PUFA NEWSLETTER

Volume 21 • Issue 2

September 2016 Contents

Letters, editorial comments and technical comments should be sent to gerard@goedomega3.com

Subscribe to the PUFA Newsletter at www.fatsoflife.com

EDITORIAL

It is really real, isn't it?

"Nothing is less real than realism. Details are confusing. It is only by selection, by elimination, by emphasis, that we get at the real meaning of things."
Georgia O'Keefe

In this issue of the PUFA Newsletter we challenge you with new realities…or at least get closer to them. Fats of Life has long shied away from discussing cancer, but recent results from long-lasting cohort studies on colorectal cancer incidence and mortality are too impressive to ignore.

An indel that contributes to your capacity to convert essential fatty acids to long-chain polyunsaturated fatty acids? Check out what an indel is and why this is really worth knowing about.

We all know that the retina is one of the places that contains the highest levels of DHA in the body, but new research provides a surprise on how it may get there in the first place.

Depression research is receiving much attention in recent years and EPA concentrates may be beneficial in major depression. Still, it has been hard to understand why some studies do not find much effect of omega-3s on depressive symptoms – why?

EPA or DHA, we often name them together in one breath - is that acceptable? Having been addressed in the past, clinical researchers remain interested in their possible individual roles in preventing atherosclerotic lesion development and progression.

A tough reality is that we live in a world contaminated with persistent organic pollutants that only very slowly disappear. Exposure to PCBs is linked to an enhanced risk for developing myocardial infarction, but are we fortunate enough to offset some of the risk? Check out how PUFAs change our world, really.

Lastly, our guest author is Tom Brenna, offering a view on long-chain polyunsaturated fatty acids that should really be present in infant nutrition.

So embrace the new reality and appreciate the future of PUFAs in a variety of health-supporting areas.

Gerard Bannenberg
Editor, PUFA Newsletter
Fats of Life
gerard@goedomega3.com

Feel free to share this issue with your colleagues by forwarding them the Subscribe link at: http://www.fatsoflife.com/subscribe/

CARDIOVASCULAR HEALTH

EPA/DHA Intake Linked to Lower Risk for Myocardial Infarction Related to PCB Exposure

THIS ARTICLE AT A GLANCE

• This is the first prospective cohort study that determined if a relationship between PCB exposure and risk for myocardial infarction exists in men, and if such an association is sensitive to the dietary intake of EPA and DHA.

• The study reports that PCB exposure is linked to a higher risk for myocardial infarction in a large group of adult Swedish men followed for 12 years.

• Adult men with a higher EPA/DHA intake had a lower incidence of myocardial infarction associated with PCB exposure.

Polychlorinated biphenyls (PCBs) are a group of chemically stable and fat-soluble substances that share a biphenyl core substituted with a variable number of chlorine groups. PCBs were produced as mixtures of congeners (structural isomers) from the 1920s onwards by industrial chlorination of biphenyl, for use in paints, sealants, inks, as plasticizers, as coolants and lubricators in electrical equipment, and in a range of household and technical products. Following the recognition that PCBs were present in human tissues, and the noxious effects on human health and terrestrial ecotoxicology by environmental exposure to PCBs became increasingly clear, their industrial production was gradually halted from the end of the 1970s onwards, and banned internationally in 1991. Given their chemical stability and widespread use for a good part of the last century, PCBs still constitute ubiquitous environmental contaminants. Inadvertent formation still occurs today as by-products of paint and dye production. Airborne PCBs derived from materials used in older buildings constitute a current source of exposure.

Food intake is a major route of entry for persistent organic pollutants including PCBs, in particular foods of animal origin, given the bio-accumulating behavior of PCBs towards higher trophic levels. The consumption of fish contributes to a significant extent to the dietary burden of PCBs, in particular from fatty fish from contaminated waters. PCBs absorbed from food are metabolized at slow rates and accumulate in fatty tissues in humans. PCBs have very large volumes of distribution in humans, nearly completely dissolving in neutral lipids of the liver and in adipose tissue (which can constitute between 20 and 50% of the body weight of lean and obese persons, respectively). The metabolism and clearance of the many individual PCB congeners are incompletely understood. Even the hydroxylated and sulfated metabolites of those PCBs that are susceptible to metabolism (PCBs that are less chlorinated) are still of sufficiently high liposolubility that they will accumulate in tissues. PCBs can also appear in breast milk due to its elevated lipid content and thus can be transferred from mother to infant. Hair growth and loss may constitute an unusual route of elimination of chlorinated biphenyls. The fat solubility, chemical stability and low clearance of PCBs likely converts adipose fat into a depot tissue for maintaining very low but persistent levels in blood. Although the levels of PCBs in foods have declined in recent years, recommendations for limiting exposure to persistent environmental contaminants are challenging to make.

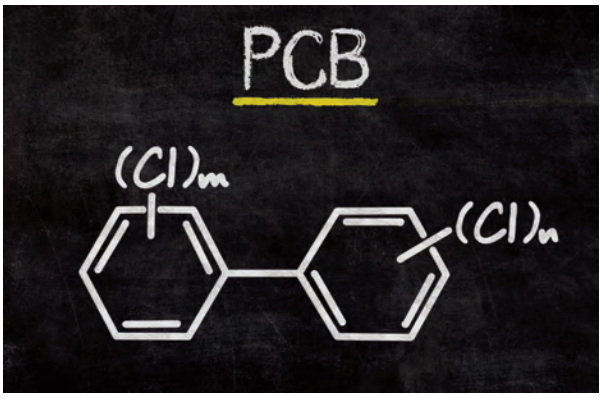
PCBs are now recognized and classified as human carcinogens. The development of specific tumor types has been associated with the occupational exposure to PCBs, but it is hard to assign the carcinogenic effects of PCBs to their individual congeners. PCBs exert developmental toxicity, induce marked changes in immune competence and act as endocrine disrupting substances that mimic and interfere with the activity of thyroid and sex steroid hormones. Exposure studies in animals and *in vitro* studies have indicated that PCBs have measurable effects on the endothelium (cells lining blood vessels) and may have long term chronic effects that predispose to atherosclerotic lesions. Epidemiological

evidence has suggested that PCB exposure is also associated with the development of atherosclerosis in humans.

The results of a recently published prospective cohort trial suggest that long-chain omega-3 polyunsaturated fatty acids (omega-3 LCPUFA) ingested from marine sources may exert a marked influence on the risk for developing coronary disease associated with PCB exposure from dietary sources. The study was performed by Bergkvist and colleagues at the Institute of Environmental Medicine, Karolinska Institute, Stockholm, and the Risk and Benefit Assessment Department, National Food Agency, Uppsala, Sweden. The principal aim was to examine the suspected association of risk for myocardial infarction in adult men that were free from cardiovascular disease at baseline (1997) with dietary intake of PCBs over a period of approximately 12 years. In addition, the effects of dietary intake of EPA/DHA and adiposity on the association between PCB exposure and myocardial infarction risk were assessed.

The exposure to PCBs through the diet is associated with an increased risk for myocardial infarction.

A total of 36,759 men were followed from 1998 up to the end of 2010 (censored observations), death, or the date of the first myocardial infarction. Dietary intake was assessed from a food-frequency questionnaire at baseline, which estimated the average food consumption over the past year. The dietary intake of PCBs, as well as of EPA/DHA, was calculated from a food items database, taking into account consumption frequency and age-specific portion sizes, and



adjusted for total energy intake. As a proxy for dietary intake of PCBs, the researchers limited the calculation to that of the PCB congener CB-153, which is considered a good indicator of total PCB level in foods, as well as in serum, and is the most abundant PCB congener present in food in Sweden. Myocardial infarction was determined from the Swedish National Hospital Discharge register and the Cause of Death register, which have registered health indices for the past 100 years.

The age range of the men at baseline was 45-79 years. The mean exposure to PCBs was calculated as 265 nanograms (ng) per day. The mean intake of EPA plus DHA was 460 milligrams (mg) per day, which is relatively high. The association between PCB exposure and primary outcome over the 12-year follow-up was evaluated after stratification of study participants by quintiles of PCB exposure (CB-153; lowest 113 ng/d, highest 436 ng/d). The intake of high fat fish, medium fat fish and low fat fish, and the average EPA/DHA intake, all increased as PCB exposure per quintile increased. There were no significant differences between the groups with respect to other factors such as family history of myocardial infarction, hypertension, aspirin use, physical activity, energy intake, or saturated fatty acid intake.

Proportional hazard analysis was used to assess the association between PCB exposure and myocardial infarction as the outcome variable, after adjustment for various covariates. The relative risk (hazard ratio) of suffering a myocardial infarction was not significantly different between the groups when adjusted for age only. However there was a trend for increased risk of myocardial infarction with increasing daily PCB exposure after adjustment for a number of potential factors that may affect risk for myocardial infarction (P=0.024). Further adjustment for EPA/DHA intake made a significant impact on the association between PCB exposure level and myocardial infarction risk, and showed that men at the highest level of PCB exposure had a 74% increased risk compared to men in the lowest quintile. The corresponding results for quintiles of EPA/DHA intake showed a statistically significant association between omega-3 LCPUFA intake and a lower risk for myocardial infarction. After adjusting for PCB exposure, the group with the highest EPA/DHA intake had a relative risk of 0.67 compared to the lowest. The relationship between PCB exposure and risk for myocardial infarction was not linear. In con-

This is the first prospective study that determined the association between estimates of dietary exposure to PCBs and myocardial infarction in men, and assessed if this relationship is sensitive to dietary EPA and DHA intake.

trast, there was no evidence that the association between lower risk for myocardial infarction with higher EPA/DHA intake was non-linear (P= 0.45; *i.e.* it is possible that this association may be linear).

A good correlation between dietary intake of EPA/DHA and PCBs was found (r=0.92). In order to better understand the relationship between EPA/DHA and PCBs, the relative risks for myocardial infarction were evaluated after stratification of EPA/DHA intake as low or high and PCB intake in tertiles. After adjustment for all covariates except for EPA/DHA, it was found that at low omega-3 LCPUFA intake levels, the highest intake level of PCBs was associated with a doubling of the relative risk for myocardial infarction (relative risk of 2.05). However, at the high EPA/DHA intake level, the risk of myocardial infarction was only 1.14 when comparing the highest PCB tertile with the lowest. In additional analyses, the association between PCB level and higher risk for myocardial infarction was only observed in men with low adiposity (possibly due to less PCBs being dissolved in fat tissue, resulting in higher chronic levels in the circulation associated with a predisposing effect on developing atherosclerotic lesions).

This study is of interest because it suggests that an inverse and protective activity may be associated with omega-3 LCUFA intake obtained from the same dietary source in which environmental contaminants such as PCBs are ingested. Limitations of the study include the challenge to accurately determine food intake from a food frequency questionnaire, and possible exposure misclassification. The validation study for PCB exposure was originally performed in women. Also, the collinearity between omega-3 LCPUFA intake and PCB exposure may introduce errors in the calculation of observed risks (as the covariates in Cox-regression modeling have a multiplicative (proportional) effect on the probability of the outcome variable). Positive aspects of the study are the nearly complete follow-up for a long time, and complete reporting of myocardial infarction.
The results of this study show an association, and thus its generalizability needs to be confirmed in other populations. The mechanisms whereby omega-3 LCPUFA may mediate a potential protective effect on the development of atherosclerosis or myocardial infarction in this setting are unknown. The good correlation between omega-3 LCPUFA and PCBs may suggest that any protective effects associated with the dietary intake of fish are associated with the lipid component of fish. Assignment of associations to any specific PCB congeners or their biologically active metabolites cannot be made at this point and other persistent environmental pollutants with suspected effects on cardiovascular disease may contribute to the observed risk. Given their persistent presence in our environment and bodies, a better understanding of the effects of environmental contaminants on human health, and of the factors that modulate their chronic toxicity, requires further studies.

In adult Swedish men, the intake of EPA/DHA had a marked effect on the relative risk for myocardial infarction associated with PCB exposure. The highest estimated EPA/DHA intake level was associated with a 33% lower risk compared to the lowest levels of intake.

Bergkvist C, Berglund M, Glynn A, Julin B, Wolk A, Åkesson A. Dietary exposure to polychlorinated biphenyls and risk of myocardial infarction in men - A population-based prospective cohort study. *Environ. Int.* 2016;88:9-14. [PubMed]

Worth Noting

Agency for Toxic Substances & Disease Registry - PCBs: http://www.atsdr.cdc.gov/PHS/PHS.asp?id=139&tid=26

Ax E, Lampa E, Lind L, Salihovic S, van Bavel B, Cederholm T, Sjögren P, Lind PM. Circulating levels of environmental contaminants are associated with dietary patterns in older adults. *Environ. Int.* 2015;75:93-102. [PubMed]

Crinnion WJ. Polychlorinated biphenyls: persistent pollutants with immunological, neurological, and endocrinological consequences. *Altern. Med. Rev.* 2011;16(1):5-13. [PubMed]

Grimm FA, Hu D, Kania-Korwel I, Lehmler HJ, Ludewig G, Hornbuckle KC, Duffel MW, Bergman Å, Robertson LW. Metabolism and metabolites of polychlorinated biphenyls. *Crit. Rev. Toxicol.* 2015;45(3):245-272. [PubMed]

Lauby-Secretan B, Loomis D, Baan R, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Grosse Y, Straif K. Use of mechanistic data in the IARC evaluations of the carcinogenicity of polychlorinated biphenyls and related

compounds. *Environ. Sci. Pollut. Res. Int.* 2016;3:2220-2229. [PubMed]

Lind PM, van Bavel B, Salihovic S, Lind L. Circulating levels of persistent organic pollutants (POPs) and carotid atherosclerosis in the elderly. *Environ. Health Perspect.* 2012;120(1):38-43. [PubMed]

McKinney JD, Waller CL. Polychlorinated biphenyls as hormonally active structural analogues. *Environ. Health Perspect.* 1994;102(3):290-297. [PubMed]

Nøst TH, Breivik K, Wania F, Rylander C, Odland JØ, Sandanger TM. Estimating time-varying PCB exposures using person-specific predictions to supplement measured values: A comparison of observed and predicted values in two cohorts of Norwegian women. *Environ. Health Perspect.* 2016;124(3):299-305. [PubMed]

Polychlorinated biphenyls: https://www3.epa.gov/region9/pcbs/faq.html

Ruder AM, Hein MJ, Hopf NB, Waters MA. Mortality among 24,865 workers exposed to polychlorinated biphenyls (PCBs) in three electrical capacitor manufacturing plants: a ten-year update. *Int. J. Hyg. Environ. Health.* 2014;217(2-3):176-187. [PubMed]

Schardein JL, Keller KA. Potential human developmental toxicants and the role of animal testing in their identification and characterization. *Crit. Rev. Toxicol.* 1989;19(3):251-339. [PubMed] FOL

Gaining Insight into the Relative Contributions of EPA and DHA to Lowering the Risk for Atherothrombotic Disease Incidence

THIS ARTICLE AT A GLANCE

- *Two recent studies have assessed whether either EPA or DHA may be more closely associated to markers of risk for the development and incidence of atherosclerotic disease.*
- *In elderly Japanese patients at high risk for acute atherothrombotic complications, the serum EPA level was associated with a lower incidence of plaque destabilization and infarction.*
- *In obese adults 18-70 years old with sub-clinical systemic inflammation, the daily intake of DHA was related to a more marked anti-inflammatory blood profile than EPA intake.*
- *The studies provide new indications that DHA and EPA may serve different roles to regulate distinct aspects in the temporal development of atherogenesis and acute atherothrombotic coronary disease. Further studies are required to replicate and refine these observations.*

When the supplemental dietary intake of omega-3 long-chain polyunsaturated fatty acids (LCPUFA) is discussed, a recommendation for the intake of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in combination is frequently considered the most prudent. In foods containing omega-3 LCPUFA, mostly of marine origin, both EPA and DHA are present. In many tissues both fatty acids are often present in considerable levels. EPA and DHA are furthermore interrelated metabolically; EPA can be further converted to DHA, although this is generally believed to occur to a very limited extent in humans, and DHA may regulate the levels of EPA by limiting the conversion of EPA to docosapentaenoic acid (DPA ω-3), and/or stimulating the retroconversion of DPA ω-3 back to EPA. On the other hand, EPA and DHA may have distinct functional effects, for example in their ability to modulate triglyceride synthesis and lipoprotein levels in the circulation. The following questions are often raised: "Why does the body employ both EPA and DHA for structural and signaling functions, do they have clearly distinct functions, is one more important than the other, and if so, in which cell types and when?"

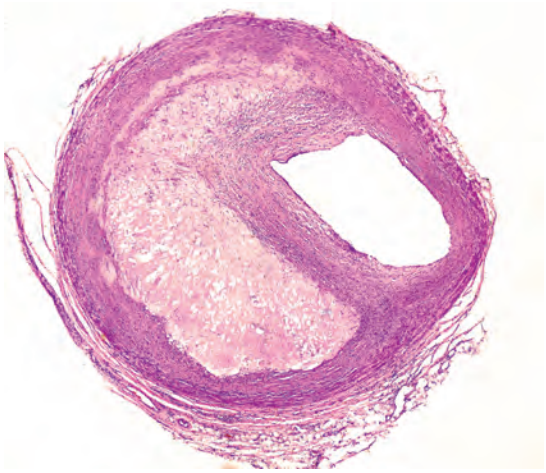
A number of intervention studies with purified EPA and DHA has previously provided evidence that both fatty acids impart selective activity on different cardiovascular functions, and which may have different effects on atherosclerosis disease development. Some of the individual hemodynamic and anti-atherogenic effects that have been reported are: *i)* both EPA and DHA supplementation reduce triglyceride levels, but they have different effects on lipoprotein subclasses, with DHA having more marked effects on increasing HDL_2 cholesterol, and increasing LDL particle size, while EPA can lower HDL_3 cholesterol (in mildly dyslipidemic individuals, and in type 2 diabetic individuals). *ii)* DHA has a stronger blood pressure-lowering effect than EPA, *iii)* DHA improves forearm microcirculatory vascular reactivity, *iv)* DHA can lower heart rate, *v)* EPA can lower platelet activation and increase platelet counts, and *vi)* EPA has a stronger tendency to increase fasting glucose in individuals with mild dyslipidemia. In type 2 diabetic individuals, EPA can increase hepatic glucose output, which may contribute to an impairment of glycemic control. Recently published research shows that questions about the individual roles of EPA and DHA remain of interest to clinical researchers studying heart disease.

The first study was carried out by Iwamatsu and colleagues at the Department of Cardiovascular Medicine at Dokkyo Medical University School in Togichi, Japan. The researchers addressed the hypothesis that in patients with advanced stages of atherosclerotic coronary disease, EPA and DHA might play a role in decreasing the risk for acute myocardial infarction. In this setting, EPA and DHA are thought to counteract the pro-coagulation and pro-inflammatory role of arachidonic acid (AA) in plaque destabilization, plaque rupture and atherothrombosis. It is not clear, however, if EPA or DHA individually are involved in reducing major coronary events in advanced atherosclerotic disease, or if both fatty acids play a role. In order to gain further insight into the relative involvement of EPA and DHA, a study was carried out to identify which circulating biomarkers of inflammation and blood lipids are associated most prominently with acute coronary syndrome,

It is currently not clear if the dietary intake of, or supplementation with, either EPA or DHA would be warranted in specific conditions of the cardiovascular system, or particular episodes of life.

i.e. the incidence of myocardial infarction and unstable angina, in elderly Japanese patients.

The study focused on a group of 369 individuals (average age 66 years) with suspected or confirmed coronary artery disease and who did not take omega-3 supplements. After diagnostic coronary angiography, patients were classified as



having acute coronary syndrome (n=24; patients that had had a ST-segment elevation myocardial infarction (STEMI), a non-STEMI, or having unstable angina), coronary spastic angina, or diagnosed with chest pain syndrome. To distinguish the latter two conditions, patients underwent an acetylcholine provocation test to determine whether the coronary vascular bed responded with a vasoconstrictor response (spastic angina), or was capable of responding with an endothelium-dependent vasodilator response (and subsequently classified as having chest pain syndrome in the absence of further diagnostic information). Next, the concentrations of several clinical diagnostic indicators were measured in venous blood samples obtained prior to the angiographic evaluation. These also comprised serum fatty acid levels (free and esterified combined), including EPA, DHA and AA, from which the ratios of EPA/AA and DHA/AA were calculated. A majority of patients were reported to have hypertension (74%), diabetes (55%) and dyslipidemia (67%), conditions that were controlled by statin treatment (82%) to lower LDL-cholesterol, and by angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (67%) to achieve a lower blood pressure.

The EPA/AA ratio (0.35) was significantly lower in patients with acute coronary syndrome than in patients with non-acute or more stable forms of coronary artery disease (stable angina pectoris, old myocardial infarction, chest pain syndrome, coronary spastic angina). A lower DHA/AA ratio was found for the acute coronary syndrome patients (0.78) only when compared to patients with stable angina (0.95). Patients with acute coronary syndrome furthermore had reduced HDL-cholesterol levels compared to the other patient groups combined. Among the various measured parameters, only the EPA/AA ratio was significantly correlated with acute coronary syndrome. The DHA/AA ratio did not discriminate acute coronary syndrome from other heart disease entities.

The results of the study suggest that in elderly Japanese, the serum EPA to AA level ratio may be predictive of the acute manifestations of coronary atherosclerotic disease in comparison to more stable forms of coronary disease and chest pain. In contrast, at this stage of heart disease development in this population, no relationship between acute coronary syndrome and the DHA/AA ratio was found. A limitation of the study is that although individuals who took omega-3 supplements were excluded from the study, no information was provided about fish consumption of the participants. It was not stated whether the patients were fasted prior to blood sampling. A further limitation is the use of ratios of EPA/AA and DHA/AA, neither of which provides information about whether it is the EPA or DHA, or in fact AA, that are altered.

A lower ratio of EPA to AA in serum was associated with a higher incidence of acute coronary syndrome in elderly Japanese with suspected or confirmed coronary artery disease. The DHA to AA ratio did not appear to display a relationship in this respect.

The second study was performed by Allaire and colleagues at the Institute of Nutrition and Functional Foods at the University Hospital Center of Québec Research Center, at the Department of Preventive and Social medicine, Laval University and at the Québec Heart and Lung Institute, in Canada. In a double-blind, randomized and placebo-controlled study with a cross-over format, a group of adult men and women (n=154, age 18-70 years) with abdominal obesity and sub-clinical inflammation were randomly assigned to one of three 10-week dietary interventions; *i)* 2.7 g/day EPA, *ii)* 2.7 g/day DHA or *iii)* corn oil (control). Obesity was defined by the International Diabetes Federation, and sub-clinical inflammation by a plasma C-reactive protein (CRP) concentration between 1 and 10 mg/l. The target study group corresponds to a sample of the population with a significantly enhanced risk of developing atherosclerotic lesions over the course of their adult life, with a significantly increased risk of stroke and acute coronary infarc-

tion. The subjects enrolled in this study were otherwise healthy.

The study design allowed to obtain information on the possible effects of either supplemental EPA or DHA, in contrast to many previous studies addressing the effects of mixtures of EPA and DHA in various ratios (often in the form of fish oil) on systemic markers of inflammation and cardiometabolic risk. The objective was to compare the effect of EPA, DHA or corn oil on circulating markers of inflammation as primary outcomes. The majority of previous studies that had addressed the effects of PUFA on cardiometabolic health documented changes in markers of inflammation as secondary outcomes. Furthermore, this study was relatively large, increasing the statistical power to detect potential changes that previous studies had not convincingly been able to document. In this study a decrease of 10% in CRP levels was considered of clinical significance.

A total of 154 participants was assigned randomly to one of three treatment phases, EPA, DHA, or corn oil, given daily for ten weeks, each with a nine-week washout period between treatments (no supplements taken). Compliance to supplementation was >96%, as determined by calculating the supplements that were returned to study coordinators at each study visit. Measurement of plasma phospholipid fatty acid composition was also used to monitor compliance, in a randomly selected subsample of participants. Twenty percent of participants dropped out during the study. The results of 123 participants who completed the intervention with DHA, and 121 participants receiving EPA, were compared to placebo.

Supplementation with DHA (2.7 g/d for 10 weeks) in adults at risk for the development of atherosclerosis had a more marked anti-inflammatory and a different modulatory effect on blood lipids compared to an equal dose of EPA.

The analysis of the results provided remarkable insight into the differences between DHA versus EPA supplementation on the studied parameters. Supplementation with EPA led to a reduction in triglycerides, a decrease in IL-6, and a very small increase in LDL cholesterol (compared to control). In contrast, the supplemental DHA intake favored changes in nearly all measured parameters compared to control: a decrease in CRP, IL-6, IL-18 and TNF-α, and an increase in plasma adiponectin. DHA increased total plasma cholesterol level (~3% increase), cholesterol associated with LDL and HDL, and decreased triglycerides. The levels of the LDL-associated apolipoprotein B remained unchanged by supplemental DHA and EPA intake, suggesting that the observed changes in LDL-associated cholesterol were related to lipoprotein size, and not to the number of circulating lipoprotein particles.

When the post-treatment effects of DHA (changes compared to control) were compared head-to-head with those after EPA intake, a significantly stronger influence of DHA was found on IL-18 (decrease), adiponectin (increase), cholesterol (increase in total cholesterol, LDL cholesterol, and HDL cholesterol), and on triglyceride levels (decrease). No evidence of an effect of sex on the studied inflammation markers was found. In men, but not in women, supplemental DHA intake promoted a significantly larger increase in LDL cholesterol level than did EPA intake.

This study suggests that during adult life the effect of dietary intake of DHA on systemic markers of inflammation may be more marked than that of an equal dose of EPA, as well as a more marked effect on blood lipids (cholesterol and triglycerides). The authors indicate that new studies are needed to evaluate how these omega-3 LCPUFA-selective activities, in particular those of DHA, may modulate cardiovascular disease development. Following current thinking that atherogenesis is to a significant extent the result of a poorly controlled and chronic inflammation of the vasculature, a sustained level of omega-3 LCPUFA intake may contribute to counteract pro-inflammatory signals associated with the development of coronary disease. However, not much information is currently available on whether both EPA and DHA are needed to modulate chronic inflammation underlying vascular dis-

ease, or if DHA may be more important than EPA to reduce atherogenesis, as suggested by the study of Allaire.

Importantly, the levels of two important mediators that were measured, IL-18 and adiponectin, displayed changes with DHA supplementation that corresponded to a shift to an anti-inflammatory profile. IL-18 is a pro-inflammatory cytokine that, among other activities, promotes myofibroblast proliferation, a hallmark of atherosclerotic lesion development. IL-18 is produced by the NLRP3 inflammasome, which is activated by diverse inflammatory stimuli. Adiponectin is an anti-inflammatory and atheroprotective adipokine that is produced by adipose tissue, for which dysregulated secretion into the circulation is often observed in obesity-associated diseases.

Interestingly, as suggested by the study of Iwamatsu and colleagues, once advanced coronary heart disease has developed and presents itself as an acute coronary syndrome, EPA may be more important than DHA to limit coronary plaque destabilization, erosion and rupture, thrombosis and/or the ensuing ischemia/reperfusion-mediated myocardial tissue damage. At least one mechanism whereby EPA acts to reduce coronary plaque instability is by its incorporation into plaque tissue and exerting a local anti-inflammatory effect, down-regulating the metalloproteases that weaken the plaque structure. The suggestion that EPA is selectively involved in these ultimate steps in acute coronary syndrome is for the moment based on an association, and its role will need be confirmed with a randomized and controlled study in patients with acute coronary syndrome.

The development of prescription omega-3 in Japan has historically focused on EPA and is based on early findings that the intake of highly purified EPA has anti-atherogenic and anti-thrombotic effects, thereby lending support to a role for EPA in lowering acute myocardial infarction risk. A later study also provided indications to the possible contribution by the dietary intake of both DHA and EPA to the lowering of atherosclerosis development in Japanese people. The JELIS study subsequently showed the efficacy of supplemental EPA intake in reducing the risk for major coronary events in hypercholesterolaemic patients, even on a background of significant fish consumption.

Indications for life- and health stage- dependent selective effects of EPA or DHA are suggested by recent studies that have addressed the modulation of risk factors for cardiovascular disease development and associations with acute manifestations of advanced disease. Future studies will provide further insight into the potential benefits of individual PUFA.

The current study by Iwamatsu and colleagues may thus been seen as a further effort to more precisely determine in which types of coronary heart disease EPA is likely to be most effective. It is not clear if the reduction in risk for major coronary events by EPA supplementation may be more pronounced in the Japanese population. A previous study has indicated that on a background of statin treatment, EPA will further decrease the incidence of major coronary events in people with established coronary artery disease, in particular those with a prior history of myocardial infarction. Higher EPA plasma levels, as well as the ratio of EPA to AA, were inversely related to risk of major coronary events, but DHA levels in the circulation were not. In contrast, a recent meta-analysis of pooled prospective and retrospective cohort studies found that both DHA and EPA levels (as well as alpha-linolenic acid and DPA) were similarly associated with a modestly lower risk of fatal coronary heart disease.

Both studies need independent replication to confirm the interesting and possibly important results. Future studies will undoubtedly provide further insight into the individual contributions of specific dietary fatty acids in primary prevention and in reducing clinical events in established cardiovascular disease. That selectivity in activity for individual omega-3 LCPUFAs may indeed exist is further supported by these studies. Supplementation with sufficiently long duration and a high enough daily dose of purified DHA leads to increases in both DHA and EPA (and a decrease in ω-3 DPA) in the circulation (as measured in phospholipids of plasma, platelet and red blood cells). In contrast, the supplementation with EPA markedly raises EPA and ω-3 DPA levels, but does not lead to a raise in DHA levels (in fact, DHA levels in plasma phospholipids may decrease). Such notable differences in fatty acid distribution point out the complexity in the mechanisms of regulation of the interconversion of omega-3 LCPUFA pathway intermediates, and remind us of the challenge to assign specific individual activities at sites of vascular inflammation where coronary vascular lesions develop and progress over long time periods. Well-founded recommendations for optimal intakes of individual fatty acids at precise stages of life and health status may still be many years away from now.

Iwamatsu K, Abe S, Nishida H, Kageyama M, Nasuno T, Sakuma M, Toyoda S, Inoue T. Which has the stronger im-

pact on coronary artery disease, eicosapentaenoic acid or docosahexaenoic acid? *Hypertens. Res.* 2016;39(4):272-275. [PubMed]

Allaire J, Couture P, Leclerc M, Charest A, Marin J, Lepine MC, Talbot D, Tchernof A, Lamarche B. Randomized, crossover, head-to-head comparison of EPA and DHA supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA Study. *Am. J. Clin. Nutr.* 2016; June 8. [PubMed]

Worth Noting

Berge RK, Madsen L, Vaagenes H, Tronstad KJ, Göttlicher M, Rustan AC. In contrast with docosahexaenoic acid, eicosapentaenoic acid and hypolipidaemic derivatives decrease hepatic synthesis and secretion of triacylglycerol by decreased diacylglycerol acyltransferase activity and stimulation of fatty acid oxidation. *Biochem. J.* 1999;343(1):191-197. [PubMed]

Cawood AL, Ding R, Napper FL, Young RH, Williams JA, Ward MJ, Gudmundsen O, Vige R, Payne SP, Ye S, Shearman CP, Gallagher PJ, Grimble RF, Calder PC. Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. *Atherosclerosis* 2010;212(1):252-259. [PubMed]

Coronary angiography: http://patient.info/health/coronary-angiography

Del Gobbo LC, Imamura F, Aslibekyan S, Marklund M, Virtanen JK, Wennberg M, Yakoob MY, Chiuve SE, Dela Cruz L, Frazier-Wood AC, et al & Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Fatty Acids and Outcomes Research Consortium (FORCe). ω-3 Polyunsaturated fatty acid biomarkers and coronary heart disease: Pooling project of 19 cohort studies. *JAMA Intern Med.* 2016;176(8):1155-1166. [PubMed]

Hino A, Adachi H, Toyomasu K, Yoshida N, Enomoto M, Hiratsuka A, Hirai Y, Satoh A, Imaizumi T. Very long chain N-3 fatty acids intake and carotid atherosclerosis: an epidemiological study evaluated by ultrasonography. *Atherosclerosis* 2004;176(1):145-149. [PubMed]

Itakura H, Yokoyama M, Matsuzaki M, Saito Y, Origasa H, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, Matsuzawa Y, JELIS Investigators. Relationships between plasma fatty acid composition and coronary artery disease. *J. Atheroscler. Thromb.* 2011;18(2):99-107. [PubMed]

Li K, Huang T, Zheng J, Wu K, Li D. Effect of marine-derived n-3 polyunsaturated fatty acids on C-reactive protein, interleukin 6 and tumor necrosis factor alpha: a meta-analysis. *PLoS One* 2014;9(2):e88103. [PubMed]

Maenhaut N, Van de Voorde J. Regulation of vascular tone by adipocytes. *BMC Med.* 2011 9:25. [PubMed]

Matsuzaki M, Yokoyama M, Saito Y, Origasa H, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, Matsuzawa Y, JELIS Investigators. Incremental effects of eicosapentaenoic acid on cardiovascular events in statin-treated patients with coronary artery disease. *Circ. J.* 2009;73(7):1283-1290. [PubMed]

Metabolic syndrome as defined by the International Diabetes Federation: http://www.idf.org/metabolic-syndrome

Mori TA, Woodman RJ. The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Curr. Opin. Clin. Nutr. Metab. Care* 2006;9:95-104. [PubMed]

Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? *J. Nutr.* 2012;142(3):614S-625S. [PubMed]

O'Brien LC, Mezzaroma E, Van Tassell BW, Marchetti C, Carbone S, Abbate A, Toldo S. Interleukin-18 as a therapeutic target in acute myocardial infarction and heart failure. *Mol. Med.* 2014;20:221-229. [PubMed]

Ohnishi H, Saito Y. Eicosapentaenoic acid (EPA) reduces cardiovascular events: relationship with the EPA/arachidonic acid ratio. *J. Atheroscler. Thromb.* 2013;20(12):861-877. [PubMed]

Ozaki E, Campbell M, Doyle SL. Targeting the NLRP3 inflammasome in chronic inflammatory diseases: current perspectives. *J. Inflamm. Res.* 2015;16(8):15-27. [PubMed]

Tamura Y, Hirai A, Terano T, Yoshida S, Takenaga M, Kitagawa H. Anti-thrombotic and anti-atherogenic action of eicosapentaenoic acid. *Jpn. Circ. J.* 1987;51(4):471-477. [PubMed]

Tanaka K, Ishikawa Y, Yokoyama M, Origasa H, Matsuzaki M, Saito Y, Matsuzawa Y, Sasaki J, Oikawa S, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K,Jelis Investigators J. Reduction in the recurrence of stroke by eicosapentaenoic acid for hypercholesterolemic patients: subanalysis of the JELIS trial. Stroke 2008;39(7):2052-2058. [PubMed]

Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, Oikawa S, Sasaki J, Hishida H, Itakura H, Kita T, Kitabatake A, Nakaya N, Sakata T, Shimada K, Shirato K, JELIS Investigators. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007;369(9567):1090-1098. [PubMed] FOL

COGNITIVE HEALTH

Identifying People with Depressive Symptoms that May Benefit Most from Omega-3 Fatty Acid Consumption

THIS ARTICLE AT A GLANCE

- *In an observational study of a cohort of Puerto Ricans, only those individuals with the highest level of urinary 8-hydroxy-2´-deoxyguanosine (8-OHdG), a marker of oxidative stress, showed an inverse relationship between the omega-3 index (combined EPA and DHA as percentage of red blood cell fatty acids) and depressive symptoms.*
- *The study suggests that it may be possible to better identify people who may benefit most from the antidepressant effect of increased omega-3 fatty acid consumption.*

Evidence is accumulating to support the use of EPA-rich omega-3 supplements to improve depressive symptoms, especially from studies with good diagnostics and controls.

Significant research activity in this area is noted, and the American Psychiatric Association recommends EPA-rich supplements in its practice guidelines as adjunctive therapy for major depressive disorder. Increased prevalence of depressive symptoms also characterizes particular stages of life, such as adolescence and during and after pregnancy. The appearance of depressive symptoms frequently accompanies specific and common health conditions, such as cardiovascular disease, or can appear as adverse effects to drug treatment, *e.g.* with interferon-gamma therapy. A number of observational and randomized controlled intervention studies have suggested that the dietary intake of omega-3 long-chain polyunsaturated fatty acids (omega-3 LCPUFA), as well as the content of these fatty acids in the circulation, is negatively related to the prevalence and severity of depressive symptoms. However, many studies have reported a lack of effect or lack of positive association, whether reporting on subclinical threshold mild depression or clinical depression. The precise reasons why the antidepressant effects of omega-3 LCPUFA are not always observed are unclear but more precise studies and intervention trials may be needed.

Depression has been associated with a disturbed balance of oxidation and reduction processes in the central nervous system. The so-called "oxidative stress" is characterized by a net increased production and/or reduced elimination of reduced oxygen species that are produced through mitochondrial respiration, by a number of oxygen-reducing enzymes, and by immune cells that become involved in chronic inflammatory processes. The assessment of the participation of specific PUFAs in the control of oxidative stress in the nervous system may provide additional insight into symptoms and levels of depression that may be associated with redox imbalances.

> *More precise stratification of patients with depressive symptoms may be needed to obtain a more refined insight when specific polyunsaturated fatty acids could play a beneficial role in preventing or treating depression.*

A recent observational study has assessed the relationship between the omega-3 index and the scale of depressive symptoms in a cohort of Puerto Rican men and women, a population with a known high prevalence of depressive symptoms. The study aimed to determine if the level of oxidative stress modifies the relationship between the omega-3 index and depressive symptoms. The study was performed by Bigornia and colleagues from the Clinical Laboratory and Nutritional Sciences, and College of Fine Arts, Humanities, and Social Sciences at the University of Massachusetts in

Lowell, MA, together with colleagues from the Department of Medicine at the Sanford School of Medicine, University of South Dakota, Sioux Falls, SD and the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA. The participants were a group of Puerto Rican men and women living in Boston, MA, USA, who participated in the Boston Puerto Rican Health Study, which is a longitudinal cohort study for which recruitment was initiated in 2004. The overall aim of the study is to better comprehend the effect of diet and other behavioral and environmental factors, genetics, and psychosocial stress on the high and increasing prevalence of cardiovascular disease risk factors in Puerto Rican adults.

At baseline and after two years, an assessment of depressive symptoms was carried out using the CES-D scale (Center for Epidemiologic Studies Depression Scale). The CES-D was designed to measure the current level of depressive symptomatology in the general population, with an emphasis on the affective component, depressed mood. It is a 20-item test that measures the frequency of symptoms of depression in nine different groups over the preceding week: sadness (dysphoria), loss of interest (anhedonia), appetite, sleep, thinking/concentration, guilt (worthlessness), tiredness (fatigue), movement (agitation), and suicidal ideation. A summed score indicates the level of depressive symptoms, with values of 16 and higher taken as a threshold for depression symptoms, and ranging up to a maximum score of 60 for a major depressive episode. The test is usually a self-administered questionnaire that can also be taken by telephone, and is one of the most widely employed tests in psychiatric epidemiology.

Blood samples were taken at baseline for the measurement of red blood cell membrane fatty acid content, from which the omega-3 index was calculated (percentage of EPA plus DHA as percentage of total fatty acid content). Oxidative stress was determined as 8-hydroxy-2′-deoxyguanosine (8-OHdG) in 12-hour urine samples and was measured using ELISA with correction for creatinine. 8-OHdG is an oxidatively modified nucleoside that is formed during the free-radical mediated oxidative reaction of the hydroxyl radical (a very reactive oxidizing form of oxygen) with DNA, and reflects single- and double-strand breaks in DNA.

This is the first study to suggest that people with higher levels of oxidative stress, as measured by the presence of a marker of oxidative degradation of polynucleotides that is excreted in urine, display a lower score for depressive symptoms as the omega-3 index increases.

Complete data sets for the current analysis were available for a total of 787 participants (aged 45-75 at baseline, 73% female). When baseline characteristics were assessed after dividing the study subjects into quartiles according to their omega-3 index, several parameters were found to significantly differ among the groups, including: age (increasing with each quartile from an average of 55 to 60 years), current smoking (lower in the highest two quartiles), healthy eating index (increasing percentage of subjects from lowest to highest quartile), and the intake of omega-3 supplements (higher in the quartile with highest omega-3 index).

No significant association could be identified between the omega-3 index and CES-D scores when the entire group of subjects was evaluated at baseline or at the 2-year follow up. However, there was evidence for effect modification of the relationship between the omega-3 index and the CES-D score at the 2-year time point by urinary 8-OHdG. No effect modification was observed for antidepressant use or *APOE* ε2 and ε4 genotypes. Interestingly, those with the highest levels of 8-OHdG in urine had a statistically significant negative relationship between the omega-3 index and the score for depressive symptoms at baseline (after adjustment for age, sex and antidepressant use). Such a relationship was not seen in people with urinary 8-OHdG levels falling in the lower three quartiles. Adjustment by a number of covariates, such as physical activity score, smoking, education, diabetes, cardiovascular disease or healthy eating index, did not modify the negative association between the omega-3 index and depressive symptoms. Estimates of the longitudinal relationship of the omega-3 index at baseline and the CES-D score at two years by multivariate linear regression analysis also suggested that people with the highest 8-OHdG levels had reduced levels of

depression with higher intake of omega-3 LCPUFA.

Urinary 8-OHdG level were positively correlated with the CES-D scores, at baseline and after two years. Urinary 8-OHdG levels were negatively related to the physical activity score and greater antidepressant use. The urinary 8-OHdG level itself was not related to the omega-3 index, or with the individual omega-3 fatty acids, EPA or DHA. Interestingly, when the assessment of the relationship between depressive symptoms and the omega-3 index was restricted to depressive symptoms that fell above the minimum threshold considered in this study for clinical relevance, *i.e.* with a CES-D score ≥16, reduced odds for depressive symptoms were found only in the tertile of individuals with highest urinary 8-OHdG levels (17-32% lower odds ratios of CES-D score ≥16). There was no evidence for a relationship between a higher omega-3 index and a lower prevalence of depressive symptoms in individuals in the tertile with lower 8-OHdG excretion. It should be pointed out that the CES-D scale should not be used for clinical diagnostic purposes, and high false-negative rates have been reported at the cut-off score of ≥16.

This observational study provides insight into new parameters that had not been specifically assessed previously and that associate with effects that are relevant in the general population. Observational studies cannot be used to attribute causal relationships, and although 8-OHdG in urine is a useful marker of systemic oxidative stress, a conclusion that oxidative stress in neuronal tissue underlies depressive symptoms cannot be deduced from this study. The value lies in the identification of a specific sub-group of individuals with depressive symptoms, that display an association between depressive symptoms and their omega-3 index, and in which higher 8-OHdG levels are found in urine.

The study suggests that in Puerto Rican adults living in the US, only those individuals with a higher measurable level of oxidative stress have more depressive symptoms if they have a lower omega-3 index. Future intervention studies will be needed to confirm this observation.

A lack of a relationship between depressive symptoms (CES-D scores) and urinary 8-OHdG in otherwise healthy adults has previously been reported. Based on the current findings, this could have been partly explained by the fact that the level of 8-OHdG excretion may not have been sufficiently high to show a relationship between omega-3 index and depressive symptoms. Additional studies that stratify subjects based on urinary 8-OHdG will be of interest to ascertain that individuals suffering with more intense levels of oxidative stress may benefit most from omega-3 LCPUFA intake.

Bigornia SJ, Harris WS, Falcón LM, Ordovás JM, Lai CQ, Tucker KL. The omega-3 index is inversely associated with depressive symptoms among individuals with elevated oxidative stress biomarkers. *J. Nutr.* 2016;146(4):758-766. [PubMed]

Worth Noting

Appleton KM, Sallis HM, Perry R, Ness AR, Churchill R. ω-3 Fatty acids for major depressive disorder in adults: an abridged Cochrane review. *BMJ Open* 2016;6(3):e010172. [PubMed]

Beydoun MA, Fanelli Kuczmarski MT, Beydoun HA, Hibbeln JR, Evans MK, Zonderman AB. Omega-3 fatty acid intakes are inversely related to elevated depressive symptoms among United States women. *J. Nutr.* 2013;143(11):1743-1752. [PubMed]

Boston Puerto Rican Health Study: http://cancercontrol.cancer.gov/populationhealthcenters/cphhd/centers-nu.html

Depressive Disorders, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition: http://dsm.psychiatryonline.org/doi/full/10.1176/appi.books.9780890425596.dsm04

Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, Lamas S. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol.* 2015;6:183-197. [PubMed]

Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. *Dyn. Med.* 2009;8:1-25. [PubMed]

Gelenberg AJ, Freeman MP, Markowitz JC, Rosenbaum JF, Thase ME, Trivedi MH, *et al*. Practice Guideline for the Treatment of Patients with Major Depressive Disorder. 2010; Third Edition: pp 51/92. [link]

Hallahan B, Ryan T, Hibbeln JR, Murray IT, Glynn S, Ramsden CE, SanGiovanni JP, Davis JM. Efficacy of

omega-3 highly unsaturated fatty acids in the treatment of depression. *Br. J. Psychiatry* 2016;209(3):192-201. [PubMed]

Lucas M, Asselin G, Merette C, Poulin MJ, Dodin S. Ethyl-eicosapentaenoic acid for the treatment of psychological distress and depressive symptoms in middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. *Am. J. Clin. Nutr.* 2009;89(2):641-651. [PubMed]

Messamore E, McNamara RK. Detection and treatment of omega-3 fatty acid deficiency in psychiatric practice: Rationale and implementation. *Lipids Health Dis.* 2016;15:25. [PubMed]

Mocking RJ, Harmsen I, Assies J, Koeter MW, Ruhé HG, Schene AH. Meta-analysis and meta-regression of omega-3 polyunsaturated fatty acid supplementation for major depressive disorder. *Transl. Psychiatry*. 2016; Mar 15. [PubMed]

Palta P, Samuel LJ, Miller ERr, Szanton SL. Depression and oxidative stress: results from a meta-analysis of observational studies. *Psychosom. Med.* 2014;76(1):12-19. [PubMed]

Radloff LS. The CES-D Scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement.* 1977;1:385-401. [PubMed]

Sontrop J, Avison WR, Evers SE, Speechley KN, Campbell MK. Depressive symptoms during pregnancy in relation to fish consumption and intake of n-3 polyunsaturated fatty acids. *Paediatr. Perinat. Epidemiol.* 2008;22(4):389-399. [PubMed]

Sublette ME, Ellis SP, Geant AL, Mann JJ. Meta-analysis of the effects of eicosapentaenoic acid (EPA) in clinical trials in depression. *J. Clin. Psychiatry* 2011;72(12):1577-1584. [PubMed]

Toyokuni S, Sagripanti JL. Association between 8-hydroxy-2'-deoxyguanosine formation and DNA strand breaks mediated by copper and iron. *Free Radic. Biol. Med.* 1996;20(6):859–864. [PubMed]

Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *J. Environ. Sci. Health C. Environ. Carcinog. Ecotoxicol. Rev.* 2009;27(2):120-139. [PubMed]

Yi S, Nanri A, Matsushita Y, Kasai H, Kawai K, Mizoue T. Depressive symptoms and oxidative DNA damage in Japanese municipal employees. *Psychiatry Res.* 2012;200(2-3):318-322. [PubMed] FOL

IMMUNE FUNCTION

The Impact of Omega-3 LCPUFA on Colorectal Cancer Development Guides Clinical Research towards Considering Immune Modulation and Regulation of Genomic Stability

THIS ARTICLE AT A GLANCE

• Large prospective cohort studies tracked colorectal cancer incidence and assessed genomic stability and immune cell composition of tumors in relation to dietary intake of omega-3 long-chain polyunsaturated fatty acids (omega-3 LCPUFA).

• The risk of developing tumors characterized by higher genomic instability and richer in infiltrates of T-regulatory lymphocyte cells was significantly reduced in people with higher dietary intakes of LCPUFA.

• In adults that develop colorectal cancer, survival is increased if LCPUFA intake is increased after diagnosis.

Colorectal cancer, or cancer of the colon or rectum, is among the most frequently-occurring types of cancer in Western society. The age-adjusted annual incidence rate of colorectal cancer in US men and women is on the order of 45 and 35 cases per 100,000 persons (2013), respectively. Death rates are approximately 10-15 cases per 100,000 persons per year. Both incidence and mortality rates are slowly decreasing. However, on a global scale, and in many countries undergoing marked economic development in particular, colorectal cancer incidence is increasing and is expected to continue to increase dramatically. Timely and periodic screening using one of several available methods is important to detect colorectal cancer early on, but only a fraction of the people who most benefit from screening (*i.e.* over 50 years) undergo this procedure. Survival from colorectal cancer that is detected and treated early is high. Treatments for colorectal cancer depend on the stage of tumor development, and generally encompass tumor excision, tissue resection, radiofrequency ablation, cryotherapy, chemotherapy, radiation therapy and targeted therapies using monoclonal therapeutic antibodies. A healthy lifestyle and diet can significantly reduce the risk for developing colorectal cancer.

DNA mismatch repair defects are an important aspect of colorectal cancer development. Higher than normal rates of mutation allow activation of oncogenes that drive tumor development further along a relatively well-characterized multistep pathway from normal cell to tumor.

Colorectal cancer is a relatively well-studied type of cancer and much is known about the sequence of molecular events occurring in the multistep progression of a normal intestinal cell to a tumor. A characteristic of both familial and sporadic colorectal cancer is the early loss of a functional gene coding for enzymes responsible for repairing DNA mismatches that occur during DNA replication (*i.e.* during cell division). Mismatch repair defects lead to so-called microsatellite instability, which is the appearance of alternative transcripts of short nucleotide repeats (microsatellites) found across the genome. Microsatellite instability corresponds to a so-called mutator phenotype, in which spontaneously occurring mutations are not repaired. Normal DNA replication has a very low error rate, and DNA repair enzymes restore any incorrectly incorporated bases, resulting in replication errors at a frequency of an estimated 1 out of every 10 billion newly incorporated nucleotides. This very low mutation rate is increased significantly, perhaps ~100-fold, if repair enzymes are inactive or absent, as frequently seen in the development of colorectal cancer. Malignant cells typically exhibit thousands of nucleotide sequence alterations, and numerous chromosomal abnormalities. The loss of one of several known mismatch repair genes leads to a variety of mutations such as nucleotide transitions, transversions, and 1-4 base pair deletions and insertions. A higher than normal rate of mutation significantly increases the possibility that oncogenes become activated, and/or lead to the inactivation of tumor suppressor genes, driving the process of tumor development further.

Microsatellite instability is observed in some 15% of sporadic colon tumors. In hereditary forms of colon cancer, which may make up 25% of colon cancers, several mismatch repair genes are known to be mutated in germ cells and predispose to the development of mismatch repair defects. It is believed that inactivation of both alleles of a mismatch repair gene is necessary to observe a mismatch repair defect. Spontaneous mutation in the remaining functional gene copy (loss of heterozygosity) can occur comparatively frequently in colonic tissue compared to other tissues because the colon epithelium has a high rate of cell division and is relatively large. Epigenetic modulation of mismatch repair gene ex-

pression can also occur, and has been related to chronic inflammation. According to the number of microsatellite markers that are observed, a classification can be made for tumors that are microsatellite-stable, have low-frequency microsatellite instability, and those that have high-frequency microsatellite instability. Both hereditary and sporadic forms of colorectal cancer can have a high level of microsatellite instability, are predominantly located in the proximal colon, and contain tumor-infiltrating lymphocytes.

In addition to the intricate genetic abnormalities that occur in the carcinogenic process, the immunobiology of cancer is another fascinating aspect of their development. Tumors circumvent parts of the immune system that normally control aberrant cell development and proliferation, which may result from the selection of the ability to co-opt the activity of specific immune cells to suppress their elimination. T regulatory lymphocytes (Treg), an important subpopulation of T cells that execute an immune-suppressive function in the immune system, have long been recognized to play a crucial role in tumor-driven immune modulation. Treg precursor cells are actively recruited into tumors and may further differentiate there to attain an immuno-suppressive phenotype. In colorectal cancer, Tregs suppress an anti-tumor response through the formation of arachidonic acid-derived prostaglandin E_2 by cyclooxygenase-2, thereby facilitating tumor growth. The involvement of polyunsaturated fatty acids is furthermore noteworthy since evidence shows that long-chain omega-3 PUFA (omega-3 LCPUFA) can reduce the risk for the development of colorectal cancer, as well as serve a positive role in its treatment. Research on the chemopreventive and chemotherapeutic activities of omega-3 LCPUFA intake in colorectal cancer development has made a noteworthy transition into clinical research in recent years.

Data from two large prospective and ongoing cohort studies were pooled to detect the potential association that omega-3 LCPUFA intake may have with the development of specific types of colorectal tumors, characterized by their genomic instability or their lymphocytic immune cell infiltrates.

In 2015, Song and colleagues at the Department of Nutrition, Harvard School of Public Health, together with colleagues from the Dana-Farber Cancer Institute, the departments of Medicine and Pathology at Brigham & Women's Hospital, the Massachusetts General Hospital, all in Boston, MA, and the National Cancer Institute at the National Institutes of Health, Bethesda, MD, unveiled a remarkable association between higher dietary omega-3 LCPUFA intake and a lower risk of colorectal tumors with genome instability. This association was not seen for colorectal cancer in general, or for microsatellite-stable colorectal tumors. The results were obtained from two large prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-up Study. From a total of 173,230 study participants, tumor tissue was available for microsatellite marker analysis from 1125 of the 2501 cases of colorectal cancer. In both studies, periodic assessments of dietary intake have been made over several decades using validated food frequency questionnaires (baseline for the first study is 1976, and for the latter 1986). Marine omega-3 intake was calculated as a cumulative average intake of EPA, DHA and DPA (ω-3) up to the end of follow-up (year 2010, participant death, loss to follow-up, or colorectal cancer diagnosis).

In order to combine the data from both cohort studies, the researchers first assessed that no significant heterogeneity existed between the two cohorts in the relationship between omega-3 intake and colorectal cancer incidence. In men, but not women, a significant trend towards lower colorectal cancer incidence was observed. Data from both cohorts were pooled and proportional hazard analysis was performed after taking into account the competing risks that the different tumor types impart on the survival of study participants. The relative risk (age-adjusted) for developing the high microsatellite instability colorectal tumor type was nearly half in people with

Daily intake by US adults of at least 350 mg omega-3 LCPUFA over periods of two to three decades was associated with a marked reduced risk to develop colorectal tumors that had a high microsatellite instability, a sign of deficient DNA mismatch repair capacity.

an omega-3 intake of at least 300 mg/d omega-3 LCPUFA compared to people with an intake below 100 mg/d, and found to be insensitive to correction for potential covariates that may affect tumor development (family history, endoscopy, physical activity, multivitamin intake, aspirin intake, alcohol consumption and a dedicated healthy diet to lower hypertension). The association between high omega-3 intake and reduced high microsatellite instable tumors was linear and statistically significantly different from the model where no differentiation of tumor types was made. The results suggested that moderate dietary omega-3 intake in recommended daily doses may be associated with a very marked effect size, *i.e.* reduction in the risk of developing colorectal tumors with a mutator phenotype in adults followed over periods of 25-35 years.

In order to gain further insight into this potential chemopreventive effect of omega-3 LCPUFA intake on the development of genome-unstable tumors, the same researchers recently assessed whether this potential modulatory effect may also be related to the immunological phenotype of colorectal tumors. Using pooled data on mortality, dietary assessments, and tumor specimens from the same large prospective cohort studies, Song and colleagues addressed the *a priori* hypothesis that omega-3 LCPUFA intake may be associated with colorectal cancer tumor subtypes characterized by specific immune infiltrates, in particular with lymphocyte populations. A similar data pooling and statistical analytical approach was followed.

Among 125,172 evaluated study participants, there were 2504 colorectal cancer cases. Of these, an evaluation of T-lymphocyte populations in colorectal tumors could be made for 614 cases. Analyses of tumor incidence were made after stratification of study participants by intake of omega-3 LCPUFA (less than 0.15 g/d, 015-0.24 g/d, 0.25-0.34 g/d and >0.35 g/d). Variables that changed in both men (Health Professionals Follow-up Study) and women (Nurses' Health Study) with increased omega-3 intake were higher physical activity, undergoing endoscopy, multivitamin use, following a healthy diet, and fewer current smokers. Lymphocyte populations were characterized by immunohistochemistry for the following immune markers using tissue microarray analysis: $CD3^+$ (invariant chain of T-cell receptors expressed on all T-cells), $CD8^+$ (expressed in cytotoxic and suppressor T-cells), $CD45RO^+$ (an isoform of CD45, expressed in activated and memory T-cells), and $FOXP3^+$ (a key transcription factor that is relatively specific for Tregs).

Trends towards a significantly decreased incidence of colorectal cancer with increased omega-3 LCPUFA intake were observed specifically for tumors with a high density of FOXP3+ Tregs ($P<0.001$). The *P*-value cut-off for statistical significance had been reduced to 0.012 to correct for multiple hypothesis testing, and a potential association with low-density CD3+ tumors was not considered of relevance ($P=0.05$ after adjusting for the various mentioned covariates). High intake of omega-3 LCPUFA (≥ 350 mg/d) was associated with a 43% lower incidence of high Treg density tumors, but not with low-density tumors. The association between intake of either EPA, DHA or DPA ω-3 and Treg-infiltrated tumors was significant as well. The relationship between decreasing incidence of Treg-dense tumors and omega-3 LCPUFA intake (as mean values for each group) was found to be linear. Microsatellite-unstable tumors contained higher densities of Tregs than microsatellite stable tumors, but a clear dose-dependent relationship between omega-3 intake and Treg-dense tumors according to genome stability proved difficult to establish and the number of cases for each combination was small.

Higher habitual intake of omega-3 LCPUFA was associated with a marked decrease in colorectal tumors harboring a class of immunosuppressive lymphocytes called regulatory T-cells, which support tumors to evade elimination by the immune system.

The authors next assessed if Treg cells isolated from the colon of mice were affected in their ability to modulate the effector function of CD4+ T-cells by exposure to omega-3 LCPUFA. Murine Treg cells were exposed for 4 h to varying concentrations of DHA (the free fatty acid form) in a cell culture medium containing bovine serum albumin. Thereafter the cells were washed and co-incubated with the effector T-cells for two days, and their proliferation in response to T-cell receptor stimulation was quantified. In the presence of Treg cells, CD4+ T-cell proliferation was markedly suppressed, as expected. Pre-exposure to DHA inhibited the immunomodulatory function of the Tregs in a concentration-dependent manner, with T-cell proliferation doubling when Treg cells had been exposed to concentrations of DHA of 50 micromolar or higher. This result is in accordance with the immune modulatory effect observed in the cohort studies. The possibility that high concentrations of free fatty acids may be present as micelles in the culture medium was not addressed (and no defatted albumin was indicated to have been employed), thereby leaving open the possibility that the observed effect may be due to a detergent effect that may have compromised Treg cell function or viability.

The insight obtained from these two studies has been recently further expanded by the first assessment of survival of patients with a diagnosis of colorectal cancer in relation to their omega-

Higher habitual intake of omega-3 LCPUFA was associated with a markedly increased survival of patients that received a diagnosis of colorectal cancer.

3 LCPUFA intake after diagnosis. The results are again drawn from the Nurses` Health and Health Professionals Follow-up Study using the dietary intake assessment of the first food frequency questionaire at least one year after diagnosis. The postdiagnostic intake was compared to the last assessment taken prior to diagnosis to assess any changes in omega-3 LCUFA intake that may have occurred. Deaths from colorectal cancer were identified from the National Death Index, and review of medical records.

Among 1659 participants diagnosed with colorectal cancer, 561 deaths were reported, of which 169 directly attributed to colorectal cancer. Those patients who consumed ≥ 300 mg/d omega-3 LCPUFA were found to have a hazard ratio (censored relative risk) of 0.59 for colorectal cancer-specific mortality, compared to patients who consumed <100 mg/d. No difference for all-cause mortality was found. The negative association between omega-3 LCPUFA intake and mortality was not different for tumors located in different parts of the intestinal tract or their level of differentiation or stage of development (60% of patients in this study had stage I or

II disease). Interestingly, patients who increased their omega-3 LCPUFA intake by at least 150 mg/d after diagnosis (compared to before diagnosis), saw a 70% reduction in mortality from colorectal cancer compared to patients that did not change their daily intake. The authors point out that increasing consumption of omega-3 LCPUFA after diagnosis may provide significant benefits to patients with a diagnosis of colorectal cancer. A limitation of the present study is that only a subsample of the patients of these cohorts was included for the analysis of survival, and further studies are necessary in a larger population with colorectal cancer.

In summary, recent results from two very large and ongoing prospective cohort studies have provided preliminary evidence that omega-3 LCPUFA intake is asociated with a reduced risk for the development of microsatellite instable and Treg dense tumors. Furthermore, patients with a diagnosis of colorectal cancer may gain marked benefit in terms of survival if their habitual omega-3 LCPUFA intake is significant, and further increased after diagnosis. These associations are observed at omega-3 LCPUFA intake levels that are easily attained by most people ingesting one or two meals of fatty fish a week, or by means of daily supplemental intake of EPA/DHA.

The recent studies provide evidence that omega-3 LCPUFA intake at doses (≥350 mg/d) that are within recommended daily dietary intake is associated with a marked reduction in the development of some colorectal cancers. In adults that do develop colorectal cancer, survival is significantly increased if omega-3 LCPUFA intake is modestly increased after diagnosis.

The chemopreventive effects of omega-3 LCPUFA intake, or unidentified covarying dietary or lifestyle factors, may be acting at multiple levels in tumor initiation and progression. Chronic inflammation is an important facet of colorectal cancer development, and it is plausible that the anti-inflammatory effects of omega-3 LCPUFA may play a role in reversibly modulating the mismatch repair deficit characteristic for a subset of tumors, as well as the immunological subversion mechanisms of tumors. Direct activity of omega-3 LCPUFA on tumor cell growth may also be important. For example, DHA was recently shown to stimulate the autocrine production of tumor necrosis factor-α by human colorectal cancer cells. Future intervention studies that are designed to address the chemopreventive activity of omega-3 LCPUFA are necessary to confirm that the emerging antitumor paradigm applies to humans, and establish that omega-3 LCPUFA intake and tissue levels play a causative role in stemming the development of certain types of tumors in the colon and rectum.

Song M, Nishihara R, Cao Y, Chun E, Qian ZR, Mima K, Inamura K, Masugi Y, Nowak JA, Nosho K, Wu K, Wang M, Giovannucci E, Garrett WS, Fuchs CS, Ogino S, Chan AT. Marine omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer characterized by tumor-infiltrating T cells. *JAMA Oncol.* 2016, May 5 [PubMed]

Song M, Zhang X, Meyerhardt JA, Giovannucci EL, Ogino S, Fuchs CS, Chan AT. Marine omega-3 polyunsaturated fatty acid intake and survival after colorectal cancer diagnosis. *Gut* 2016, July 19. [PubMed]

Song M, Nishihara R, Wu K, Qian ZR, Kim SA, Sukawa Y, Mima K, Inamura K, Masuda A, Yang J, Fuchs CS, Giovannucci EL, Ogino S, Chan AT. Marine omega-3 polyunsaturated fatty acids and risk of colorectal cancer according to microsatellite instability. *J. Natl. Cancer Inst.* 2015;107(4). [PubMed]

Worth Noting

Agency for Healthcare Research and Quality (US). Screening for Colorectal Cancer: A Systematic Review for the U.S. Preventive Services Task Force. In: Lin, J.S., *et al.*, editors, *U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews*. Report No.: 14-05203-EF-1. Rockville (MD); 2016 [PubMed]

Beyer M, Schultze JL. Regulatory T cells in cancer. *Blood* 2006;108(3):804-811. [PubMed]

Calviello G, Serini S, Piccioni E, Pessina G. Antineoplastic effects of n-3 polyunsaturated fatty acids in combination with drugs and radiotherapy: preventive and therapeutic strategies. *Nutr. Cancer* 2009;61(3):287-301. [PubMed]

Colorectal cancer. Center for Disease Control: http://www.cdc.gov/cancer/colorectal/statistics/race.htm

Colorectal and rectal cancer treatments - National Cancer Institute: http://www.cancer.gov/types/colorectal/patient/colon-treatment-pdq#section/_162

http://www.cancer.gov/types/colorectal/patient/rectal-treatment-pdq#section/_135

Bishehsari F, Mahdavinia M, Vacca M, Malekzadeh R, Mariani-Costantini R. Epidemiological transition of colorectal cancer in developing countries: environmental factors, molecular pathways, and opportunities for prevention. *World J. Gastroenterol.* 2014;20(20):6055-6072. [PubMed]

Cho KR, Vogelstein B. Genetic alterations in the adenoma-carcinoma sequence. *Cancer* 1992;70(6 Suppl):1727-17231. [PubMed]

Cockbain AJ, Toogood GJ, Hull MA. Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* 2012;61(1):135-149. [PubMed]

Eshleman JR, Markowitz SD, Donover PS, Lang EZ, Lutterbaugh JD, Li GM, Longley M, Modrich P, Veigl ML, Sedwick WD. Diverse hypermutability of multiple expressed sequence motifs present in a cancer with microsatellite instability. *Oncogene* 1996;12(7):1425-1432. [PubMed]

Eshleman JR, Markowitz SD. Mismatch repair defects in human carcinogenesis. *Hum. Mol. Genet.* 1996;5:1489-1494. [PubMed]

Fluckiger A, Dumont A, Derangere V, Rebe C, de Rosny C, Causse S, Thomas C, Apetoh L, Hichami A, Ghiringhelli F, Rialland M. Inhibition of colon cancer growth by docosahexaenoic acid involves autocrine production of TNFα. *Oncogene* 2016;8 Feb:1-12. [PubMed]

Health Professional Follow-up Study: https://www.hsph.harvard.edu/hpfs/

Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics 1995;51(2):524-532. [PubMed]

Nurses' Health Study: http://nurseshealthstudy.org/

Mutations: http://www.biology-pages.info/M/Mutations.html

Peltomäki P. Deficient DNA mismatch repair: a common etiologic factor for colon cancer. *Hum. Mol. Genet.* 2001 10(7):735-740. [PubMed]

Prevention of colorectal cancer – American Cancer Society: http://www.cancer.org/cancer/colonandrectumcancer/moreinformation/colonandrectumcancerearlydetection/colorectal-cancer-early-detection-prevention

Prindle MJ, Fox EJ, Loeb LA. The mutator phenotype in cancer: molecular mechanisms and targeting strategies. *Curr. Drug Targets* 2010;11(10):1296-1303. [PubMed]

Richman S. Deficient mismatch repair: Read all about it. *Int. J. Oncol.* 2015;47(4):1189-1202. [PubMed]

Tissue microarrays for immunohistochemical analysis: http://www.jove.com/video/3620/production-tissue-microarrays-immunohistochemistry-staining

Yaqub S, Henjum K, Mahic M, Jahnsen FL, Aandahl EM, Bjornbeth BA, Taskén K. Regulatory T cells in colorectal cancer patients suppress anti-tumor immune activity in a COX-2 dependent manner. *Cancer Immunol. Immunother.* 2008;57(6):813-821. [PubMed]

FOL 21

VISUAL FUNCTION

EPA for Retinal Neuron DHA Synthesis

THIS ARTICLE AT A GLANCE

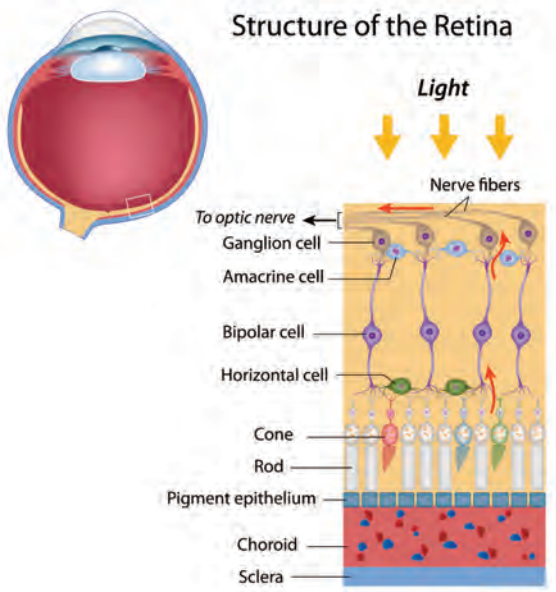
• Photoreceptor cells contain high levels of DHA in their outer light-sensitive segment. Past research had shown that the DHA in photoreceptors was obtained from hepatic synthesis transported via the circulation, as well as from local synthesis in the retinal pigment epithelial cells that support photoreceptor function.

• New research indicates that photoreceptor neurons themselves can also import EPA and convert it to DHA to serve a role in their differentiation and protection from oxidative damage.

The photoreceptor cell is a neuronal cell type specialized in sensing light and transducing photons into electrical signals. Photoreceptor cells form the sensory part of the retina, capturing photons in rod photoreceptors to allow dim light vision (monochromatic vision in the dark), in cone photoreceptor cells that measure color, and photosensitive retinal ganglion cells that are involved in diurnal regulation and initiate the pupillary reflex response to incident light. Phototransduction by photoreceptor cells allows our brain to construct a visual image of our surroundings, an ability that is vital for survival of the human organism as a whole. The retina is characterized by an immune privileged status to avoid that immune cell infiltration disturbs the physical configuration and cellular health of the photosensitive retinal tissue. Docosahexaenoic acid (DHA) plays a central structural and functional role in both the sensory function of the photoreceptor cell as well as in mechanisms that protect retinal tissue from being damaged.

DHA plays a fundamental structural and functional role in photoreceptor physiology.

The stacked membranes in the outer segments of rods that hold the light-sensitive opsin proteins are composed of phospholipids containing very high levels of DHA. DHA-containing lipids physically interact with rhodopsin, the opsin of rod photoreceptors, to allow proper phototransduction. Sufficient dietary DHA intake, along with the macular pigments lutein



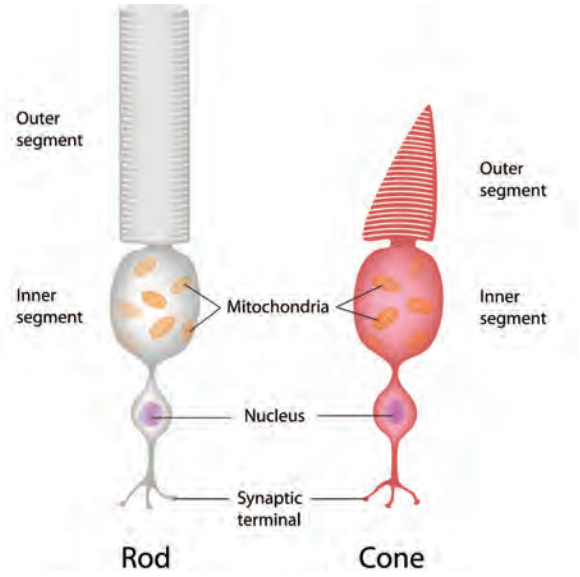
and zeaxanthin, is required to maintain the sensitivity and recovery of photoreceptor rods. The high concentrations of polyenic fatty acids together with the exposition to light make the retina a tissue that is also sensitive to photo-oxidation. To sustain lifelong functioning, the retina is endowed with a sophisticated mechanism of protection from tissue damage that may ensue from photo-oxidation. Unique carotenoids, such as lutein, zeaxanthin and *meso*-zeaxanthin, divert free electron radical species that may form within photoreceptor membranes. During outer segment turnover by retinal pigment epithelial (RPE) cells, necessary for the recycling of bleached retinal photopigment, DHA is released from phagocytosed membranes to activate cytoprotective mechanisms that protect the photoreceptor from oxidative damage and cell death, while maintaining inflammatory responses at bay. This is achieved by activation of gene transcription of tissue protective proteins by DHA itself, as well as via the enzymatic oxygenation of DHA to the docosanoid neuroprotectin D1, which activates pro-survival signaling in the face of increased oxidative stress and outer segment turnover.

During early post-natal development, the large demand for DHA by photoreceptor cells is covered by DHA synthesized from alpha-linolenic acid (ALA) in the liver, as well as preformed DHA obtained from breast milk and carried via lipoprotein-mediated transport to the developing retina. RPE cells have recently been shown to express the Mfsd2a transporter that takes up DHA-containing lysophosphatidyl-choline (lysoPC), which may be derived from the circulation. The maintenance of photoreceptor DHA levels is furthermore supported by cell types in close proximity to the photoreceptor

This study formally addressed whether EPA might serve as a metabolic substrate for the formation of DHA by retinal neurons themselves, which would support the hypothesis that the retina may not solely rely on DHA import from the circulation.

cells. RPE cells not only play an important role in recycling the active opsin pigment, 11-*cis*-retinal, back to photoreceptor cells, but they can also synthesize DHA from precursor fatty acids such as ALA and docosapentaenoic acid (ω-3). Likewise, microvascular endothelial cells in the retina have this biosynthetic capacity. In other words, various cell types in retinal and choroidal tissue may contribute to form DHA locally and supply this fatty acid to photoreceptor cells. Furthermore, RPE cells actively phagocytose membrane fragments from the outer photoreceptor segment, and shuttle DHA-containing phospholipids back to the inner segment, thereby contributing to the active concentration of DHA within photoreceptor cells.

Compared to DHA, which makes up over half of all fatty acids esterified in phospholipids of the outer segments of photoreceptor cells, EPA is present in very low amounts. Interestingly, EPA can be converted rapidly into DHA when administered intravitreally. Other metabolic precursors of DHA can also be actively transformed into DHA when added to mixtures of retinal cells. Both EPA and DHA levels in



serum are associated with lower risks of certain retinopathies, but how EPA derived from the circulation contributes to retinal health and function is unclear. Various omega-3 polyunsaturated fatty acids can clearly serve as metabolic precursors for DHA formation within the retina, but it has not been specifically addressed if neural cells of the retina may be capable of DHA synthesis from EPA.

A recent study by Simón and colleagues from the Bahía Blanca Institute of Biochemical Investigations (INIBIBB), at the Universidad Nacional del Sur-CONICET, in Buenos Aires, Argentina, together with colleagues at the University of Oklahoma, OK, USA, addressed if EPA is able, just like DHA, to promote the survival and differentiation of photoreceptor cells, and if these cells could synthesize DHA using EPA as a biosynthetic precursor. The researchers made use of primary cultures of pure retinal photoreceptor neuronal cells isolated from rat retinas. Retinal cells were obtained from the retinas of 2-day old rat pups, and placed in cell culture conditions that give rise to pure neuronal cultures, composed mainly of photoreceptor cells and amacrine cells (retinal interneurons). Under these conditions, the photoreceptor cells start dying spontaneously after several days in culture by apoptosis, but supplementation with a low concentration of DHA can selectively inhibit this process and stimulates differentiation into photoreceptor cells, with the formation of new apical processes, an increased expression of opsin, and the localization and concentrating of opsin in the apical processes of the cells.

The researchers first showed that, similar to the effect of DHA, the addition of low concentrations of EPA (3 μM) protected the retinal neurons from apoptosis induced by oxidative stress (experimentally generated by the redox cycling agent paraquat, or by hydrogen peroxide). Cells received EPA two days before exposure to oxidative stress, and were followed for another day. Mitochondrial membrane potential, a measure of the capacity of the mitochondria to generate ATP and energy, was also maintained by EPA. None of the other fatty acids that are present in reasonable levels in the retina, palmitic acid, oleic acid and arachidonic acid, shared the cytoprotective activity of EPA and DHA.

Exposure of retinal neurons to EPA also stimulated further differentiation into photoreceptor neurons with the expression of opsin, and the formation of apical processes with an intense opsin labeling. These effects were accompanied by a marked increase in DHA levels in cellular lipids. Esterified EPA levels did not increase, indicating that all of the EPA was directly converted to longer chain metabolites *en route* to DHA. In order to synthesize DHA from EPA, a desaturation reaction catalyzed by the fatty acid desaturase FADS2 is required as one of the biosynthetic steps. Indeed, the neuronal cells expressed FADS2, independent from being exposed to EPA or not. Using an inhibitor (CP-24879) of both Δ-5 and Δ-6 fatty acid desaturases the increase in DHA for-

mation from EPA was abolished. Interestingly, the antiapoptotic effect and the cell differentiation induced by EPA were abolished by blocking DHA synthesis. These results suggest that retina neuronal cells are capable of using EPA to form DHA. Furthermore, this ability allows the use of EPA to support a cytoprotective function in retinal neurons, and aids in the differentiation of photoreceptor cells, at least under cell culture conditions. This study is the first to indicate that not only DHA, but also EPA, may directly support the synthesis of DHA in retinal neurons to serve photoreceptor function.

The study was carried out with cells from rat retinas, and confirmation would be needed with human retinal neurons (if at all possible). At present it is not confirmed that EPA imported from the circulation will actually be used by neuronal cells *in vivo* to synthesize DHA, and to what extent EPA may contribute to DHA levels in the retina. In addition to retinal neurons, microvascular endothelial cells, RPE cells, and glial cells all appear capable of forming DHA from precursors, and it may be challenging to define if DHA synthesis *in vivo* predominates in one cell type, and under which specific conditions. These results reveal the redundant and widespread multicellular capacity to form DHA in the choroid and retina, in addition to the uptake of preformed DHA in the form of lysoPC-DHA from the circulation, so that the supply of DHA is guaranteed from multiple sources to safeguard its critical function in vision.

The study indicates that neuronal cells of the retina have the capacity to convert EPA into DHA, necessary for photoreceptor differentiation and viability. Studies to confirm whether this also applies to human retinal cells are necessary.

Simón MV, Agnolazza DL, German OL, Garelli A, Politi LE, Agbaga MP, Anderson RE, Rotstein NP. Synthesis of docosahexaenoic acid from eicosapentaenoic acid in retina neurons protects photoreceptors from oxidative stress. *J. Neurochem.* 2016;136(5):931-946. [PubMed]

Worth Noting

Bazan HE, Careaga MM, Sprecher H, Bazan NG. Chain elongation and desaturation of eicosapentaenoate to docosahexaenoate and phospholipid labeling in the rat retina in vivo. *Biochim. Biophys. Acta* 1982;712(1):123-128. [PubMed]

Bazan NG, Calandria JM, Serhan CN. Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. *J. Lipid Res.* 2010;51(8):2018-2031. [PubMed]

Delton-Vandenbroucke I, Grammas P, Anderson RE. Polyunsaturated fatty acid metabolism in retinal and cerebral microvascular endothelial cells. *J. Lipid Res.* 1997;38(1):147-159. [PubMed]

Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. *Prog. Lipid Res.* 1983;22:79–131. [PubMed]

Jeffrey BG, Neuringer M. Age-related decline in rod phototransduction sensitivity in rhesus monkeys fed an n-3 fatty acid-deficient diet. Invest. *Ophthalmol. Vis. Sci.* 2009;50(9):4360-4367. [PubMed]

Rotstein NP, Aveldaño MI, Barrantes FJ, Politi LE. Docosahexaenoic acid is required for the survival of rat retinal photoreceptors in vitro. *J. Neurochem.* 1996;66(5):1851-1859. [PubMed]

Rotstein NP, Pennacchiotti GL, Sprecher H, Aveldaño MI. Active synthesis of C24:5, n-3 fatty acid in retina. *Biochem J.* 1996;316 (Pt 3):859-864. [PubMed]

Rotstein NP, Politi LE, Aveldaño MI. Docosahexaenoic acid promotes differentiation of developing photoreceptors in culture. *Invest. Ophthalmol. Vis. Sci.* 1998;39(13):2750-2758. [PubMed]

Scott BL, Bazan NG. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Natl. Acad. Sci. U.S.A.* 1989;86(8):2903-2907. [PubMed]

Soubias O, Teague WE, Gawrisch KJ. Evidence for specificity in lipid-rhodopsin interactions. *J. Biol. Chem.* 2006;281(44):33233-33241. [PubMed]

Wang N, Anderson RE. Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. *Biochemistry* 1993;32(49):13703-13709. [PubMed]

Wong BH, Chan JP, Cazenave-Gassiot A, Poh RW, Foo JC, Galam DL, Ghosh S, Nguyen LN, Barathi VA, Yeo SW, Luu CD, Wenk MR, Silver DL. Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid (DHA) in eye and is important for photoreceptor cell development. *J. Biol. Chem.* 2016;291(20):10501-10514. [PubMed] FOL

NEW DEVELOPMENTS

Positive Selection of an Indel Polymorphism in the FADS Gene Cluster May be Driving Long-Chain PUFA Biosynthetic Capacity in Specific Human Populations

THIS ARTICLE AT A GLANCE

- *This study reports a 22-bp nucleotide insertion-deletion (indel) genetic polymorphism that may be causally related to the control of gene expression of the fatty acid desaturases, enzymes that control the biosynthesis of long-chain polyunsaturated fatty acids (LCPUFA) from 18-carbon PUFA.*
- *The population frequency of the indel (rs66698963) is remarkably different among human populations with the insertion being far more frequent in South Asians, Africans and some East Asian populations, and far less common in European and other East Asian populations.*
- *The polymorphism has a significant effect on baseline arachidonic acid levels, and on the product-precursor relationship for the omega-6 LCPUFA biosynthesis pathway. The effects on omega-3 LCPUFA homeostasis remain to be reported, but further exploration of indel frequency in populations may significantly augment our understanding of the link between diet and PUFA status in health and disease.*

The appearance of genetic polymorphisms, *i.e.* differences in the DNA nucleotide sequence between individuals, contributes to the opportunity for functional adaptation to specific environments that organisms may encounter. Over many generations, the selection of beneficial traits associated with particular polymorphisms can lead to gradual changes in the frequency of specific genetic polymorphisms in a genetically-isolated population. When a specific genetic variant reaches all members of the population, the trait becomes fixed. In contrast with the positive selection of favorable adaptive polymorphisms, a genetic variant may also gradually disappear from a population if there is no survival advantage in having it.

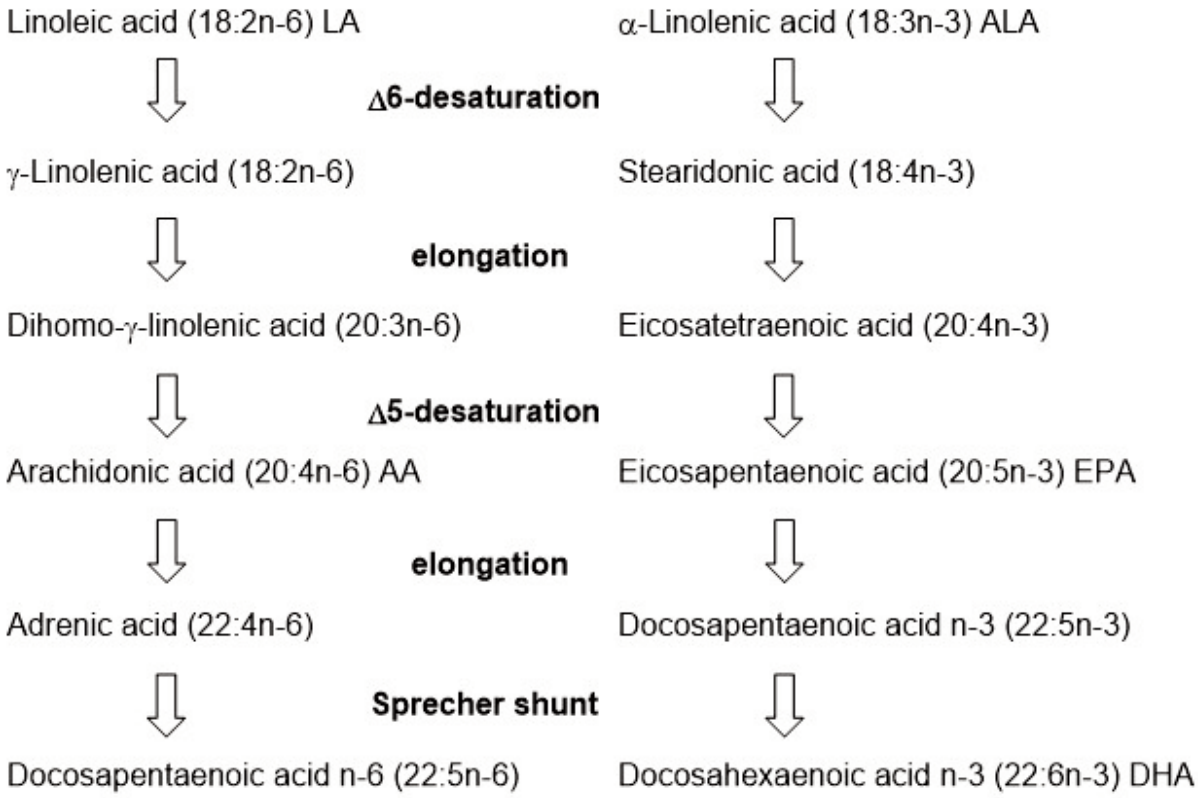
A 22-base pair insertion-deletion mutation in the fatty acid desaturase gene cluster was recently identified. This study expands our understanding of its relevance by comparing the allele frequency in different populations, provides evidence for positive selection in diverse human populations, and made an assessment of the biochemical phenotype associated with the indel genotype.

In addition to constituting fascinating signatures of the population genetic history, diverse types of genetic variance are also of importance to human health and chronic disease susceptibility. Several single-nucleotide polymorphisms (SNPs) that may affect long-chain polyunsaturated fatty acid (LCPUFA) biosynthesis, the distribution of dietary PUFA, and their functional effects have been described in recent years (see this Guest Article). Studies on polymorphisms found in one cluster of closely-located genes coding for fatty acid desaturases (FADS), enzymes that play an important role in the biosynthesis of both omega-6 and omega-3 LCPUFA, are providing an interesting perspective on the interaction between traditional diets and the population genetic history of humans.

Several SNPs located in the FADS gene cluster are now known to be related to changes in risk for complex and chronic diseases. None of these small polymorphisms can be clearly assigned to changes in the amino acid sequence of the FADS enzymes, and are believed to mostly influence the regulation of gene expression. In 2012, a much larger, 22-base pair long stretch of nucleotides was identified within a part of the FADS-2 gene that is not translated to protein (an intron). The polymorphism was present as an insertion in cells derived from a group of Japanese individuals, but was completely absent (a deletion) in others, albeit with a lower frequency (hence called the minor allele).

This genetic variant, termed an indel (insertion/deletion mutation), was found to influence the regulation of the expression levels of both *FADS-1* and *FADS-2* in a cellular system, with the minor allele in Japanese people being associated with lower expression of the *FADS-1* and *FADS–2* genes. The 22-base pair sequence (named rs66698963) is likely to affect the activity of a nearby sterol response element (a binding site for sterol response element binding protein, which is a class of DNA-binding transcription factors involved in sterol and fatty acid homeostasis).

Figure 1: The formation of highly unsaturated fatty acids from the essential fatty acids linoleic acid and α-linolenic acid involves fatty acid desaturation at two enzymatic steps, as well as in the Sprecher shunt. Competition for conversion to longer fatty acid forms occurs as the same enzymes can convert both omega-6 and omega-3 fatty acids.



The same group of researchers, working at Cornell University, Ithaca, NY, in collaboration with colleagues at the University of Kansas, KS, USA, and the Sinhgad College of Engineering, at the University of Pune, India, recently reported on a more comprehensive assessment of the frequency of this insertion-deletion polymorphism in several different ethnic human populations, and determined if positive selection of this genetic variant may have occurred. Furthermore, the biochemical implications of the indel polymorphism were determined by evaluating the fatty acid composition of red blood cell membrane phospholipids as a long-term marker for LCPUFA formation.

The population frequency of the allele corresponding to the rs66698963 insertion (allele named I) or deletion (allele named D) was determined from genomic DNA extracted from human samples (blood, breast milk, and placenta) obtained from several participating institutions in the US and Canada (n=211, nearly all from Kansas City), as well as from a group of Asian Indians (n=76). The observed allele frequency in North Americans was 0.38 for I and 0.62 for D, whereas for the Asian Indians it was 0.82 I and 0.18 D. The striking difference in allele frequency distribution prompted a more comprehensive assessment of genotype variation, as the results suggested that the minor allele (D) originally identified in Japanese and then in Asian Indians might well constitute a major allele in other populations.

To that end, whole-genome sequencing data from the 1000 Genomes Project was accessed to estimate the global genotype frequency distribution of rs66698963. Frequency distributions of genetic variation in ethnic populations of African (7 different populations), European (5), East Asian (5), and South Asian (5) origin were estimated. The I/I genotype was

the genotype with highest frequency in African and South Asian populations. In European and East Asian populations, the I/D heterozygous genotype was most abundant, except in Japanese (Tokyo) and Han Chinese (Beijing), where also the I/I genotype was present to a major extent. Among people of South Asian ancestry, in particular Gujarati and Telugu Indian people, the D/D genotype was nearly absent and approximately 80% of the population was homozygous for I/I. Finnish people had the highest frequency of the D/D genotype (approx. 40%), and people from the United Kingdom had the lowest frequency of the I/I genotype.

This remarkable population-associated genotypic variation encouraged testing the hypothesis that positive selection of this fatty acid desaturase gene cluster indel may be at play. First, the degree of population differentiation in allele frequencies was quantified. A higher than expected differentiation of population structure for the indel was recognized using the F_{ST} statistic, a test for co-ancestry of alleles among individuals. Employing pair-wise comparisons of the F_{ST} statistic between the ethnic populations grouped according to the four continental regions, it was found that significant genetic divergence for the indel has occurred between South Asians and Europeans, Africans and Europeans, and between South Asians and East Asians. Together with results from a second test for population differentiation, the researchers report that positive natural selection of the rs66698963 insertion may have occurred in several of the studied populations.

Further evidence for positive selection of the insertion in different populations was obtained by site frequency spectrum (SFS) analysis, and by tests for natural selection based on haplotype frequency. SFS analysis was carried out with three approaches that assess (loss of) genetic diversity (π test), an excess of rare variants (Tajima's D test), and an excess of high-frequency derived alleles (Fay and Wu test). SFS tests compare DNA sequences from individuals from different populations and make estimations of the differences in sequence by gradually stepping through the sequence in 1,000 bp steps and comparing 5,000 bp-long sequences. A probability scoring is performed that calculates the likelihood that the frequency of sequence variants in a population is statistically significantly different from surrounding DNA sequences that are on average not under selective evolutionary pressure. The results from these tests showed statistically significant (strong) positive selection of the insertion in South Asian populations, and a less significant one for the African populations, but not in European or East Asian populations. Strong positive selection of the insertion was detected in all the South Asian populations individually as well, in particular in Gujarati and Telugu Indian.

Haplotype-based tests for positive selection provided evidence of a strong selective pressure for the stretch of DNA that encompasses the rs33398963 indel in South Asians. Haplotypes are stretches of DNA (containing genes and regulatory sites) that are inherited as a unit. To detect a higher than expected inheritance of particular haplotypes within populations, three approaches were employed: the integrated Haplotype Score (iHS), the number of segregating sites by length (nSL), and the Cross-Population Extended Haplotype Homozygosity (XPEHH). These tests evaluate all sequence variants that occur with a frequency >5% against the overall genome-wide variation, and a normalized probability of occurrence is calculated. Positive selection of the haplotype carrying the insertion was subject to positive selection in South Asians, and also in African populations. In East Asian populations analyzed separately, significant selection for the insertion was found to have occurred in the Chinese Han of Beijing and in the Japanese population of Tokyo, but not the other three populations (Southern Han Chinese, Chinese Dai and Vietnam Kinh).

Based on these results, the authors propose that the rs66698963 indel, or a DNA sequence located very closely to it, constitutes a so called "adaptive allele", *i.e.* a genetic element that is subject to positive selection related to some

The study indicates that the FADS2 indel genotype influences baseline arachidonic acid levels, and the activity of the omega-6 PUFA pathway. How omega-3 LCPUFA levels are affected by this indel remains to be documented.

specific advantageous function in that human population. The presence of the insertion was found to represent the "ancestral" allele, having much stronger extended homozygosity (forming part of larger haplotypes, and having lower recombination frequency) than the "derived" allele, which is the evolutionary loss of the insertion (*i.e.* the deletion).

Since the indel is closely located to a regulatory sequence that controls expression of both FADS1 and FADS2, the authors next assessed if basal PUFA levels, as well as the substrate-product relationships for several omega-6 LCPUFA that can be formed from linoleic acid (LA: 18:2), were different between individuals with the I/I, I/D, and D/D genotypes of the original North American subjects. The biochemical phenotypes turned out to be remarkably distinct. As assessed from the composition of omega-6 PUFA esterified within erythrocyte phospholipids, the conversion of 20:3 to arachidonic acid (AA) (and onwards to 22:4) was significantly increased in carriers of the insertion (both in hetero- and homozygotes). Linoleic acid levels were unchanged, suggesting that in the tested individuals more than enough linoleic acid is available that can be converted to longer chain omega-6 PUFA, and that it is the FADS-1 and FADS-2 enzyme levels that are rate-limiting. In rs66698963 insertion homozygotes, the difference in the level of AA minus that of LA was 30% higher than the difference measured in deletion homozygotes. Several other product-precursor relationships all suggested that the insertion plays a measurable role in increasing baseline FADS-mediated LCPUFA biosynthesis. At this stage this has formally only been shown to occur for omega-6 LCPUFA, but it is expected that the same will apply to omega-3 LCPUFA biosynthesis given that the biochemical pathways for both LCPUFA families are shared.

In summary, this study has provided new indications that in different human populations rs66698963 constitutes an advantageous mutation that leads to an enhanced endogenous transformation of LA to longer-chain omega-6 PUFA. In some populations, such as the South Asian, a selective "sweep" of this insertion has led to near fixation. In other populations, the insertion does not appear to have undergone positive selection, and a high frequency of deletion is found. These results are in line with the consideration that people who over a period of many generations traditionally ingest a vegetable-based diet, have experienced a population fitness benefit from increasing the conversion of plant-derived LA to omega-6 LCPUFA. In populations where traditional diets already provide LCPUFA, such as fish and meat, no selective advantage is associated with retaining the insertion, which has to a significant extent been lost from the human genome and has resulted in a polymorphic distribution among members of European and some East Asian populations.

Divergence in human diets: A thali, a traditional vegetable dish from India (left); Arctic char, a staple marine food of the Inuit (right)

Expanding the analysis to further ethnic and geographically-defined population will provide an even better idea of the polymorphic distribution of this important indel. In the extreme case of the Greenlandic Inuit, reliance over many generations on a marine diet that is rich in preformed LCPUFA has obviated the need for maintaining the insertion, which has consequently been nearly totally lost from the Inuit genome. The latter has not been formally shown, but the ancestral form of a SNP (rs174570) that is nearly fixed in Inuit segregates closely to the indel reported here, and was also found in South Asians.

Positive selection of various genetic polymorphisms in the FADS gene cluster that are associated with an increased efficiency to convert medium chain PUFA to LCPUFA had previously been reported in African populations. It is thought that positive selection for increased conversion may have allowed African populations to establish themselves across the continent using predominantly plant-based diets as substrate for LCPUFA synthesis. Why these polymorphisms are not represented in populations that migrated out of Africa is not clear. The information in the present study suggests that the

ancestral allele containing the insert has been present in the human genome for a long time: the 22-bp indel is absent in other primates but it was found in the DNA of Denisovan and Neanderthal humans.

This study has a number of potentially important implications that have been clearly outlined by the authors. *FADS2* indel genotypes contribute to the variability in response to PUFA consumption. For vegans and vegetarians that predominantly consume LCPUFA precursors produced by plants, tissue LCPUFA composition will depend on the relative proportions of LA and alpha-linolenic acid consumed. For vegans/vegetarians with the I/I genotype and who ingest a diet with a dominance of LA, higher baseline AA levels are expected to result, likely associated with higher risk for developing chronic diseases related to inflammation. Direct consumption of preformed omega-3 LCPUFA, such as EPA and DHA from marine sources, may be required for these people to a much greater extent than those with a D/D genotype to balance the formation of prostanoids produced during inflammatory responses. D/D carriers maintain lower AA levels and may be less susceptible to chronic inflammatory disease, and less susceptible to excess LA intake. D/D individuals may however benefit more from supplemental EPA/DHA intake in periods of development dependent on omega-3 LCPUFA (*e.g.* during pregnancy).

Further studies of the FADS2 indel will likely significantly expand our insight into how genotype can predict our susceptibility to chronic inflammatory disease in relation to precursor PUFA biotransformation and the dietary need for preformed LCPUFA.

Testing these implications in concordance with *FADS2* indel screening holds promise to provide us with new insights into the question of who is most likely to benefit from the ingestion of which specific LCPUFA and/or its precursors, and their doses. Indels are inaccurately detected and annotated with current whole genome sequencing algorithms and the authors note that the D/D frequency estimated from whole genome sequencing efforts may in reality be even higher, and the I/D frequency lower, than currently determined from whole-genome sequencing projects. This may imply that the frequency of deletion homozygotes is even higher in populations with significant frequency of the D allele than estimated. Further research into this *FADS2* indel is likely to provide very interesting insight into the role of PUFA in the health of humans living in a modern society that is increasingly disconnected from locally-sourced traditional food sources, and in some ethnically-mixed societies with a high diversity of indel genotypes.

Kothapalli KS, Ye K, Gadgil MS, Carlson SE, O'Brien KO, Zhang JY, Park HG, Ojukwu K, Zou J, Hyon SS, Joshi KS, Gu Z, Keinan A, Brenna JT. Positive selection on a regulatory insertion-deletion polymorphism in FADS2 influences apparent endogenous synthesis of arachidonic acid. *Mol. Biol. Evol.* 2016;33(7):1726-1739. [PubMed]

Worth Noting

Fumagalli M, Moltke I, Grarup N, Racimo F, Bjerregaard P, Jorgensen ME, Korneliussen TS, Gerbault P, Skotte L, Linneberg A, Christensen C, Brandslund I, Jorgensen T, Huerta-Sanchez E, Schmidt EB, Pedersen O, Hansen T, Albrechtsen A, Nielsen R. Greenlandic Inuit show genetic signatures of diet and climate adaptation. *Science* 2015;349(6254):1343-1347. [PubMed]

Li Y, Wang DD, Ley SH, Howard AG, He Y, Lu Y, Danaei G, Hu FB. Potential impact of time trend of life-style factors on cardiovascular disease burden in China. *J. Am. Coll. Cardiol.* 2016;68(8):818-833. [PubMed]

Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, Biscuola M, Cavallari U, Galavotti R, Martinelli N, Guarini P, Girelli D, Olivieri O, Corrocher R, Heinrich J, Pignatti PF, Illig T. SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* 2008;43(4):289-299. [PubMed]

Mathias RA, Fu W, Akey JM, Ainsworth HC, Torgerson DG, Ruczinski I, Sergeant S, Barnes KC, Chilton FH. Adaptive evolution of the FADS gene cluster within Africa. *PLoS One* 2012;7(9):e44926. [PubMed]

Minihane A-M. The Impact of Common Gene Variants on EPA and DHA Status and Responsiveness to Increased Intakes PUFA Newsletter, December 2015.

1000 Genomes Project: http://www.1000genomes.org/about#1000G_PROJECT

Reardon HT, Zhang J, Kothapalli KS, Kim AJ, Park WJ, Brenna JT. Insertion-deletions in a FADS2 intron 1 conserved regulatory locus control expression of fatty acid desaturases 1 and 2 and modulate response to simvastatin. *Prostaglandins Leukot. Essent. Fatty Acids* 2012;87(1):25-33. [PubMed]

Weir BS. Estimating F-statistics: A historical view. *Philos. Sci.* 2012;79(5):637-643. [PubMed]

FOL

GUEST ARTICLE

Is Arachidonic Acid Required to Balance Docosahexaenoic Acid in Infant Feeding for Proper Development?

J. Thomas Brenna
Division of Nutritional Sciences
Cornell University
Ithaca, NY 14853 USA

The long chain polyunsaturated fatty acids (LC-PUFA) docosahexaenoic acid (DHA) and arachidonic acid (AA, or ARA) are conserved components of the breast milks of all well-nourished mothers worldwide. Since at least the mid-1990s, they have been permitted as components of infant formulas in Europe, and since 2001 in the USA. Prior to about 1990, the safest sources of fats for infant formulas were vegetable oils with no LCPUFA, or dairy fat with negligible

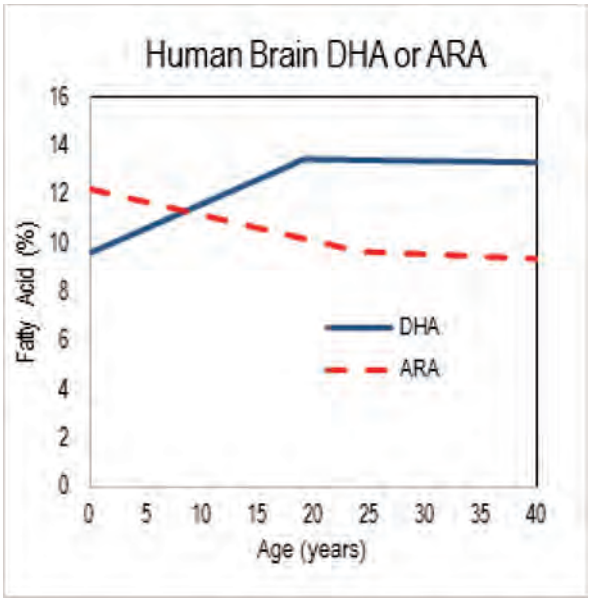
DHA. Concerns over the ability of infants, and particularly preterm infants, to endogenously biosynthesize all LCPUFA needed to supply the rapidly growing brain surfaced around 1990 and were directly primarily to DHA. Since then, research has focused very much on DHA and not on ARA. However, the science undergirding the need for ARA has come into focus recently as a result of the European Food Safety Authority (EFSA) implicit assertion that ARA is an optional nutrient for the term infant even when docosahexaenoic acid (DHA) is present [1]. Following this advice, manufacturers would be permitted to make infant formulas that have DHA but no ARA. The action has been challenged by some researchers [2], including me, as without firm basis clinically or pre-clinically [3, 4].

What is a required nutrient?

Establishing nutrient requirements has evolved from a century ago in the era of vitamin discovery to modern times. Early on, animal experiments were the leading guide to nutrient deficiency symptoms, suggesting human studies which identified acute deficiency symptoms and in some cases attempting to recapitulate symptoms experimentally. For more than 20 years, the nutrition research community's understanding of "requirement" now extends to chronic conditions, as in the case of cardiovascular disease and, importantly, to acute symptoms that are detectable only with specialized tests. Clinical studies go hand in hand with animal studies because the former are highly restricted in the control and sampling that can be exerted, and the latter are inherently imperfect models for humans. Indeed, in formal settings, animal studies are not considered evidence in the sense of evidence-based medicine, a swing of the pendulum too far for comfort in my opinion [5]. Regardless, all can agree that for maximal confidence, clinical evidence developed in a sampling of the relevant population and indications, with relevant comparator (controls), and looking at relevant outcomes, is needed.

Nutrition in development

The relevance of DHA nutrition to infant development is suggested by DHA accretion during the human brain growth spurt, when the brain increases size dramatically from about 27 weeks of gestation to 2 years of life [6]. The brain is uniquely rich in DHA, and its increase in size implies an unusually high demand for DHA. DHA continues to accumulate



to about 18 years of age where it levels off until about 90 years, as shown in the nearby chart drawn from data from a cross-sectional autopsy study [7]. Less remembered is that at birth, ARA is at substantially greater concentration in the brain than DHA. ARA concentrations remain high, though unlike DHA, they gently drop in childhood.

Direct Clinical Evidence

What is the clinical evidence that DHA alone without ARA is safe? The universally agreed upon optimal food for infants is breast milk. Simply put, data from studies around the world show that breast milk conserves ARA concentrations. The amount of ARA in breast milks across the globe appears in a tight window of about 0.47% ± 0.13%, compared to DHA (0.32% ± 0.22%) [8].

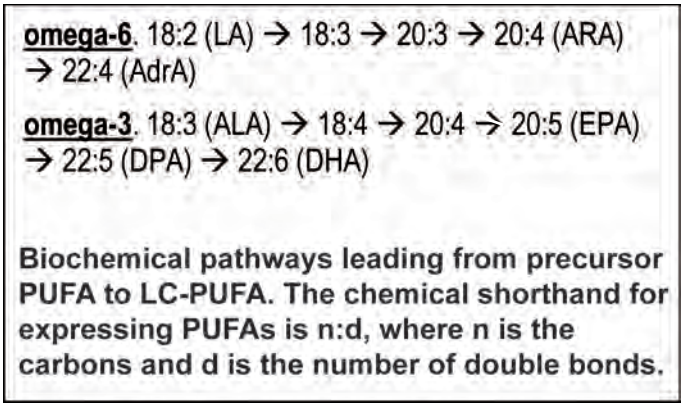
Both of these LCPUFA are major structural components of all neural tissue in all mammalian species, and perinatal metabolic deficiency of either causes acute deficiency symptoms. On this basis, the default assumption should be that they are required components of formulas requiring compelling evidence that they can be omitted. Moreover, the underlying biochemical shows that they interact with one another, one inhibiting the other's access to enzymes and with them, synthesis of signaling molecules. Unfortunately, convenience in specifying individual nutrient levels for legal clarity often omits the known relationships between nutrients, thereby oversimplifying the basic biology. In the case of DHA and ARA, some regulations require ARA and DHA proportions in a specific range, while others state minimal levels of each without regard to the other.

Dozens of clinical trials have been conducted, starting in the 1990s, of LCPUFA formulas compared to formulas that have only omega-6 linoleic acid and omega-3 alpha-linolenic acid as precursors for the LCPUFA. Most of these trials have two characteristics in common: 1) the "LCPUFA" group has both DHA and ARA; 2) the outcomes are neural in nature, typically visual or cognitive, apart from general "failure to thrive" indexes, particularly weight [9-14]. Studies with formulas that include DHA with and without EPA have been conducted. No studies have been reported that use ARA alone in a formula, and it is unlikely that one will be conducted in normal infants in the foreseeable future because of concerns over acute effects of ARA, e.g. in clotting or inflammatory response.

A proper clinical study design for establishing need for ARA in the presence of DHA would be RCTs that include treatment arms with DHA only and with DHA and ARA (DHA+ARA), and measuring a functional endpoint that is likely to be limited by ARA intake, for instance vascular or immune outcomes. In fact, only three cohorts of human infants, all apparently healthy full term infants, have ever been studied: the Retina Foundation of the Southwest study [15-18], the Adelaide study [19, 20], and the Ross Pediatric Research Study [21-23]. None of them considered endpoints characteristic of ARA. Growth was considered as an ARA endpoint, for which some early evidence was presented in preterms [24]; growth measured by weight gain is not a general characteristic of ARA and data obtained since that pioneering early work has obviated concerns [25, 26]. Considering these studies together [3], one of three trials, the Retina Foundation trial with the highest ARA and among the two with adequate DHA (Ross used low DHA), found significant effects on neural outcomes for DHA+ARA compared to DHA only. These experimental studies of ARA provide scant evidence to delete ARA from the diets of infants when they are consuming DHA, and on the contrary suggest it may be important even to DHA-dominated outcomes. Indeed, a recent study of very preterm infants (<1500 g and/or 32 weeks gestation at birth) comparing two levels of ARA with constant DHA showed that infants consuming formulas with greater ARA (2 fold) had better psychomotor development at two years of age, as well as significantly greater plasma ARA [27]. As a general matter, the weight of existing clinical evidence favors ARA inclusion with DHA and not the other way round.

Preclinical/Basic science

From the preclinical perspective, nutritional sources of ARA and DHA behave differently. In LCPUFA-free diets, all ARA and DHA must be obtained from biosynthesis in the body, via biochemical pathways that interact in the most intimate of



Biochemical pathways leading from precursor PUFA to LC-PUFA. The chemical shorthand for expressing PUFAs is n:d, where n is the carbons and d is the number of double bonds.

ways. The very same endogenous enzymes mediate the conversion of 18 carbon omega-6 linoleic acid (LA) to ARA and 18 carbon alpha-linolenic acid (ALA) to EPA and DHA (see box above). The structural difference in the omega-6 and omega-3 PUFA is subtle, only one double bond, and explains why they have common pathways. All these fatty acids and others interact to modulate tissue levels of fatty acids. This observation is the reason that the "ratio" of omega-6 to omega-3, or better, LA to ALA, is a key nutritional factor defining tissue composition. The pathway also shows that ARA is an intermediate for the formation of adrenic acid (AdrA), which also accumulates in the brain albeit at lower levels than ARA and DHA. The precise role of AdrA is not known but it also

is structurally similar to DHA, and rises when DHA is low, implying that it may have some common functions.

In industrialized diets, the 20th century emphasis on PUFA for control of serum cholesterol led to an excess of omega-6 LA and relatively low omega-3 ALA [28]. In part as a result, diets are replete with omega-6, while DHA is suppressed. Data from animal studies controlled far more precisely than any human study can be controlled demonstrate these effects are highly reproducible [29] and have consequences for functional endpoints, especially neural function [5]. Neural DHA levels are highly dependent on diet DHA levels, while ARA levels are much less so [30]. This does not mean, however,

that ARA metabolism is not affected, or that its role as a precursor of other metabolites is not inhibited when diet DHA is high and dietary ARA is absent. Indeed, animal data support the hypothesis that brain ARA metabolism is altered and inhibited in omega-6 deficiency, which would imply high DHA relative to ARA, albeit confounded by low ARA.

The proposal to routinely feed artificial formulas with DHA but without ARA has little support from animal studies designed specifically to test it. Alarmingly, feeding pregnant rats marine oil with high EPA/DHA and some small amount of ARA causes severe depression of ARA, to about half (-50%) of controls, in brain structural lipid in late gestation fetuses and in pups fed from weaning to 7 weeks of age [31]. The direction of such changes can be expected for human newborns, with severity of depression depending on many factors including the overall diet content of marine oil, neonate fatty acid status at birth, and duration of feeding. An artificial formula of unprecedented composition that causes radical changes in composition should not be in widespread use without clear evidence of its long term safety and that it supports infant development similar to breast milk.

Medically, ARA has long been known as a precursor for potent signaling molecules with diverse chronic and acute actions, such as regulators of inflammation, immune response, blood clotting, and parturition. Indeed, ARA's effects on heart disease are best known. Legacy drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, as well as newer blockbusters such as montelukast that enhance breathing in asthmatics, target inhibition of the synthesis or action of ARA products. These functions are thought to require only small amounts of ARA but they necessarily consume it, requiring a continuous supply.

The molecular and biochemical processes that control DHA and ARA synthesis are more complex than the net DHA and ARA that are eaten in the diet. Tools of modern biology are revealing how specific fatty acids interact with the molecular mediators and human genetics to modulate particular tissue compositions. Recent detailed genetic data indicates that the relative amounts of LA and ALA vs the LCPUFA drove evolutionary adaptation in humans [32], a result long suspected from now-classic studies [33].

Conclusions

Humans are among the most adaptable mammals. Many, and perhaps most, well-nourished infants will thrive and develop apparently normally without a source of preformed DHA and ARA, or with DHA only and provided with sufficient precursor omega-6 PUFA. However, large numbers of infants whose metabolic resilience is compromised due to illness, or whose genetics are tuned to intakes of preformed DHA and ARA, may well require a balance of DHA and ARA for optimal development. The infant nutrition research community has a model for optimal nutrition, human breast milk of well-nourished mothers. Its well-understood composition should be a heavily weighted guide for formulation of breast milk substitutes. Formula with DHA but no ARA is possible only with formulation of manufactured components leading to a fat composition that does not exist in nature. The standard must be clear and convincing evidence compared to the proper standard-of-care, human breastmilk, to move to such a composition.

Declaration of Interest. The author accepts research funding from DSM, a maker of ARA for infant formulas.

References

1. "EFSA_Panel_on_Dietetic_Products_Nutrition_and_Allergies", Scientific Opinion on the essential composition of infant and follow-on formulae. *EFSA Journal*, 2014. 12(7): p. 3760.

2. Koletzko, B., S.E. Carlson, and J.B. van Goudoever, Should Infant Formula Provide Both Omega-3 DHA and Omega-6 Arachidonic Acid? *Ann Nutr Metab*, 2015. 66(2-3): p. 137-8.

3. Brenna, J.T., Arachidonic acid needed in infant formula when docosahexaenoic acid is present. *Nutr Rev*, 2016. 74(5): p. 329-36.

4. Crawford, M.A., et al., The European Food Safety Authority recommendation for polyunsaturated fatty acid composition of infant formula overrules breast milk, puts infants at risk, and should be revised. *Prostaglandins Leukot Essent Fatty Acids*, 2015. 102-103: p. 1-3.

5. Brenna, J.T., Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life. *Matern Child Nutr*, 2011. 7 Suppl 2: p. 59-79.

6. Dobbing, J. and J. Sands, Comparative aspects of the brain growth spurt. *Early Hum Dev*, 1979. 3(1): p. 79-83.

7. Carver, J.D., et al., The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Res Bull*, 2001. 56(2): p. 79-85.

8. Brenna, J.T., et al., Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr*, 2007. 85(6): p. 1457-64.

9. Fleith, M. and M.T. Clandinin, Dietary PUFA for preterm and term infants: review of clinical studies. *Crit Rev Food Sci Nutr*, 2005. 45(3): p. 205-29.

10. Gibson, R.A., W. Chen, and M. Makrides, Randomized trials with polyunsaturated fatty acid interventions in preterm and term infants: functional and clinical outcomes. *Lipids*, 2001. 36(9): p. 873-83.

11. Hoffman, D.R., J.A. Boettcher, and D.A. Diersen-Schade, Toward optimizing vision and cognition in term infants by dietary docosahexaenoic and arachidonic acid supplementation: a review of randomized controlled trials. *Prostaglandins Leukot Essent Fatty Acids*, 2009. 81(2-3): p. 151-8.

12. Morale, S.E., et al., Duration of long-chain polyunsaturated fatty acids availability in the diet and visual acuity. *Early Hum Dev*, 2005. 81(2): p. 197-203.

13. Uauy, R., et al., Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. *J Pediatr*, 2003. 143(4 Suppl): p. S17-25.

14. Campoy, C., et al., Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *Br J Nutr*, 2012. 107 Suppl 2: p. S85-106.

15. Birch, E.E., et al., Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr Res*, 1998. 44(2): p. 201-9.

16. Birch, E.E., et al., A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev Med Child Neurol*, 2000. 42(3): p. 174-81.

17. Birch, E.E., et al., Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev*, 2007. 83(5): p. 279-84.

18. Hoffman, D.R., et al., Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *J Pediatr Gastroenterol Nutr*, 2000. 31(5): p. 540-53.

19. Makrides, M., et al., Dietary long-chain polyunsaturated fatty acids do not influence growth of term infants: A randomized clinical trial. *Pediatrics*, 1999. 104(3 Pt 1): p. 468-75.

20. Makrides, M., et al., A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. *Pediatrics*, 2000. 105(1 Pt 1): p. 32-8.

21. Auestad, N., et al., Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long chain polyunsaturated fatty acids for one year. Ross Pediatric Lipid Study. *Pediatr Res*, 1997. 41(1): p. 1-10.

22. Scott, D.T., et al., Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? *Pediatrics*, 1998. 102(5): p. E59.

23. Auestad, N., et al., Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics*, 2003. 112(3 Pt 1): p. e177-83.

24. Carlson, S.E., et al., Arachidonic acid status correlates with first year growth in preterm infants. *Proc Natl Acad Sci*

U S A, 1993. 90(3): p. 1073-7.

25. Makrides, M., et al., Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. *Am J Clin Nutr*, 2005. 81(5): p. 1094-101.

26. Simmer, K., S.K. Patole, and S.C. Rao, Long-chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev*, 2011(12): p. CD000376.

27. Alshweki, A., et al., Effects of different arachidonic acid supplementation on psychomotor development in very preterm infants; a randomized controlled trial. *Nutr J*, 2015. 14(1): p. 101.

28. Holman, R.T., S.B. Johnson, and P.L. Ogburn, Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. *Proc Natl Acad Sci* U S A, 1991. 88(11): p. 4835-9.

29. Holman, R.T., The slow discovery of the importance of omega 3 essential fatty acids in human health. *J Nutr*, 1998. 128(2 Suppl): p. 427S-433S.

30. Diau, G.Y., et al., The influence of long chain polyunsaturate supplementation on docosahexaenoic acid and arachidonic acid in baboon neonate central nervous system. *BMC Med*, 2005. 3(11): p. 11.

31. Yonekubo, A., et al., Dietary fish oil alters rat milk composition and liver and brain fatty acid composition of fetal and neonatal rats. *J Nutr*, 1993. 123(10): p. 1703-8.

32. Kothapalli, K.S., et al., Positive selection on a regulatory insertion-deletion polymorphism in FADS2 influences apparent endogenous synthesis of arachidonic acid. *Mol Biol Evol*, 2016. 33(7): p. 1726-39.

33. Gibson, R.A. and A.J. Sinclair, Are Eskimos obligate carnivores? *Lancet*, 1981. 1(8229): p. 1100. FOL