8-14 year old boys. It is possible that measurable emotional problems may be avoided in children if the mother attains sufficiency in omega-3 PUFA during pregnancy, in particular with respect to DHA. Further research is necessary to confirm these important findings.

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BRAIN AND CNS

Omega-3 LCPUFA Status may Determine the Effectiveness of Supplemental B-Vitamin Intake in Decreasing the Rate of Cognitive Decline in Elderly People

THIS ARTICLE AT A GLANCE

• *Alzheimer's Disease is an accelerated form of age-related cognitive decline characterized by brain atrophy.*

• *This study has uncovered a previously unknown association between higher omega-3 LCPUFA status and the potential for inhibition by supplemental B-vitamin intake of progressive brain atrophy in mild cognitive impairment in elderly people.*

• *The results of this study set the stage for controlled trials that will be able to confirm if omega-3 LCPUFA sufficiency is determining the potential for inhibition of cognitive decline by the combination of vitamin B-6, vitamin B-12 and folate, as well as a novel possibility for treatment of Alzheimer's Disease.*

Age-related cognitive decline is a normal process that affects people as they get older. However, Alzheimer's Disease is characterized by an acceleration of the rate of brain atrophy and cognitive impairment leading to dementia and brain atrophy. Life style factors such as diet modify the development of AD. Recent research is highlighting the potential for modifiable outcomes in cognitive decline anteceding AD development if the precise dietary factors that are involved are identified.

Introduction

Alzheimer's Disease (AD) is an accelerated form of age-related cognitive decline characterized by brain atrophy. It is the most common cause of dementia, and one of the most common diseases in the elderly that is currently untreatable. Brain structures that are affected in AD patients include the medial temporal lobe of the cortex, important for memory formation and storage, a general decrease in cortex volume, enlargement of the ventricles, and degeneration of the locus caeruleus. Several potential mechanisms that may underlie AD have been or are currently being investigated that relate to neuroinflammation, disturbed neurotransmitter release and removal, the formation of neurotoxic peptides and insoluble protein aggregates, and disturbed metal and redox homeostasis. AD relates furthermore to disturbances in metabolism, and its prevalence is associated with some genetic and environmental risk factors. AD is characterized as dementia accompanied by the presence of specific protein aggregates (neurofibrillary tangles and senile plaques) in brain tissue. Vascular alterations in the brains of elderly people can also bring about dementia, and mixed forms of vascular dementia and AD (mixed dementia) are particularly common.

The prevalence of AD is increasing as many Western societies have a growing percentage of elderly people. Worldwide an estimated 115 million people will suffer AD by 2050 if no treatment or preventive approach is found. The burden on personal and institutional care for AD patients is very high. Recent research has offered some progress indicating that progressive cognitive decline in established AD can be slowed down using drugs like memantine. Several pharmacological approaches that selectively target the formation of protein aggregates and amyloid-derived peptides associated with AD are ongoing.

Epidemiological evidence suggests that life-style related factors affect mild cognitive impairment and the progressive cognitive decline that antecedes development to dementia. Some of the focus is changing to non-pharmacological approaches aimed at restoring biochemical deficiencies that underlie the atrophy of neuronal tissue, and preventing the cognitive decline preceding AD. The role of the diet is thought to be important, but it is not known which dietary components have an effective role in prevention of AD or might halt disease in established AD. Research from the Oxford Project to Investigate Memory and Ageing (OPTIMA), which has been ongoing since 1988, has

offered numerous important insights in AD. OPTIMA draws upon a very large number of patient records and biobanked tissues. Recent research has provided important evidence that sub-optimal low-normal vitamin B12 and folate levels are strongly linked to AD development.

When specifically tested in a randomized clinical trial (VITACOG), supplementation with a high dose of B-vitamins during two years (0.8 mg/d of folic acid, 20 mg/d of vitamin B6 and 0.5 mg/d of vitamin B12) was found to significantly slow down cognitive decline and brain atrophy rate in elderly (>70 yrs) people with mild-cognitive impairment. Of interest, the VITACOG trial revealed that the efficacy of the B vitamins in lowering cognitive decline was better in people with higher levels of homocysteine. B vitamins control homocysteine levels by stimulating the conversion of homocysteine to methionine. Homocysteine is a known risk factor for vascular damage and associated with hypoperfusion of the neocortex and atrophy of the medial temporal lobe in AD patients. The results suggested that differences between individuals existed in the effectiveness and response to B-vitamin intake. In order to achieve a better understanding of which fundamental changes may underlie cognitive decline, adequate intervention trials and analyses have to be performed that can stratify outcomes on those factors that relate to the speed of cognitive decline.

Retrospective Study Shows Promise

A recently published retrospective analysis of results of the VITACOG trial has investigated to what extent the effect of B-vitamin supplementation relates to the omega-3 LCPUFA status of the study participants. The study was performed by Jernerén and colleagues from the Department of Pharmacology at the University of Oxford, together with researchers from the Oxford Project to Investigate Memory and Aging at Oxford University, the University of Alexandria, Egypt, the United Arab Emirates University, and the University of Oslo, Norway. The primary objective was to evaluate the rate of brain atrophy as a function of the total plasma fatty acid concentrations of EPA, DHA, and both combined. There is some evidence that omega-3 LCPUFA are inversely correlated with homocysteine levels in blood. Furthermore, omega-3 LCPUFA are structural components of polar lipids in brain tissue, and are important for neurocognitive health. Intake of omega-3 PUFA could reduce cognitive decline in people with minor cognitive impairment. The central nervous system imports DHA at least in part through the phospholipid phosphatidylcholine (PC) present in the circulation, and B-vitamins play an important role in the biosynthesis of this lipid. A valid hypothesis is that cognitive decline leading to AD may be due to inadequate supply of circulating PC-contained DHA to the central nervous system, in combination with a lack of control over risk factors such as elevated homocysteine.

The analysis evaluated the rate of brain atrophy in 168 elderly people, of which 85 were in the B-vitamin group and 83 in the placebo group. The mean age of the study participants

was 76.6 yrs, with 61% women. There were no baseline differences in a range of anthropomorphic, physiological and biochemical variables between the placebo and B vitamin-treatment groups. A small elevation in the placebo group was noted for the GDS Depression Score, and body mass index. No changes in plasma EPA, DHA, and combined EPA±DHA levels were found compared to baseline or between groups, indicating that B-vitamins at this daily dose do not affect omega-3 LCPUFA homeostasis (at least the component measured in the circulation). A significant decrease in homocysteine level was found in the treatment group (from 11.3 to 8.7 µM). As expected, folate and vitamin B-12 levels in the circulation were significantly higher in the supplementation group.

There was an inverse relationship between blood levels of EPA, DHA, and both combined, with the yearly brain atrophy rate in the people that had received B-vitamins, but not in the placebo group. Statistically significant differences in the slope of these relationships were found for DHA and for EPA±DHA combined in the B-vitamin group

The study reveals statistically significant reductions in rates of brain atrophy of ca. 40-50% in B-vitamin supplemented elderly people when their omega-3 status was high. Having higher blood EPA, DHA, or both combined, without concomitant supplemental B-vitamin intake was not associated with any decrease in brain atrophy.

compared to placebo. The effect of B vitamin supplementation on reducing brain atrophy rates was statistically significant for plasma DHA levels above 245 µM, above 139 µM for EPA, and above 390 µM for EPA±DHA combined. In the two highest tertiles of omega-3 LCPUFA status, statistically significant reductions in atrophy rates were noted in the order of 40-50% in the B-vitamin supplemented people when compared to placebo. When further stratified by people having low (<11.3 µM) or high homocysteine levels (≥11.3 µM), higher DHA and EPA±DHA levels in plasma were associated with marked reductions in brain atrophy rates (between 59 and 73%) in those people with high homocysteine levels. However, the sample size per group was small for the latter analyses.

The results show that brain atrophy rates in elderly people with mild cognitive impairment can be significantly modified by B vitamin intake, and that this strongly depends on endogenous levels of EPA and DHA. Without supplemental B vitamin intake, the basal omega-3 LCPUFA status does not relate to the rate of brain deterioration. These results are important with a view to the possibility to attenuating the transition from mild cognitive impairment to AD, and they raise questions on the nature of this inter-relationship. A combined action for both B-vitamins and omega-3 LCPUFA in the regulation of neuro-inflammation and brain atrophy is currently not described. The results also strongly indicate the need for a well-designed intervention trial that aims to demonstrate the dependence of the B-vitamin effect on omega-3 status and intake in prevention and potentially treatment of AD.

A Pilot Study

A recent pilot study by Fiala and colleagues at the Departments of Surgery and Neurology, University of California School of Medicine, Los Angeles, CA, has studied the effect of supplementation with an omega-3 LCPUFA-containing drink in patients with minor cognitive impairments and with AD. Supplementation during a period of 4-17 months increased the capacity of monocyte-derived macrophages to phagocytose amyloid- ß, a protein precursor to plaque-forming aggregates in AD brains. The biosynthesis of RvD1, a DHA-derived proresolving lipid mediator with phagocytosis-stimulating activity, was increased in macrophages isolated from those patients that received the omega-3 LCPUFA-enriched drink. These preliminary indications obtained *in vivo* in people with cognitive impairment suggest that promotion of biochemical processes that process amyloid-ß and impart anti-inflammatory activity may be helpful to slow down progression towards AD. This pilot dietary intervention study was not controlled, and the results need to be confirmed in a properly designed clinical trial.

New insight in the interactions between multiple essential nutrients and vitamins in the slowing down of brain atrophy that underlies cognitive deterioration before Alzheimer's Disease onset now offers solid possibilities to test preventive strategies with reasonably well-explained biochemical mechanisms.

Discussion

The recent VITACOG trial provides impetus to further study the potential benefits in optimizing vitamin B and omega-3 LCPUFA status in people with mild cognitive impairment to slow down, and perhaps bring to a halt, the abnormal accelerated cognitive decline that precedes AD development. A previous clinical trial with high dose B-vitamin (5 mg/d of folate, 25 mg/d of vitamin B6, 1 mg/d of vitamin B12) in patients with established mild to moderate AD did not show improvements in cognition although homocysteine levels were reduced. It is now important to establish to what extent omega-3 status might determine an effect of B-vitamins in established AD disease. Since differences in the protective effects of omega-3 LCPUFA in sub-groups of people with cognitive impairment have been observed, further definition of patient groups needs to be examined to properly understand the effectiveness of omega-3 intake and status.

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Oral Omega-3 Long-Chain PUFA Intake to Improve Comfort of People with Dry Eye

THIS ARTICLE AT A GLANCE

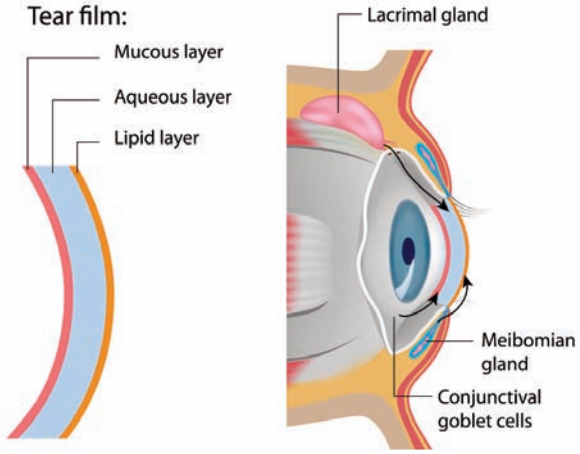
- *There is much ongoing research to assess if omega-3 LCPUFA intake can provide relief in different forms of dry eye disease.*
- *Three recent studies indicate that omega-3 LCPUFA supplementation can improve dry eye symptoms alone or in combination with other means.*
- *The relief provided is indicated to be useful for people with dry eye disease in contact lens wearers and in people who are exposed to computer screens for many hours every day.*

Introduction

Dry eye, or keratoconjunctivitis sicca, is a quite common ailment affecting the cornea and conjunctiva of the eye. It is characterized by insufficient wetting and lubrication of the cornea and interior of the eyelid, made worse by accumulation of particulate material derived from sloughed cells and dirt. The definition of dry eye disease (or syndrome) is from multiple origins as distinct lipid and aqueous tear film layers covering the corneal and interior of the eyelid epithelial surfaces can become compromised. Aqueous deficient dry eye is caused by reduced lacrimal function, due to reduced production or alterations in tear fluid. Lipid-deficient dry eye disorder is caused by hyposecretion of meibum, a type of sebaceous lipid secretion that forms the lipidic film, due to loss of function and number of Meibomian glands. In addition, a reduced number or function of goblet cells can cause changes in tear fluid composition. Ocular surface inflammation is an integral part of dry eye disease, and is accompanied with damage to the corneal epithelium and conjunctiva. Dry eye syndrome may be underdiagnosed as dry eye symptoms are not always discerned properly by patients, while inflammatory changes and deteriorated tear film stability and composition can already be measured.

Dry eye disease is a multifactorial disorder or spectrum of disorders with compromised wetting of the cornea and interior parts of the eyelids. The prevalence can be high and is geographically variable. There is much ongoing research to assess if omega-3 LCPUFA can contribute to providing relief in different forms of dry eye disease.

The prevalence of dry eye disease worldwide is surprisingly high (estimates range from a few percent of the population to ~35%), but also varies substantially geographically. Dry eye disease is a multifactorial disorder that advances with age, and leads to a significantly reduced quality of life. A relationship between the dietary intake of omega-3 LCPUFA and dry eye incidence has been identified, and several intervention studies have indicated that dry eye disease can be alleviated by increased dietary or supplemental intake of EPA/DHA. Three new recent studies have focused on assessing the potential effectiveness and practical applications of omega-3 LCPUFA in alleviating symptoms and discomfort in people with dry eye. Of interest, the three studies approach these applications from different angles. The first study addresses combining several therapeutic approaches that together significantly reduce discomfort, a second study shows that omega-3 LCPUFA supplementation improves comfort in wearing contact lenses in people with dry eye, and the third study shows that computer vision syndrome-related dry eye can be alleviated taking omega-3 LCPUFA.



Symptom Relief in Lipid-Deficient Dry Eye Disease

In the first study, Korb and colleagues in Boston, MA, with support from Alcon Research, Fort Worth, TX, addressed

whether the combination of several physical and nutritional types of intervention that have hitherto been employed separately can provide better support to people with dry eye syndrome when used in combination. The study compared whether the combined use of lid wipes, eye drops, and an omega-3 supplement together is superior to the use of warm wet compresses alone. The study consisted of a single-center, open-label, investigator-masked, randomized study with adult patients that had diagnosed lipid-deficient dry eye disease. Twenty-six study subjects with less than seven functional Meibomian glands and with dry eye symptoms were randomized into two groups. The reference group applied warm compresses once-daily for eight minutes, during three months. The use of warm wet compresses is a common home-applied treatment that alleviates dry eye symptoms, but is variable in its effectiveness depending on a multitude of factors (duration, temperature, humidity, frequency). The treatment group self-applied eyelid hygiene with hypoallergenic lid cleansing wipes, followed by installation of a drop of lipid emulsion lubricant that was formulated to restore lipid, aqueous, and mucin components of the tear film, four times daily. These patients also took a daily supplement of two capsules containing 1 gram of omega-3 LCPUFA in the form of ethyl esters, comprising a daily dose of 528 mg EPA and 278 mg DHA.

The primary end-point was Meibomian gland functionality, which has previously been demonstrated to correlate with symptoms of lipid-deficient dry eye disease, and can be related to the number of functioning glands. Meibomian gland functionality was tested by applying a controlled force that mimics deliberate blinking of the eye. Ocular and dry eye symptoms were assessed as secondary end-points using two validated questionnaires. The OSDI (Ocular Surface Disease Index) questionnaire assessed symptoms, and the contexts in which these may be exacerbated, over the preceding week. The SPEED (Standard Patient Evaluation of Eye Dryness) questionnaire records the frequency and severity of symptoms over the previous three months in a context-independent fashion. Additionally, eyelid status was measured according to several parameters, *i)* the presence and degree of collarettes (aggregates of keratin and dead epidermal cells at the edge of the eyelids and eyelashes) and debris present on the epidermis and eyelashes, *ii)* the degree of itching and eye rubbing, and *iii)* the percentage of partial Meibomian glands assessed by meibography by a single observer.

This study shows that people with lipid-deficient dry eye could employ a combination of lid wipes, lipid emulsion eye drops, and an omega-3 supplement to relatively quickly improve their symptoms, itching, corneal desquamation, and regain functionality of meibomian glands that produce the lipid film that is compromised in these patients.

Results

Meibomian gland functionality significantly improved over the course of the combined treatment period, whereas no change was observed in those patients only using warm wet compresses. The number of functional Meibomian glands increased from approximately four functioning glands per eye at baseline, to approximately nine functioning glands after three months. After three months both the warm compress group and the combination treatment had improved SPEED scores, and OSDI scores progressively improved from month 1 onwards in both groups. However, no significant differences in these patient-reported improvements in symptoms were found between the two groups. With respect to itching eyes and eye rubbing, in both treatment groups marked improvements were reported during the course of the study. In the combination treatment group, after only one month a large proportion of patients were free of itching eyes, and eye rubbing improved progressively from one month onwards in both groups. The number of people that were free of collarettes and desquamated debris on eyelid epidermis and eyelashes increased progressively over the course of the treatment period, and much faster in the combined treatment group. Improved Meibomian gland functionality could not be assessed by meibography, which did not reveal significant changes in the number and morphology of Meibomian glands during the treatment period or between treatment groups.

In summary this study provides useful guidance to people with lipid-deficient dry eye to employ a combination of lid wipes, lipid emulsion eye drops, and an omega-3 supplement to relatively quickly improve their symptoms, itching, corneal desquamation, and regain functionality of Meibomian glands that produce the lipid film that is compromised in these patients. The study does not explicitly demonstrate or attribute any specific activities to the presence of the omega-3 supplement in this combination approach, but suggests that it may possibly play a role in improved Meibomian gland activity and reduced corneal epithelial cell damage.

A Controlled Trial on Dry Eye in Contact Lens Wearers

A second recent study reports on the potential use of omega-

3 LCPUFA supplementation for dry eye in people wearing contact lenses. The study was carried out by Bhargava and Kumar, from the Laser Eye Clinic, Noida, and the Department of Pathology, Santosh Medical College, Ghaziabad, India. The study involved 496 women, all contact lens wearers, which were allocated in a randomized fashion to two groups, a treatment group receiving an omega-3 LCPUFA supplement, and a control group receiving placebo (corn oil). The omega-3 group received two capsules of fish oil composed of 180 mg EPA and 120 mg DHA per 300 mg capsule twice daily, amounting to a daily dose of 720 mg EPA and 480 mg DHA. The study was carried out in a double blind fashion at multiple clinical centers for a period of six months. The study subjects were recruited from regional workplaces and medical schools from the Northern central part of India where baseline fish consumption is low, and people mostly tend to have a vegetarian diet.

The primary objective of the study was to determine a decrease in subjective symptoms of dry eye and lens wear discomfort at six months after intervention. Symptoms that were recorded included itching or burning, sandy or gritting sensation, redness, blurring of vision, ocular fatigue, and excessive blinking. A combined symptom score was calculated, reflecting mild, moderate, and severe dry eye. Lens wear comfort was assessed separately with patient-rated scoring on a 6-point scale ranging from no discomfort to severe discomfort. Secondary outcome measures were a change in tear production (measured with the Schirmer test), tear film stability (measured by tear film breakup time), and conjunctival epithelial cell morphology and goblet cell density were measured by conjunctival impression cytology of the inferior bulbar conjunctiva.

In Indian people wearing contact lenses and who had dry eye disease, marked improvements were measured, both in symptoms and in objective assessment of dry eye characteristics, after a six month supplementation period with daily EPA/DHA.

Results

The combined symptom score improved markedly from 7.9 at baseline to 3.3 after supplementation, reflecting a change of moderately severe (73%) and severe (5%) symptomatic persons, to no individuals with severe symptoms and 5.5% of individuals with moderately severe symptoms. After six months, a small improvement was also noted in the control group, but nearly 60% of individuals remained having moderately severe or severe symptoms. Statistically significant improvements were observed for all secondary outcomes in the contact lens wearers that received fish oil during six months, whereas no changes were noted in the control group. The number of goblet cells increased from 892 to 1051 cells/mm^2 conjunctival surface area. The subjective rating of contact lens comfort also improved markedly in the omega-3 group, but did not change in the control group.

Improving Dry Eye Symptoms in Computer Vision Syndrome

The third study is also from Bhargava and colleagues, and focuses on dry eye symptoms that result from prolonged exposure to visual display terminals, *i.e.* computer screens. A relatively new development over the past two decades is the increasing time that people all over the world spend watching displays, mostly by working at personal computers, but also during leisure time. Computer vision syndrome encompasses several negative health aspects of prolonged visual display exposure, and is a growing occupational health challenge. Long and frequent exposure to displays has been shown to reduce the rate of eye lid blinking, and changes blink amplitude and the so-called blink quality. This can lead to reduced tear film quality, and a high percentage of people experience symptoms that correspond to dry eye. In addition, people who spend much time at screens frequently develop a number of other problems related to eye strain and irritation, headaches and postural problems.

The study addressed whether omega-3 LCPUFA supplementation might have an effect on dry eye symptoms and objective measurements of tear film quality in computer vision syndrome. Thereto, 478 symptomatic computer users were recruited for a three month trial in which they were assigned at random to a placebo group and an omega-3 group. The study subjects had worked for at least a year with a computer for at least three hours a day. They were recruited from universities and technological institutes in the central-northern part of India. The average age was ~23 years. People in the omega-3 group received a daily dose of 720 mg EPA and 480 mg DHA (as fish oil taken as two capsules twice daily). The control group received encapsulated olive oil that was matched in appearance. Participants and investigators were masked to the intervention. Compliance was assessed from capsule counts at monthly eye center visits. The primary outcome was the symptomatic improvement in dry eye symptoms, and tear film quality and stability and conjunctiva impression cytology scoring were used as secondary outcomes.

Table 1: Improvements in dry eye symptoms, tear film status, and conjunctiva health after three months of fish oil supplementation in Indian young adult workers that are exposed to at least three hours of daily work at a computer screen.

Function	Control group	Omega-3 LCPUFA supplemented group
Dry eye symptoms (score value)	6.8 ± 2.2	3.9 ± 2.2 *
Schirmer score (mm)	20.5 ± 4.7	21.4 ± 4.0 *
Tear breakup time (seconds)	12 ± 2.2	15 ± 1.8 *
Goblet cell density (cells/mm2)	899 ± 375	1018 ± 281 *
Epithelial and goblet cell density (Nelson grade score value)	0.9 ± 0.9	0.5 ± 0.6 *

*Values are mean +/- S.D.; *P<0.01*

Results

Baseline characteristics between the two groups were comparable, although the placebo group had lower symptoms and conjunctival impression cytology scores. The three month supplementation with EPA/DHA resulted in marked changes between the two groups (**Table 1**). The symptom score was markedly lower in the omega-3 group. The Schirmer and tear breakup time scores were significantly higher in the omega-3 group. The number of goblet cells had significantly increased after 3 months of fish oil supplementation. A scoring of epithelial morphology and goblet cell number (Nelson grading) showed a significant improvement of conjunctival health.

The three new studies suggest that people with different origins and types of dry eye may find symptom relief and improved tear and lipid film formation from increasing their intake of EPA/DHA by oral supplementation.

Discussion

The three highlighted studies provide new indications that omega-3 supplementation may be of practical use in alleviating dry eye symptoms in contact lens wearers, and in people who need to spend many hours working at computer screens. Omega-3 LCPUFA may also be used to provide relief in combination with other approaches in common use by patients with lipid-deficient dry eye symptoms, such as cleansing of eyelids and the topical application of lipid emulsion eye drops. The studies reported here need replication to unequivocally demonstrate their more wide generalizability in different geographies, in particular in people that do not meet recommended intake levels of 250-500 mg EPA/DHA per day.

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SPEED (Standard Patient Evaluation of Eye Dryness) test for dry eye symptoms: http://optometrytimes.modernmedicine.com/optometrytimes/news/using-speed-questionnaire-identify-dry-eye?page=full FOL

CLINICAL CONDITIONS

Omega-3 LCPUFA Supplementation; Activating a Protective Cardiovascular Response in Patients with Advanced Chronic Kidney Disease

THIS ARTICLE AT A GLANCE

• *In this report the authors present the changes in plasma levels of EPA- and DHA-derived lipid mediators in response to an eight-week supplementation with omega-3 LCPUFA in patients with advanced chronic kidney disease.*

• *Importantly the study shows that even in an advanced disease state the body remains capable of mounting a protective tissue response with an increased level of resolvin D1.*

• *This is one of the first studies showing lipid mediator formation in response to omega-3 LCPUFA supplementation in a randomized placebo-controlled double-blinded trial of a patient group with a defined disease.*

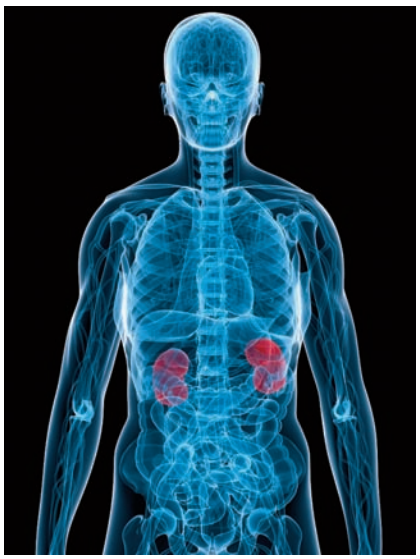
• *This study provides a new basis to start addressing improving the maladaptive cardiovascular changes and renal fibrotic state that characterizes advanced CKD.*

Renal failure is a final stage of chronic kidney disease (CKD), a disorder in which kidney structure and function progressively degenerate. Patients with advanced CKD depend on periodic hemodialysis or peritoneal fluid dialysis for survival. However, death due to end-stage renal disease is to a major extent the result of the downfall of the cardiovascular system. The development of maladaptive left ventricular hypertrophy, to overcome chronically increased vascular resistance, and arteriosclerosis both contribute to cardiac failure (heart failure). Mortality from cardiac failure in CKD is estimated to be up to 20 times higher than in the healthy population. Underlying the progressive renal tissue damage in CKD is a non-resolving inflammation of the kidneys accompanied by gradual tubulo-interstitial and glomerular fibrosis. The origins of sustained renal injury can lie with several factors such as diabetes, sustained elevated blood pressure, metabolic inflammation caused by poor dietary habits, infectious diseases, and immune-complex and auto-immune disorders, converging on perpetuating low-grade tissue inflammatory damage and progressive tissue fibrosis.

Patients with advanced stages of chronic kidney disease display high mortality rates due to heart failure. Beyond dialysis and kidney transplantation, few effective therapeutic options are available for these patients. Recent research interest is focusing on assessing if the body is still capable of mounting a protective tissue response that may help in potentially improving the function of the cardiovascular system.

Current treatment approaches in the early stages of CKD aim at halting further disease progression by reducing blood pressure, achieving lower blood sugar levels, and taking up a healthy lifestyle. However, effective treatments that reverse end-stage renal disease are not available. Theoretically, anti-inflammatory and anti-fibrotic approaches should be beneficial in halting the development of CKD and the secondary effects on the cardiovascular system. Anti-inflammatory activities of fish oil supplementation in CKD patients have recently been documented.

A clinical trial that was carried out several years ago in non-diabetic patients with advanced kidney damage (CKD stage 3-4) assessed the effect of omega-3 LCPUFA supplementation on cardiovascular physiology. That study, carried out by researchers from the School of Medicine and Pharmacology, at the University of Western Australia, in Perth, Australia, showed significant improvement in blood pressure (ca. 3 mmHg decrease), heart rate (ca. 4 beats per minute decrease), and serum triglycerides (ca. 40% decrease), independent from any effects on renal function. The results suggested that possibly end-stage renal disease patients may find benefit from omega-3 LCPUFA,



even if the underlying renal disease was not measurably improved.

The tissue-protective, anti-inflammatory and inflammation resolving actions of omega-3 LCPUFA are attributed in part to their conversion to SPMs, Specialized Pro-resolving lipid Mediators that are enzymatically oxygenated derivatives of EPA, DHA, and other LCPUFA. The SPMs include resolvins, protectins and maresins, and they act at picomolar/low nanomolar concentrations at G protein-coupled membrane receptors to reprogram immune cells and bring about tissue responses that decrease inflammation. The formation of SPMs in humans as a result of augmented omega-3 LCPUFA substrate provision and in relation to endogenous omega-3 LCPUFA tissue levels is an increasingly active field of research. The formation of EPA- and DHA-derived lipid mediators that regulate vascular tone, tissue perfusion, and cellular excitability, are increasingly appreciated. Of relevance to advanced CKD, recognized anti-fibrotic actions of resolvins are of particular interest.

Plasma Analysis Reveals Promising Changes

The analysis of plasma samples from the clinical trial in CKD patients mentioned above has now permitted a first evaluation of the formation of SPMs in response to omega-3 LCPFA supplementation in people with a well-characterized disease state. The study, published by Mas and colleagues of the same research group, tested the hypothesis that EPA- and DHA-derived SPMs might be detected in CKD patients after taking an omega-3 PUFA supplement. Eighty-five patients with moderately to severely impaired kidney function (stage III and IV; glomerular filtration rate between 15 and 60 ml/min x 1.73 m^2, and serum creatinine below 350 mM) were allocated in a double-blind randomized fashion to four groups. Patients with a diagnosis of cardiovascular disease were excluded. A control group received 4 g encapsulated olive oil daily, the omega-3 LCPUFA group received 4 g daily of n-3 PUFA ethyl ester oil (containing 460 mg EPA, 38 mg docosapentaenoic acid ω-3 (DPA), and 380 mg DHA per capsule; 4 capsules daily). A third group received coenzyme Q10 (CoQ), and a fourth group received the combination of omega-3 LCPUFA and CoQ. The duration of the intervention period was eight weeks, during which participants maintained their regular daily habits. The mean age of the volunteers was ~57 yrs (range 25-75 yrs) years. The groups were well matched with respect to age, body mass index, blood pressure, and a range of blood biochemical and renal variables and drug intake. The number of patients that completed the study in each group were 15, 20, 21 and 18 patients, respectively.

This double-blinded placebo-controlled and randomized trial addressed if patients with advanced chronic kidney disease responded to daily supplementation with a relatively high dose of omega-3 LCPUFA or coenzyme Q10 with the formation of SPMs (specialized proresolving mediators). This would indicate whether the body in this advanced diseased state would still be capable of mounting an endogenous tissue-protective, anti-inflammatory and anti-fibrotic response.

The changes in the tissue levels of fatty acids and blood levels of SPMs were measured before and after the intervention period. The level of individual fatty acids was measured in the phospholipid fraction of platelets isolated from plasma, by gas chromatography. SPMs were extracted from plasma and the following analyzed by liquid chromatography-tandem mass spectrometry; resolvin D1 (RvD1), RvD2, 17*R*-RvD1, 10*S*,17*S*-di-hydroxy-docosahexaenoic acid (10*S*,17*S*-diHDHA,) protectin D1, and the SPM precursors 17-hydroxy-DHA (17-HDHA) and 18-hydroxy-eicosapentaenoic acid (18-HEPE).

Results

Baseline levels of EPA, DHA, and arachidonic acid within platelet phospholipids were not different between the patients in the four groups. After eight weeks of supplementation, significant increases in the levels of EPA and DHA were found in the patients that had received the omega-3 LCPUFA supplement, and a decrease in arachidonic acid. There was no effect of CoQ on fatty acids within platelet phospholipids. Main effects analysis was used to determine the statistical significance, taking into account post-intervention values adjusted for baseline in individual patients. Alongside the increases in EPA and DHA, marked increases in the plasma levels of

The patients that received omega-3 LCPUFA during eight weeks had higher plasma concentrations of 18-HEPE, a derivative of EPA and precursor for E-series resolvins, and the derivatives of DHA, including 17-HDHA (a resolvin precursor) and resolvin D1.

18-HEPE and 17-HDHA, and small increases in RvD1 were found in patients that received the omega-3 LCPUFA supplement. No significant changes in plasma levels of 17*R*-RvD1 and RvD2 were noted. Protectin D1 and 10S,17S-diHDHA were undetectable in plasma. CoQ did not affect the SPM levels in plasma.

Discussion

Of interest, the study showed a small but significant increase in the level of RVD1, a change that is not observed in healthy volunteers supplemented with omega-3 LCPUFA. In healthy volunteers, RvE1 is the only resolvin (thus far) that increases in response to a few days of omega-3 LCPUFA supplementation. This suggests that in a condition of advanced CKD, activation of the biosynthetic pathway for at least RvD1, involving both 15-lipoxygenase and 5-lipoxygenase, is operative when sufficient DHA is available. The marked increase in 18-HEPE levels in plasma, suggest that RvE1 may be increased (note, E-series resolvins were not measured in this intervention trial in CKD patients). It is currently unclear in which tissue the RvD1 that is detectable in plasma is being formed. The results might also reflect a selective down-regulation of the metabolic inactivation of RvD1, effectively permitting higher concentrations to build up.

The hypothesis of this study was that the concentration of circulating SPMs may be responsive to supplementation with omega-3 LCPUFA, a first step in demonstrating that such a protective response could potentially have a role in mediating a cardiovascular benefit in CKD patients. Resolvins have potent anti-inflammatory actions that may contribute to reducing cardiovascular pathologies such as atherosclerosis and tissue remodeling. To date SPMs have not been clearly shown to date to have direct effects on cardiovascular physiological parameters such as regulating blood pressure, cardiac output, or vascular compliance, but they do have potent modulatory roles on platelet reactivity that are likely beneficial in pathobiology of cardiovascular system. It will be of interest to determine if additional lipid mediators such as the EPA- and DHA-derived epoxides, for which the understanding of their effects on cardiovascular physiology are more developed, are formed under the conditions studied here.

Progress

A recent study by the same research group has expanded the information on advanced CKD patients with the demonstration that a significant reduction in the plasma levels of the vasoconstrictor 20-hydroxy-eicosatetraenoic acid (20-HETE) is found in these same CKD patients supplemented with omega-3 PUFA. This effect was accompanied by a significant decrease in blood pressure. 20-HETE is a key mediator of autoregulation in the kidney that helps stabilize glomerular capillary pressure to maintain a constant kidney blood flow and hence constant glomerular filtration rate. Elevated 20-HETE in CKD may constitute an important driver of disturbed renal fluid homeostasis, and may contribute to the chronic injury and tissue remodeling of the kidney in CKD.

The results of this clinical study in CKD patents are important since this is the first demonstration in a well-designed clinical trial that omega-3 PUFA-derived anti-inflammatory mediators are formed as a result of omega-3 LCPUFA supplementation in patients with a defined disease state. Crucially, even in advanced CKD, the body apparently remains capable of activating a protective tissue response. Whether the observed SPM formation following omega-3 LCPUFA supplementation equals a response that offers sufficient protection to stall further deterioration of the kidneys, exerts a beneficial effect on the mal-adaptive cardiovascular functioning, or both, will undoubtedly be addressed in upcoming research.

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GUEST ARTICLE

Metabolic Effects of Long Chain n-3 Fatty Acids: Implications for Prevention of Diabetes

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Type 2 diabetes (T2D) is a worldwide increasing non-communicable disease characterized by the association with insulin-resistance and defects in insulin secretion. The main factors explaining this increasing prevalence beyond polygenic predisposition are obesity and sedentariness. The basic mechanisms sustaining insulin-resistance (IR) and defects in insulin-secretion are becoming better known. Insulin-resistance relates to the liver, muscle and adipose tissue (AT). Hepatic glucose production is excessive, due to increased gluconeogenesis, and uptake of glucose by muscle – that contributes the most to whole body glucose uptake – and to a lesser degree by AT, is severely impaired at stage of overt diabetes. In addition, the ability of insulin to inhibit lipolysis is also impaired, which contributes to the increased flux of non-esterified fatty acids (NEFAs). This increased flux associated to other factors such as: defect in fatty acid oxidation in muscle because of insufficient physical activity, lower mitochondrial oxidative capacity (in aged people), defect in fat storage into AT because of its defect of expandability (hypertrophy of adipocytes in obese people) lead *in fine* to ectopic fat storage into muscle, liver and ß cells (1). This ectopic fat storage associated with increased NEFAs flux leads to "lipotoxicity."

Lipotoxicity is a term that refers to the impairment of insulin-signaling by metabolites of fatty acids (e.g. ceramides, diacylglycerol ...). In ß cells (2), these metabolites induce apoptosis of ß cells leading to a progressive reduction of ß cell mass, oxidative stress and endoplasmic reticulum stress, which leads in turn to progressive impairment of insulin-secretion, which can no longer adapt to IR. Lipotoxicity has been advocated also as a possible mechanism leading to non-alcoholic steatohepatitis from liver steatosis in patients with T2D (3). The second major factor that contributes to IR is "glucotoxicity." Glucotoxicity is a term that refers to both the further impairment of glucose uptake through the decrease in GLUT4 transporters number in muscle and insulinaemic response to glucose by ß cells. It develops progressively when glycaemia increases. Inflammation also participates in IR (4). Besides IR and a defect in ß cell function, the other abnormalities that characterize T2D are a defect of incretin effect (GIP, GLP-1 secretions), increase in glucagon secretion and increased tubular glucose reabsorption.

The great interest in scientific literature towards the potentiality of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) to prevent T2D relates to the pioneering epidemiological study of Bang *et al.* in Eskimos from Greenland, relating high consumption of LC n-3 PUFA to the quasi absence of diabetes in this population (5) and to the experimental work of Storlien *et al.* showing that fish oil protected rats from high fat diet-induced insulin-resistance (6). Since then, a considerable number of studies in rodents and numerous epidemiological studies, randomized control trials (RCTs), meta-analyses and reviews have been carried out. Here, our objective is to summarize as best as possible the conclusions of these studies, to highlight some very recent ones and to try to draw a perspective. We will limit our review to prevention of T2D.

Summary of Animal Research

In animal models of dietary-induced IR (i.e. high fat, high fructose or high sucrose diets), it can be concluded that high amounts (non-extrapolatable to human diet or supplementation) of LC n-3 PUFA, mainly given as fish oils, are very efficacious to prevent IR. Although the overall basic mechanisms of this preventive effect remain incompletely understood, many mechanisms have been proven or advocated (reviews in 7-9).

In muscle, LC n-3 PUFA (20% FO substitution into a 60% safflower oil fat diet) completely prevent the decrease in phosphatidyl 3' kinase activity (a key enzyme of insulin

signaling) and the decrease of GLUT4 transporters abundance (10). This effect may be mediated by an alleviation of lipotoxicity inasmuch as many studies have shown that LC n-3 PUFA prevented the increase in triacylglycerol (TAG) in muscle (11). This effect of LC n-3 PUFA could be mediated through adiponectin, which is increased. Adiponectin increases fatty acid oxidation through the activation of AMPK, p38 MAPK and PPAR-α (12). In addition, adiponectin increases glucose transport in muscle and insulin sensitivity (12). It appears that LC n-3 PUFA do not act via PPAR-delta but additional studies are required.

Of importance, Kopecky's group has shown in mice that LC n-3 PUFA amplified the preventive and reversive effect of thiazolinediones towards insulin-resistance induced by a high fat diet (13-15), which suggests that PPAR-ɣ could be implicated in the effects of LC n-3 PUFA. Indeed, thiazolinediones are ligands of PPAR-ɣ as well as LC n-3 PUFA. In liver of rodents, LC n-3 PUFA prevent IR through many mechanisms such as activation of PPAR-α, which stimulates FA oxidation, suppression of the nuclear abundance of SREBP-1c, ChREBP, and MLX, which depresses *de novo* lipogenesis and stimulates FA oxidation (16,17), activation of AMPK and alleviation of inflammation and oxidative stress.

Human Trials Overview

In humans, works of Ebbesson's group in Eskimos from Alaska demonstrated that reintroduction of LC n-3 PUFA (traditional diet) in Westernized Eskimos drastically reduced components of metabolic syndrome and incidence of diabetes (18-20). We observed that 1.8 g/d EPA + DHA given as fish oil decreased the insulinaemic response to oral glucose in healthy subjects (21) and partially prevented the hyperinsulinaemic response during dexamethasone-induced IR in healthy subjects (22). These data strongly suggested the ability of LC n-3 PUFA to increase insulin sensitivity and to at least partially prevent IR induced by a glucocorticoid.

Many meta-analyses have been performed with contradictory conclusions (23-34). It appears that LC n-3 PUFA has a preventive effect towards T2D in Asian populations but may not in Western populations. The contradictory data about a preventive effect of LC n-3 PUFA is likely to be explained by many confounding factors: a) the duration and dose consumed; b) the type of n-3: fish oils, ethyl esters, phospholipids, oily fish; c) the concomitant amount of other fatty acids such as LC n-6 PUFA, which may counteract their effects; d) the counteracting effect of whole diet e.g. Western diet rich in saturated fat, n-6 PUFA, sugars; e) the background consumption of n-3 (from childhood or more recently; f) the genetic background of populations; g) the specific effects of EPA vs. DHA, which may differ or could be antagonistic towards some biochemical pathways.

Meta-analyses are of course useful but their conclusions should not be systematically taken as the truth, especially when their conclusions are different from one meta-analysis to one other. On illustration is the very recently published Finnish Diabetes Prevention Study carried out in 407 overweight patients with glucose intolerance (pre-diabetes) followed over 11 years, which concludes that serum LC n-3 PUFA concentrations at baseline predicted lower T2D incidence (– 28%) (35). The persistent controversy advocates for conducting other well-designed mechanistic studies in human models of reversible induced IR (for evident ethical reasons). The availability of very high concentrates (≥ 90%) of EPA and DHA would also permit the comparison of their potential benefit. The studies in animal models, although not always extrapolative to humans, continue to be required to better define the targets of the effects of LC n-3 PUFA and help to better focus the objectives of human trials. It would also be useful to better delineate in cross-sectional and intervention studies the phenotype of the subjects as well as the environmental factors such as composition of diet, physical activity, degree of overweight, etc.

It is also of interest to note that recommendations of daily amount of LC n-3 PUFA vary between countries and expert panels (see in: 36), which relates to the difficulty of drawing firm conclusions and proposing adequate amounts of LC n-3 PUFA in the specific field of T2D prevention.

In conclusion: animal models are very concordant in demonstrating that LC n-3 PUFA prevent diet-induced IR, although generally a high dose does not extrapolate to humans. They have permitted a better understanding of the basic mechanisms sustaining this preventive effect. Data in humans (mechanistic studies, interventional studies in Alaskan Eskimos, some longitudinal studies in Asian and in Finnish) are also in accordance with these results in animal models. Other studies are negative; one meta-analysis also found an increased risk in US people. LC n-3 PUFA must be considered only as an adjuvant dietary mean to prevent T2D; the best-established means is lifestyle intervention (physical activity plus maintenance of normal weight and prevention of excesses of Western diet).

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INVITED OPINION

A Balanced Approach to Omega-3 Benefits

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Similarities and differences between the omega-3 (n-3) and omega-6 (n-6) essential nutrients have moved in and out of discussions over the past 50 years as the biomedical community explored their diverse actions in human health. Evidence-based misunderstandings and apparent contradictions easily occur whenever a portion of the whole is discussed without a broader context to keep a balanced perspective (e.g., 1). Current paradoxes about benefits and harm from n-3 and n-6 nutrients prompt me to offer links to some recent open-access articles to give readers a convenient context that may help them review and resolve some apparent contradictions and lead to more constructive interpretations with positive outcomes for the use of omega-3 nutrients in human health.

1. Balancing just enough and not too much

The pharmaceutical industry has provided much evidence of harmful actions of n-6 arachidonate derivatives, which became profitable targets for drug development and marketing. Hundreds of national and international meetings have discussed ways to decrease unwanted signaling by many n-6 derivatives and receptors in the arachidonic cascade (2; Figs. 1 and 2). The large number of successful drugs leaves no doubt that human harm comes from excessive n-6 eicosanoid actions, and the two figures show much less intense actions occur with some n-3 compared to n-6 derivatives. Health conditions made worse by excessive n-6 mediator actions include heart attacks, atherosclerosis, thrombosis, stroke, immune-inflammatory disorders, asthma, arthritis, cancer proliferation, obesity, psychiatric disorders, depression, suicide, homicide, oppositional behavior, unproductive workplace behaviors and length of stay in hospitals (see also 2; Table 1).

In the course of developing drugs for treatments, dose-response studies showed the range of effective dose (therapeutic window) that had enough drug to decrease the "target process" without suppressing other desired n-6 functions. Aspirin-related non-steroidal anti-inflammatory drugs (NSAID) that inhibit cyclooxygenase-1 plus coxib drugs that inhibit cyclooxygenase-2 all give health benefits with a finite therapeutic window of efficacy (2; Fig. 1). Whenever a therapeutic window is narrow, the drug should be used at the lowest effective dose and reviewed regularly (3). This issue continues to disrupt NSAID and coxib marketing (4), and it has importance in section 3 below. While the pharmaceutical industry continues to focus on creating new patented materials that diminish n-6 arachidonate-based conditions, the extensive evidence they have provided about bioactive derivatives and receptors can be applied to designing nutrition strategies that decrease the need for such treatments.

2. Food controls the accumulated balance of n-3 and n-6 highly unsaturated fatty acid (HUFA)

A nutrition-based approach to diminish unwanted actions of n-6 derivatives is to decrease the availability of the n-6 precursor of the "arachidonic cascade" by displacing it competitively with similar naturally occurring n-3 precursors (5; Figs. 1, 2 and 3). This approach expands awareness beyond n-6 arachidonate and its products to include the competing n-3 20- and 22-carbon n-3 highly unsaturated fatty acids (HUFA) that share space with n-6 HUFA in tissue lipids and compete for release by the phospholipase, cPLA2. Both types of HUFA influence each other and are released during tissue responses.

HUFA balance is easily monitored from finger-tip blood-spot samples and described as proportions of competing n-3 and n-6 in the total HUFA as proposed at the 2004 ISSFAL Congress in Brighton, UK (6). Figure 3 in 5 shows that in apparently healthy people arachidonate (20:4n-6) can range from 30% to 70% of HUFA while the competing n-3 HUFA range from 65% to 15% of HUFA. People in Japan and Mediterranean countries have lower %n-6 in HUFA and lower risk for heart attack mortality than Americans and Northern Europeans (7; Fig. 1). The mortality rates for diverse groups closely associate (r^2=0.99) with the observed proportions of n-6 in HUFA (8), indicating that blood HUFA balance is a useful health risk assessment biomarker.

3. Choosing foods that give a healthy HUFA balance

HUFA balance is also a useful measure of average daily intakes of competing n-3 and n-6 nutrients in foods (9). An Omega 3-6 Balance Score uses data on 11 n-3 and n-6 acids in a food, expressing them as a single Score (10). People's observed HUFA balances from 81% to 30% n-6 in HUFA correspond to average daily food scores from –7 to +2. Estimates of the impact of food items on tissue HUFA balance are made easy by free "apps" that can be downloaded from a wellness website to personal mobile devices and computers. Omega Foods shows omega 3-6 balance scores for over

5,000 food items, and Omega Meals helps people plan daily menu plans that will give a desired HUFA balance. The apps are useful in personal decisions for menu planning, food shopping or discussing food choices with friends.

4. A narrow therapeutic window for dietary n-6 linoleate is widened by n-3 nutrients

We learned long ago that dietary n-6 linoleic acid is very effective in forming 20- and 22-carbon highly unsaturated fatty acids (HUFA) that accumulate in tissues and prevent deficient growth (11). In the absence of dietary n-3 nutrients, a dietary n-6 linoleic supply as low as 0.5% of food energy gives accumulated arachidonate more than 50% of HUFA (7). Knowing risk of heart attack is progressively greater for people with more than 50% n-6 in HUFA (8), we begin to see how benefits from eating omega-3 (n-3) nutrients come from widening the very narrow therapeutic window for dietary essential omega-6 (n-6) linoleic acid. Adding 2,000 mg of n-3 HUFA to typical daily USA intakes of n-3 and n-6 nutrients (which include 17,000 mg of n-6 linoleate; far above the lowest effective dose) can lower the HUFA balance from 80% to 45% n-6 in HUFA (12). Fortunately, supplemental intakes of the competing n-3 HUFA (EPA and DHA) at doses up to 5 g/day do not raise safety concerns (13).

Importantly, the n-3 linolenic nutrient competes effectively with n-6 linoleic with similar dynamics during accumulation in tissue HUFA (14). Those similar dynamics were not readily apparent in a large meta-analysis of diet-tissue studies, which all had much more competing n-6 than n-3 nutrients (15). Rather, the study emphasized the greater efficacy of n-3 HUFA over n-3 linolenate in balancing HUFA accumulated in tissues when n-6 linoleate is far above its lowest effective dose. If typical daily USA intakes of n-6 linoleate were lowered from 17,000 mg to 4,000 mg (1.6 % of food energy), it would allow as little as 400 mg added n-3 HUFA to lower the HUFA balance from 80% to 45% (12). Clearly, typical USA daily intakes of n-6 linoleate greatly exceed their lowest effective dose and need to be reviewed (7).

5. Viewing a biologically significant range of HUFA balance

Knowing of n-6-mediated pathophysiology and using biomarker values for %n-6 in HUFA, three apparent contradictions in the current biomedical literature were re-examined (5). For example, the lack of association of depression with %n-6 in HUFA in a large longitudinal study of American women (16) may appear to contradict the across-national association of depression with low intakes of n-3 HUFA (17). However, estimates of the likely HUFA balance in the cross-national groups had a wide range from 30% to 80% n-6 in HUFA, whereas it ranged from only 69% to 73% n-6 in HUFA for the quintiles in the longitudinal study. Such small differences are not likely biologically significant.

Similarly, a report of greater prostate cancer mortality with higher %n-3 in plasma in two clinical studies (18, 19) appeared to contradict the strong association of prostate cancer mortality rates with the % n-6 in HUFA for five different countries (20). Deaths from prostate cancer associated with estimates of likely HUFA balance ranging from 35% n-6 in HUFA for "traditional" Japanese and 50% n-6 in HUFA for "modern Japanese" compared to 60% n-6 in HUFA for Italy and about 70–80% n-6 in HUFA for UK and USA. In contrast, HUFA proportions in the clinical study were 77% n-6 in HUFA in controls compared to 76% in cases (18), and 72% n-6 in HUFA in controls compared to 71% in cases (19). Such a very narrow range of HUFA balance has little biological significance for interpreting omega-3 benefits.

Finally, a 2001 study reported no benefit of n-3 HUFA on the number of pain attacks. However, a 2013 report (21) used concepts mentioned in Section 2 to design biologically significant lowering of the competing n-6 nutrients to allow increased intake of omega-3 nutrients to lower the %n-6 in HUFA from 77% to 61% and greatly reduce pain and the need for vasoactive medications, acute opioids, and non-steroidal anti-inflammatory drugs. Clearly, investigators should be cautious about over-interpreting negative results when their study examines only a small range of HUFA balance. The HUFA balance for individuals worldwide can range from 20% to 85% n-6 in HUFA depending on voluntary food choices. Scientific studies should plan to examine a much broader set of possibilities than the 75-80% n-6 in HUFA common in the USA.

6. Overview:

A balanced view of benefits from omega-3 (n-3) nutrients is that dietary n-6 linoleic acid has a very narrow therapeutic window, which is widened by n-3 nutrients (7). Whenever a therapeutic window is narrow, the material should be ingested at the lowest effective dose. Eating fewer n-6 nutrients allows n-3 nutrients to be more effective in improving HUFA balance and preventing unwanted health conditions. A HUFA balance above 60% n-6 in HUFA is associated with higher risk for unwanted health conditions. Eating more n-3 nutrients keeps the HUFA balance low and broadens the window of safety for n-6 nutrients. A simple wellness program can use "apps" with over 5,000 different Omega 3–6 Balance Scores plus semi-annual monitoring of personal HUFA balance values from finger-tip blood-spot assays to effectively decrease health conditions made worse by HUFA balances above 50% n-6 in HUFA.

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