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EDITORIAL

All is Well that Ends Well – Looking Ahead to 2015

Dear readers of Fats of Life,

I am pleased to present you with a new issue of the PUFA Newsletter. Since the last issue in August, the field has moved on and many new and interesting studies on the roles of polyunsaturated fatty acids in health and disease have been published. I hope you will enjoy the summaries on a number of recent and newsworthy studies.

First of all, we highlight a study that has taken a detailed look at the potential different roles of long-chain omega-3 PUFA in women and men in the differential regulation of components of the coagulation pathway, based on previous observation that platelet aggregation and fibrinolysis in women and men are susceptible to EPA and DHA supplementation in different ways. Two experimental studies reflect the scientific progress in the relatively new understanding of how DHA and EPA bring about their blood-pressure lowering actions through the formation of epoxide derivatives. A large prospective study exposes interesting and complementary associations between the plasma phospholipid level of linoleic acid and long-chain omega-3 PUFAs and the reduced risk of different cardiovascular causes of death in elderly people.

Furthermore, we discuss an intervention study that indicates that in a large group of women with a history of atopy, there is evidence that a modest daily dose of DHA during the second trimester of pregnancy can lower the incidence of respiratory symptoms in their infants until 18 months of age. In a cross-over intervention trial an important dose-dependent reduction has been revealed in the frequency of epileptic seizures in adults with partial-onset epilepsy by fish oil.

In a study of women participating in the Nurses' Health Study II, the researchers have found a significant reduction in the risk of hearing loss in adult women with the highest intake of long-chain omega-3s. And finally, a small double blinded randomized clinical intervention trial has now shown that moderate periodontitis can be halted and partially reversed using a combination of DHA supplementation together with low dose aspirin intake.

This PUFA Newsletter also features an Invited Review by Dr. William Harris summarizing the collected scientific views on a study that was published last year purporting a positive association between omega-3 levels in blood and risk of prostate cancer, and which has been impactful among physicians and scientists, as well as consumers.

As always, the studies offer new insights and also bring up new questions. We hope that highlighting different aspects of research on omega-3s and other PUFAs will keep you informed on how the multiple areas of basic research, experimental clinical research and nutritional epidemiology are advancing.

With the last issue of this year at hand, I invite you to stay tuned next year for new and important research on omega-3s and other polyunsaturated fatty acids. There are plans to update the Fats of Life website to make the site more user-friendly and refresh its appearance. This year we had steady growth in the number of subscribers to the PUFA Newsletter and in 2015 we hope to reach even more people as we see Fats of Life as an interesting way to stay informed on the field and the importance of essential fatty acids for health. Feel free to share this and future issues with your colleagues by forwarding them the Subscribe link at: http://www.fatsoflife.com/subscribe.

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CARDIOVASCULAR HEALTH

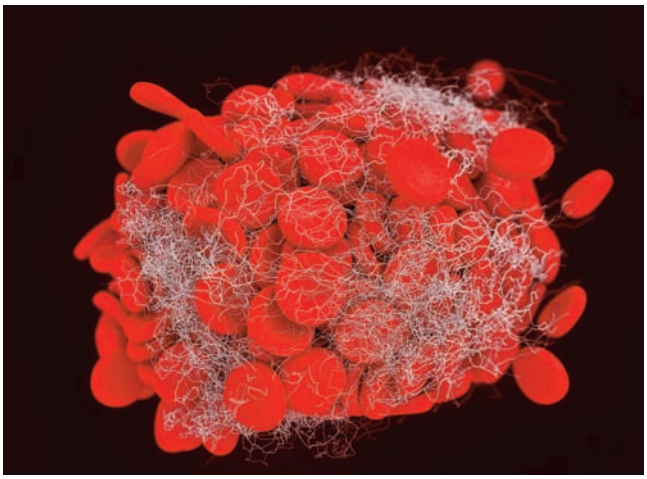
Sex Differences in the Effects of Omega-3s?

In biomedical research, metabolic and functional differences between the sexes for specific nutrients are often not specifically addressed and fully appreciated. Fundamental physiological processes that underlie the functioning of most organs are thought to be more similar than different between the sexes. So an interesting question is to what extent the function of essential fatty acids in health and disease is sex-dependent. Intuitively, dose-response differences may be expected, but do omega-3s and other PUFA serve qualitatively different purposes in male and female physiology?

Long chain omega-3 PUFA play an extremely important role during the perinatal period, with integral clinical implications for women and their infants. In male reproductive health, there is early evidence that omega-3s adequacy may be important for male fertility. But for the proper functioning of organs and biochemical processes that are common to women and men, do qualitative differences exist in the roles of omega-3s and other PUFA? This is a topic that has received relatively little attention thus far, yet is likely very relevant. Conditional indispensability is a term that indicates that the need for specific essential fatty acids changes during the life cycle, from conception to death. Would sex constitute another condition that determines the requirement for a specific PUFA? And how would that translate into the needs of women vs. men?

Are there any qualitative differences in the roles of essential fatty acids between men and women? In this study the researchers investigated the sex-specific changes in factors important in hemostasis and platelet aggregation that occur upon supplementation with EPA or DHA.

Researchers from the Nutraceuticals Group at the University of Newcastle, and the Haematology Research group at the Calvary Mater Hospital in Waratah, New South Wales, Australia made an interesting observation in 2009. They documented the aggregation properties of platelets from volunteers that were exposed to EPA or to DHA prior to exposing them to a stimulus which triggers their aggregation. Platelets are anucleated cells in the blood that sense damage to tissues by contacting damaged tissue structures that are normally not exposed to the blood in which they circulate, as well as to stressed endothelial cells lining the blood vessel lumen. Upon activation the platelets clump together to form an aggregate within a network of fibrin, initiating the formation of a blood clot that helps to confine an infectious stimulus and limit blood



loss upon vascular injury. Platelet aggregation and coagulation are also central to heart infarcts and ischaemic stroke, occurring after many years of vascular damage and plaque buildup. Long chain omega-3 PUFAs have been shown to reduce platelet aggregation, and increased intake of EPA/DHA is associated with a decreased risk of atherothrombotic mortality. The researchers reported that in platelets isolated from women, a short exposure to a relatively low concentration of EPA or DHA (1 micromolar) reduced platelet aggregation in whole blood stimulated with collagen (a common stimulus which mimics a damaged blood vessel). In contrast, in platelets isolated from men the external provision of DHA inhibited aggregation to about the same proportion as in women, but EPA did not inhibit aggregation.

The researchers thereafter undertook two studies where the effect of supplementation with EPA-rich oil and DHA-rich oil on platelet aggregability was determined in healthy men and women. The first study addressed the relatively fast effects of supplementation on the aggregatory activity of platelets in blood taken at time points up to 24 hours after supplementation. The study was carried out in a double-blinded fashion with randomized distribution of the volunteers, with a placebo group receiving sunflower oil instead of EPA or DHA. The EPA-rich oil consisted of 1 g of EPA and 200 mg DHA as triglyceride, and the DHA-rich oil consisted of 1 g DHA and 200 mg EPA as triglyceride, both given in the form of two capsules. Thereafter, platelet aggregation in the presence of collagen in whole blood samples was studied in identical fashion to the original study. Platelet

aggregation in men was reduced by up to 21% by intake of EPA, whereas DHA had no effect. In women the opposite effect was observed with DHA supplementation leading to a 14% decrease in platelet aggregability, but no effect of EPA supplementation.

In the second intervention study published in 2013, the same research group returned to study the effect in groups of healthy men and women that received supplementation of their diet with either the EPA-rich oil or the DHA-rich oil, at the same daily dose as in the first 24 hour study. In the latter study, the effect on platelets was determined after four weeks of supplementation. Again, a clear difference between men and women was found in their platelet-aggregating ability; while in men the supplementation with an EPA-rich oil led to a decreased ability to aggregate, in women it was the supplementation with the DHA-rich oil that induced a decrease in the ability of their platelets to aggregate. *Vice versa*, supplementation with DHA or EPA did not have an effect of platelet aggregation in men compared to women, respectively. Clearly, a sex-dependent difference between the effect of EPA and DHA on platelet aggregation was found, but a direct translation of the previously observed *in vitro* responses did not seem to hold for the situation *in vivo*, neither within 24 hours nor after 4 weeks.

In both studies marked correlations between the levels of sex hormones and platelet aggregation were found. Following DHA supplementation, the plasma level of testosterone correlated positively with platelet aggregation, whereas in the context of EPA supplementation this association was negative. Of interest, in the four week study, men that received EPA displayed a modest but significantly reduced level of plasminogen-activator inhibitor-1 (PAI-1) in plasma compared to women who displayed a small increase in PAI-1 levels. PAI-1 constitutes an important control over fibrinolytic activity, critical for the timely dissolution of blood clots, through (negatively) regulating the activity of urokinase and tissue plasminogen activator. This study suggested that men may receive more benefit from EPA, and women more from DHA, to lower thrombotic disease risk. However, given that the *in vivo* sex-specific responses are qualitatively different from the direct *in vitro* responses on platelet aggregation, selective effects on different components of the hemostatic system in addition to fibrinolysis were possible. Precisely over which components of the hemostatic system EPA and DHA exerted their principal and differential actions remains to be addressed in further detail.

To document in further detail the sex-specific effects of EPA and DHA on different coagulation factors involved in hemostasis, the authors recently reported the results of further research. Citrated blood samples taken at the end of the same four week intervention described above were analyzed for a number of important coagulation factors. The plasma levels of factors II (prothrombin), V, VII, VIII, IX and X were determined using coagulometric methods employing respective coagulation factor-deficient plasma samples. Fibrinogen (factor I), von Willebrand factor (factor VIII) activity and antigen, and the capacity to form thrombin (endogenous thrombin potential) were also measured. There were no significant differences between men and women at baseline for the tested coagulation factors, or the plasma levels of DHA, docosapentaenoic acid or arachidonic acid. Women had a lower baseline level of EPA, a higher platelet count and aggregatory activity, compared to the men. As expected, baseline average levels of the sex hormones, testosterone and oestradiol, were significantly different between men and women.

After the four week supplementation period, plasma EPA and DHA levels had increased measurably in all subjects taken together in comparison to the control group. AA levels remained unchanged. As had been reported before, supplementation with EPA-rich oil or DHA-rich oil decreased platelet aggregation (by 11.8 and 14.8%, respectively). However, no measurable changes were found in any of the coagulation factors. Only when the test subjects were stratified by sex did any significant differences become apparent. EPA reduced

The study indicates that in men supplementation with EPA may reduce prothrombin, factor V and von Willebrand factor in plasma, but not in women. Such changes may contribute to the reduced platelet aggregation observed in men upon supplementation with EPA.

platelet aggregation in men significantly more strongly (18.4% reduction) compared to women (5.5% reduction) and the control group. In DHA-supplemented subjects, the effect was much stronger in women (18.9% reduction) compared to men (9% reduction) and the control group (2.1% reduction).

In men supplemented with EPA, prothrombin levels were reduced by 7.9%, which was a significant effect when compared to males that had received DHA (3% increase in prothrombin) and the control group (5.5% increase). No major changes in prothrombin were observed in women. Factor V levels were significantly decreased in men receiving EPA compared to the control group, but no significant changes were detected in women, or in men receiving DHA. Furthermore, a decrease in the plasma level of von Willebrand factor was found in the plasma of EPA-supplemented men compared to EPA-supplemented women. Another significant difference was that women who received the DHA supplement had higher incorporation of DHA into plasma lipids than men receiving DHA. Von Willebrand factor facilitates the binding of collagen to platelets, and a decrease may be involved in reduced platelet aggregation. Decreased levels of factor V and pro-thrombin lower the potential for coagulation through decreasing both thrombin formation and thrombin-stimulated formation of fibrin. The activity of EPA and DHA themselves with decreased platelet aggregation may also be related to the formation of microdomains in the platelet membrane that lower inter-platelet interactions, or through the formation of EPA- and DHA-derived lipid mediators with agonist-specific anti-platelet actions.

The researchers examined the relationships between the level of sex hormones, platelet aggregation, and the identified coagulation factors that were affected by omega-3 intake after stratification by sex. An inverse association was found between testosterone levels and platelet aggregation after supplementation with EPA. Interestingly this association was positive after supplementation with DHA. Lower oestradiol levels were associated with lower prothrombin and factor V levels after EPA supplementation. Lastly, a positive association between the decrease in von Willebrand factor and reduction in platelet aggregation after EPA supplementation was observed.

This study suggests there may be differences in the way that hemostasis is regulated by long chain omega-3 PUFA in men and women. Although there were no significant differences at baseline between men and women for prothrombin, factor V, von Willebrand factor, and DHA level, the statistical significance of the measured supplementation-induced changes in coagulation factors, platelet aggregation and plasma fatty acids was determined for the percentage changes in the values in blood samples collected at four weeks post-intervention with baseline values. An alternative and possibly more precise manner to determine statistically significant changes would have been to use absolute values of the various variables of interest and examine post-intervention results adjusted for baseline differences.

Differential regulation of hemostasis by EPA and DHA in men and women was found to be strongly determined by the levels of progesterone and oestradiol. In men a negative association between platelet aggregation and testosterone levels was also associated with a negative association of the level of testosterone with DHA distribution into platelet phospholipids. Estrogens have been shown to increase plasma DHA levels in men, suggesting that the distribution and transfer of DHA to platelet membrane phospholipids is regulated by sex hormones, or that endogenous biosynthesis of DHA from omega-3 precursors is increased.

The results may indicate that differential regulation of hemostasis by EPA or DHA supplementation in men and women is strongly related to the levels of the sex hormones progesterone and oestradiol.

The results also imply that in men, improving DHA delivery to platelets may have a thromboprotective effect, but at higher testosterone levels, the male sex hormone dominates over oestradiol, and it is EPA that exerts control over the aggregability of platelets. These anti-aggregatory effects of EPA may be associated with measurable reductions in prothrombin, factor V, and von Willebrand factor. In women, the association appears to be the reverse, with DHA having anti-aggregatory effects in the context of DHA distribution into platelets. However, in women there were no specific changes in any of the tested coagulation factors that associated with the inhibitory effects of DHA.

The study by Phang and colleagues suggests there may be differential regulation of hemostasis between the sexes by EPA and DHA. Intrinsic differences in the way that EPA and DHA are distributed and incorporated into platelet membranes appear to occur in men and women, likely under direct control of male and female sex hormones. But fundamental differences in the way that EPA and DHA regulate the levels of several components of the coagulation cascade, platelet reactivity itself, as well as the process of fibrinolysis, may also be at play. With chronic cardiovascular diseases frequently

responsible for fatal infarcts and the debilitating disorders resulting from thromboemobolic events, taking into account the fundamental differences between men and women is of high relevance. It is hoped that these differences are further delineated, particularly in the diseased state, and may one day provide more precise recommendations for the consumption of long chain omega-3s in the population at risk.

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Epoxides of DHA and Blood Pressure Lowering

High blood pressure is a major risk factor for developing cardiovascular disease. A significant benefit for cardiovascular health can be achieved through normalizing blood pressure. High blood pressure corresponds to a systolic pressure above 140 mm Hg and a diastolic blood pressure greater than 90 mm Hg. Globally, the prevalence of adults with elevated blood pressure is approximately 40%. The consequences of chronically elevated blood pressure are various; coronary artery plaque formation leading to coronary heart disease, stroke due to arterial plague disruption and atherothrombosis in ascending arteries, heart failure, peripheral vascular disease, damage to the kidneys, retinal haemorrhage and visual impairment. A number of risk factors for hypertension are known although often the mechanisms behind a person having an elevated blood pressure are not always clear.

Blood pressure is primarily determined by resistance vessels in healthy people. Chronically elevated blood pressure is often caused by decreased vascular patency (potential to dilate) of the arteries, leading to an increased vascular resistance that the heart has to work against. Localized changes in blood flow and chronically in-

An average daily intake greater than or equal to 2 grams a day of EPA and DHA can lower both systolic and diastolic blood pressure in individuals with hypertension who do not receive anti-hypertensive drugs.

creased vascular pressure lead to hemodynamic injury of the endothelial cells lining the blood vessels, creating a sustained activation of these cells that favors their interaction with white blood cells as well as sub-endothelial fluid accumulation and early signs of inflammation. These initial events can ultimately lead to localized inflammation of the blood vessels and atheroma formation (vascular plaque formation that precedes atherosclerosis).

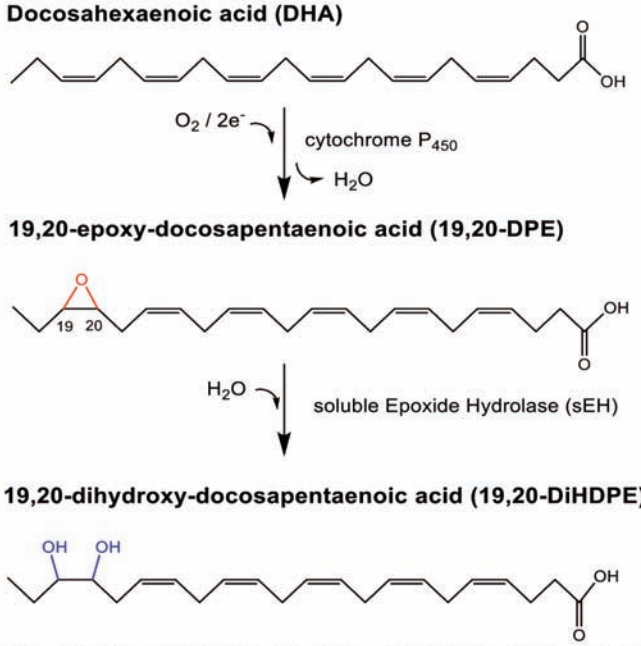
Although blood pressure increases with age, high blood pressure can be controlled through lifestyle changes such as modification of the diet, reduced alcohol consumption and increased physical activity, as well as by pharmacological treatment. A high need for sustainable and cost-effective interventions that can halt and reverse the rising prevalence of hypertension has been acknowledged. The intake of long chain omega-3s is now recognized as one option to reduce blood pressure. An average daily intake greater than or equal to 2 grams a day of EPA and DHA lowers both systolic and diastolic blood pressure in individuals with hypertension who do not receive anti-hypertensive drugs. The extent of blood pressure lowering is clinically meaningful, and at least as effective as other interventions such as sodium and alcohol restriction or physical exercise. The mechanisms whereby long chain omega-3s reduce blood pressure include improved functioning of the vascular endothelium leading to reduced vascular resistance, and a reduction in heart rate and improved filling of the heart. A recent study in mice showed that blood pressure-lowering by an omega-3-enriched diet depends to a large extent on the activation of nitric oxide-dependent vasodilation of resistance vessels.

Two recent studies address novel mechanisms whereby EPA and DHA may induce blood pressure-lowering effects through the formation of epoxide-group-containing derivatives of EPA and DHA.

Two recent studies provide new information on possible mechanisms how DHA and EPA contribute to blood pressure lowering. The first study by Ulu and colleagues from the University of California in Davis is an experimental exploratory study carried out in mice to identify new mediators derived from DHA that have hypotensive actions. The second study is from Schuchardt and colleagues at the Leibniz University and the University of Veterinary Medicine in Hannover, Germany, and researchers in the first study.

The study by Ulu et al. was undertaken as a result of earlier observations that a diet rich in EPA and DHA lowers systolic blood pressure in hypertensive mice. A diet rich in omega-6 PUFA does not offer this anti-hypertensive effect. This research group has long-standing experience in delineating the physiological role of an enzyme called soluble epoxide hydrolase (sEH) and inhibition of this enzyme was found to enhance the anti-hypertensive effect of the omega-3-enriched diet. The formation of a derivative of DHA called 19,20-epoxy-docosapentaenoic acid (19,20-EDP; Figure 1) accompanied the reduction in blood pressure. In the present study, the direct hypotensive action of 19,20-EDP was determined. 19,20-EDP was infused

Figure 1. What is a DHA epoxide? Shown here is the chemical structure of 19,20-epoxy-docosapentaenoic acid (19,20-EDP: *middle*) a derivative of DHA (*top*). 19,20-EDP is formed by an enzyme called a cytochrome P450, and is degraded by another enzyme called soluble epoxide hydrolase (sEH). Inhibitors of sEH have been developed and limit the degradation of PUFA epoxides, thereby potentiating their activities in vivo.



The epoxide derivative of DHA, 19,20-EDP, that Ulu and colleagues have studied has been shown to mediate at least part of the blood pressure-lowering activity of DHA. 19,20-EDP is also produced in humans after a single dose of EPA and DHA, as shown in the study by Schuchardt and colleagues. An *epoxide* (red color) is an oxygen atom bound in a cyclic configuration to a carbon chain. After hydrolysis (incorporation of water) by sEH it converts to a *vicinal* (neighboring) *diol* (two hydroxyl groups located next to each other; blue color). The diol formed from 19,20-DPE is named 19,20-dihydroxy-docosapentaenoic acid (19,20-diHDPE)

from a subcutaneously implanted minipump, and in order to reduce potential fast metabolic degradation, 19,20-EDP was also infused with an inhibitor of the sEH enzyme that breaks down 19.20-EDP. Angiotensin-II (Ang-II) was employed to induce hypertension, also administered subcutaneously. When determined over a 6-day period, administration of 19,20-EDP resulted in a significant reduction (approximately 30%) in Ang-II-increased systolic blood pressure. The hypertensive effect of Ang-II was further reduced to approximately half in the presence of the sEH inhibitor.

Measurement of 19,20-EDP in the circulation demonstrated that blood plasma levels were increased and stabilized by the sEH inhibitor. In the kidney, the inhibitor raised 19,20-EDP levels without the need for co-infusion of 19,20-EDP, pointing out that endogenous formation of 19,20-EDP in Ang-II-induced hypertension is already activated in the kidney and protected from metabolism when the inhibitor is present. A fatty acid epoxide is hydrolyzed by epoxide hydrolases to the corresponding diol, a substance that has two hydroxyl groups bound on neighboring carbon atoms (Figure 1). The expected diol derivative of 19,20-EDP was found in plasma, but not in plasma of those mice that had also received the sEH inhibitor. Of interest, mice treated with 19,20-EDP had decreased expression of the message for the receptor of Ang-II, *AT1a*, in kidney tissue, which correlated with the anti-hypertensive effect. These results suggest that 19,20-EDP may mediate part of the anti-hypertensive actions of DHA, not excluding additional mechanisms of action, such as formation of other mediators derived from DHA and EPA. Further studies will be required to demonstrate that specific epoxide derivatives of DHA and EPA are responsible for blood pressure-lowering activity in humans.

To obtain evidence for the formation of such derivatives in humans, Schuchardt and colleagues studied six healthy male volunteers that took an EPA/DHA supplement followed by measurement of the formation of the omega-3 PUFA epoxides. The participants were slightly overweight (average BMI of 25) and had a normal lipid profile. The study subjects avoided ingestion of foods rich in omega-3 PUFA during the preceding four weeks (no fish, seafood, or alpha-linolenic acid-rich vegetables) in order to minimize variability in long chain omega-3 PUFA status and blood levels. After an overnight fast they ingested four capsules of EPA and DHA in the form of re-esterified triglycerides amounting to a total dose of 1008 mg EPA and 672 mg DHA. The capsules were taken with a standardized breakfast with a defined energy content, as well as protein and lipid content. The subjects were followed for 48 hours, during which standardized meals were eaten at precise times. Blood samples were taken before supplementation and 6, 8, 24 and 48 hours after.

A significant increase in the level of EPA within plasma phospholipids was detected as early as six hours after taking the oral supplementation. Maximal levels were reached at eight hours, at which point the level had approximately doubled from baseline to nearly 2% of total fatty acids. In contrast no significant change in the plasma phospholipid level of DHA was observed.

Various classes of derivatives of EPA, DHA and arachidonic acid were measured in EDTA plasma samples, such as monohydroxylated derivatives (containing a single hydroxyl group), di-hydroxylated derivatives, as well as the derivatives containing an epoxide group. Individual members of each of these broad compound classifications have been shown to possess biological activity, *e.g.* in the regulation of inflammation and blood pressure. The authors focused on a sub-group of lipid mediators that are primarily formed through the action of several enzymes called cytochrome P_{450}. Specific cytochrome P_{450} enzymes have been shown to readily oxygenate EPA and DHA even more efficiently than arachidonic acid when presented to the enzyme. All lipid mediators were prepared for analysis by means of alkaline hydrolysis to release any plasma lipid-bound mediators.

The study reveals that a number of lipid mediators derived from EPA were present in plasma at increased concentrations six hours after supplementation. These included several epoxide derivatives of EPA, as well as the corresponding vicinal diols. Whereas no measurable change in the level of the DHA-derived lipid mediator 19,20-DPE was found

A number of epoxide derivatives of EPA and DHA are formed and measurable in blood plasma several hours after a single dose of EPA/DHA by human volunteers, including the blood pressure-lowering DHA derivative 19,20-DPE.

in plasma, supplementation led to a 25% increase in the level of the corresponding diol 19,20-diHEPE at eight hours. The results suggest that the potentially anti-hypertensive DHA derivative 19,20-DPE is generated upon supplementation with DHA, but that rapid metabolism by sEH (Figure 1) metabolizes the substance to the diol. The results also indicate that EPA undergoes significantly more extensive conversion than DHA to epoxide-containing lipid mediators measurable in plasma, or that the EPA-epoxides are more resistant to further metabolism, after a single dosing in adults.

The two studies together provide new insight into the plausible mechanisms whereby the anti-hypertensive actions of EPA and DHA are mediated. The fact that basal levels of 19,20-DPE are detectable in the circulation, and that small increases in formation may occur after a single oral dose of DHA in human volunteers, suggests that different pools of epoxygenated lipid mediators are present in plasma, and that supplementation may transiently increase the level in one pool. It will be interesting to see future studies in normotensive and hypertensive people in which different blood cells and specific lipids are measured for their content and changes in 19,20-DPE and other epoxygenated derivatives of EPA and DHA upon supplementation.

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Independent Cardiovascular Disease Risk Reduction Associated with Linoleic Acid and Long-Chain Omega-3 Fatty Acids

Linoleic acid is recognized as an essential fatty acid for human health because it cannot be synthesized by humans and needs to be ingested through the diet. It is a precursor of the polyunsaturated fatty acids (PUFA) of the omega-6 type, of which arachidonic acid is the most well-known. Arachidonic acid is the substrate for a large number of local hormones in the body, the eicosanoids, that regulate many aspects of tissue physiology. Linoleic acid itself is needed for proper formation of the skin epithelium, facilitating the formation of the corneocyte lipid envelope consisting of lipids cross-linked to protein to form a relatively impermeable barrier to water and exogenous pathogens.

Dietary intake of linoleic acid has increased considerably in Western societies over the past decades, and has become the most abundant polyunsaturated fatty acid in our diet. The average consumption of n-6 polyunsaturated fatty acids exceeds nutritional requirements, and it has been recognized that daily intake of linoleic acid can be lowered substantially, and intake of long chain omega-3 PUFA (omega-3 LCPUFA) increased, to achieve better health. The reason that ingesting lower amounts of linoleic acid may be beneficial is two-fold. Firstly, linoleic acid biotransformation to arachidonic acid uses the

The precise roles of the relative contributions of essential fatty acids that we ingest as well as the relevance of their levels in different compartments of the body (organs, tissues and cell-types) is a topic of ongoing research.

same enzymes as those needed to convert the essential omega-3 fatty acid alpha-linolenic acid to EPA and DHA. With high intake levels of linoleic acid, this "competition" would significantly suppress the already low endogenous formation of EPA and DHA. Secondly, linoleic acid is the precursor of arachidonic acid, and high levels of linoleic acid intake support the formation of relatively high tissue levels of arachidonic acid. Too high levels of arachidonic acid are considered negative for good health since they may favor the formation of the arachidonic acid-derived eicosanoids that are largely (but not uniquely) inflammation-promoting, increasing the risk for chronic inflammatory and thromboembolic disorders.

The precise roles of the relative contributions of essential fatty acids that we ingest as well as the relevance of their levels in different compartments of the body (organs, tissues and cell-types) is a topic of ongoing research. The easy division between the health-promoting actions of omega-3 versus "inflammation-promoting" omega-6 PUFA is not as straightforward and simple to make as one may expect, since arachidonic acid serves as a precursor to both anti-inflammatory and pro-inflammatory local hormones, and also plays different roles in different organs, under healthy or pathological conditions. Nevertheless, too high absolute and relative levels of arachidonic acid compared to

EPA and DHA are associated with the risk for chronic inflammatory disease that trace back to fundamental dietary imbalances. Whereas linoleic acid is recognized as an essential fatty acid, the association of high "omega-6" intake and arachidonic acid tissue levels with a predisposition to the development of chronic inflammatory disease, has led to the situation where relatively little attention has been paid to the possible potential benefits of linoleic acid itself, either as found in our diet or in our tissues, may have to health.

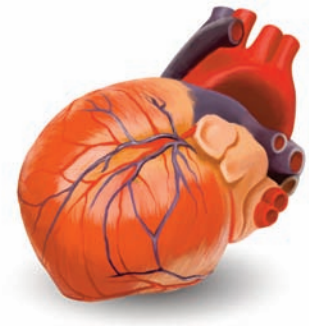
A prospective study carried out by Wu and colleagues from The George Institute of Global Health at the University of Sydney, Australia, and a number of universities in the US, has examined the associations of the levels of individual PUFA in plasma phospholipids with the risk of total mortality, and mortality from a number of cardiovascular disease causes. The multi-center study (named Cardiovascular Health Study) employed standardized protocols and annual in-clinic evaluations and telephone contact, to follow a cohort of elderly (≥65 yrs) men and women - a total of 2792 participants - who were free of cardiovascular disease (CVD) at the onset of the study, for a period of eighteen years. The composition of PUFA in subjects' plasma phospholipids was measured at the beginning of the study, and again between 15 years and the end of the study. Mortality was followed throughout the 18 years that the study was ongoing. Specifically the nature of mortality was determined and classified as "all-cause", or by its cause. Furthermore, the incidence of fatal and non-fatal coronary heart disease (CHD) and stroke, and arrhythmic CHD deaths were measured.

The study determined the associations between the plasma levels of individual polyunsaturated fatty acids and the risk of total mortality and mortality from a number of disease causes in elderly American men and women.

The associations of the plasma phospholipid level of a specific PUFA with the observed risk were determined by regression analysis. Stratification was applied using age (<median, ≥median), gender, race, and plasma omega-3 PUFA (<median, ≥median). During the study period, 1994 deaths occurred, of which 678 deaths were related to cardiovascular cause (427 fatal and 418 non-fatal coronary infarctions, and 154 fatal and 399 non-fatal strokes occurred). Estimated dietary intake of individual fatty acids had been assessed in the subjects prior to study initiation using a validated semi-quantitative picture-sort food-frequency questionnaire.

The results of the study indicate that a higher level of linoleic acid in plasma phospholipids is associated with a statistically significant lower total mortality. The highest quintile of linoleic acid (elderly people who had a median of 22.9% linoleic acid as % of total fatty acids) was associated with a 13% reduced risk, when compared to those elderly people who had the lowest levels (median of 16.6% of total fatty acids). The trend in the

level of risk reduction with increasing levels of linoleic acid in plasma phospholipids was also found to be statistically significant. In study subjects with higher levels of linoleic acid, the reduced mortality risk was largely associated with cardiovascular disease causes, and in particular with a decrease in death not attributable to arrhythmias. In fact, the highest level of linoleic acid was associated with a 22% reduced mortality risk if restricted to death from CVD. The highest level of linoleic acid was associated with a 49% reduced risk of non-arrhythmic CHD mortality, and a 45% reduced risk of mortality from congestive heart failure. However, linoleic acid was not associated with arrhythmic CHD mortality. Among non-cardiovascular causes of death, a 54% risk reduction for respiratory death was associated with the highest plasma phospholipid level of linoleic acid. Median intake of linoleic acid by the study participants was 6% of total energy.



An additional finding of interest was that the highest levels of both linoleic acid and omega-3 PUFAs predicted a further risk reduction for total mortality. Elderly people with the highest levels of linoleic acid and omega-3 PUFA had a 54% lower risk of total mortality and a 64% lower risk of CVD mortality, compared to study subjects with the lowest levels of both. None of the other PUFAs in plasma phospholipids was associated with reduced or increased risk in mortality. Careful inspection of baseline characteristics of the subjects indicated that statistically significant trends could be observed between quintiles with increasing percentages of linoleic acid in plasma phospholipids; lower average age, higher % males, higher % white Americans, increased annual income, decreased % diabetes mellitus and treated hypertension, increased physical activity, decreased body mass index, decreased waist circumference, lower number of daily servings of fruit, and increased usage of vegetable oil and butter for cooking.

The results are the findings of a prospective study that has been executed over a period of several years. The surprising result is important for our understanding of the specific associations, and potential contributions, that different essential fatty acids have in disease prevention through lowering the risk for particular disease etiologies. This study is a prospective study and only associations can be offered, which in addition are limited to elderly American men and women. Of interest, the study points to complementary associations for omega-3 LCPUFA and linoleic acid in the primary prevention of cardiovascular pathologies. In this scenario higher circulating levels of linoleic acid play a more marked role in reducing the risk for non-arrhythmic CVD, whereas omega-3 LCPUFA appear to associate more strongly with a reduced risk for arrhythmic disorders leading to death. The combined and additive risk reduction appears to give a compelling indication that both omega-6s and omega-3s are important for health. This study furthermore indicates that the highest plasma phospholipid linoleic acid levels are actually associated with a reduced total and CVD mortality in healthy older adults.

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The levels of PUFA in plasma phospholipids may not perfectly follow a close relationship with their long-term dietary intake, and could be a reflection of transient concentration changes occurring after recent food intake. Basing correlations on a single baseline measurement of plasma phospholipid levels may have limited value for reflecting long-term intake of specific fatty acids. The levels of linoleic acid in plasma phospholipids has been reported to follow the changes in red blood cell membranes, considered a pool that is more strongly associated with longer term intake patterns. The authors state the reported associations may even be underestimations of true associations, and that inter-individual correlations between plasma phospholipid composition of omega-6 PUFA and measured risk factors are comparable to associations for other cardiovascular risk factors such as blood pressure. Also the fasting plasma phospholipid levels of omega-3 LCPUFA reflect their habitual dietary intake, and offer some indication of long-term intake behavior. In any case, it will be of interest to see confirmation that the associations observed in the present study between PUFA levels in plasma phospholipid and mortality will also be observed when linoleic acid and omega-3 LCPUFA are measured in red blood cell membranes and, if possible, tissue biopsies, which may be more confident read-outs for longer term intake.

The publication discusses a number of other studies that have indicated beneficial associations for linoleic acid and mortality across distinct populations. A recent meta-analysis of prospective cohort studies also points out a meaningful reduced risk in coronary heart disease by linoleic acid in a dose-dependent

fashion. Notably, changes in dietary intake of one fatty acid are often linked to concomitant changes in other lipid components of the diet. Currently, the debate on the potential beneficial effects of dietary linoleic acid is open, as meta-analyses of intervention studies have pointed to both protective and deleterious effects of increased dietary intake of omega-6 PUFA and linoleic acid on coronary heart disease and death. A more detailed analysis of the effects that can be uniquely attributed to changes in dietary linoleic acid compared to other PUFA and other fatty acids is highly desirable, to extricate the interdependent effects present in intervention trials.

The results of this study indicate that linoleic acid in plasma phospholipids may be a predictor for the risk of non-arrhythmic type of cardiovascular death, such as heart failure, in elderly Americans. Omega-3 LCPUFA levels appear to be associated with reducing the mortality caused by arrhythmias. Future studies that explore the mechanism whereby linoleic acid itself might be involved in lowering CVD, and in combination with omega-3 LCPUFA EPA and DHA, are clearly of interest, to understand such potential complementary actions on different aspects of cardiovascular disease. It is interesting to see that we can gain further appreciation for the "essentiality" of both the omega-6 and omega-3 fatty acids, which are not necessarily competing to achieve better health. Especially in the elderly, where risk of CVD is greatest, this seems worthy of further examination and scrutiny.

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MATERNAL AND INFANT HEALTH

DHA Supplementation of Atopic Mothers during Pregnancy Reduces Respiratory Symptoms in Their Children

Since omega-3 fatty acids are known to have anti-inflammatory activity, and allergic disease is typified by the active release of inflammatory mediators, several studies have investigated whether omega-3 fatty acid intake can lower the incidence of allergies. Within this line of thought, clinical researchers have investigated the possibility that the omega-3 status of the mother during pregnancy affects the incidence of allergic reactions in her offspring. This refers to eczema, asthma, and rhinitis, with symptoms including bronchial hyper-responsiveness (wheezing and difficulty breathing), coughing, irritated skin and rash, and nasal discharge. Atopy is the collective description of a heritable portion of allergies, and is linked to the spontaneous increased production of one type of antibodies called immunoglobulins type E (IgE), which bind to allergens and trigger mast cell-mediated inflammatory responses. Heritability is important, but does not follow a simple model; it is likely that multiple genetic traits contribute to increased total IgE as well as allergen-specific antibodies. Increased IgE predisposes to allergic reactions, with illness from the respiratory symptoms of atopy being particularly disabling for the infants and a burden for the families. Studies which have investigated the effect of an increased intake of EPA/DHA or fish oils containing long chain omega-3s, or the importance of omega-3 status of the expecting mother, on the incidence of allergic disorders in the offspring have produced equivocal results.

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A research group led by Dr. Isabelle Romieu from the National Institute of Public Health (INSP) in Cuernavaca, Mexico, in collaboration with the World Health Organization and the International Agency for Research in Cancer in Lyon, France, has now reported the results of a double-blind, randomized, placebo-controlled trial in pregnant women who received a modest daily dose of DHA during the second pregnancy trimester and whose children were monitored for respiratory symptoms. This study is part of an ongoing collaborative effort between the INSP in Cuernavaca, and Emory University in Atlanta, USA, and is called the Prenatal DHA (n-3) Fatty Acid Supplements on Infant Growth and Development (POSGRAO) Study. The women were aged 18 to 35 years and received supplemental DHA between 18 and 22 weeks of gestation. In total 1094 eligible pregnant women were recruited from the Mexican Social Security Institute hospital system. As specific eligibility criteria the women were asked to breastfeed for three months after birth and indicate their intent to remain in their area of current residence for two years after delivery. Women with high-risk pregnancies, lipid absorption disorders, or who regularly consumed fish oil or DHA supplements or certain medications, were not eligible for the trial.

The women were randomly assigned to one of two groups. Women in the first group received 400 mg of DHA (in two capsules) daily during weeks 18 to 22 of gestation, whereas those in the second group received a placebo composed of a blend of corn oil and soybean oil. There were no notable differences in baseline characteristics of the mothers in the treatment groups. Over half of all the women were overweight (59%) and 32.6% of mothers were atopic. Median intake of dietary omega-3 fatty acids prior to entry into the trial was low at 55 mg/day. The changes in fatty acid status of plasma and breast milk in the DHA-supplemented and control groups have previously been reported; small and statistically significant increases in plasma DHA and total omega-3 level were achieved by the 5-week DHA-supplementation, as well as increased levels of DHA and alpha-linolenic acid in breast milk at one month post-partum. Furthermore, there were no significant differences in base-

This study determined the effect of a daily 400 mg DHA supplement taken by pregnant women during weeks 18 to 22 of gestation on the incidence rate of a number of respiratory symptoms that developed in newborns during the first 18 months after delivery.

line dietary intake and intake of individual fatty acids between the two groups. It is important to note that basal DHA level in plasma (1.3% of all fatty acids) was relatively low, even after supplementation (1.7%). The DHA level in breast milk, even after supplementation, also remained relatively low (0.22% of all fatty acids).

After delivery, the children were monitored every month up to 12 months and again at 18 months of age. Information on the presence or absence of respiratory signs and symptoms, as well as the number and duration of episodes, was determined using a clinical questionnaire. The following variables were scored: *i)* coughing, *ii)* wheezing, *iii)* difficulty breathing, *iv)* wheezing and difficulty breathing, *v)* coughing with wheezing and/or difficult breathing, *vi)* coughing with phlegm, *vii)* phlegm, nasal discharge, and/or stuffy nose, *viii)* fever with phlegm and nasal discharge or nasal congestion, *ix)* coughing with fever, and *x)* wheezing with fever. Maternal atopy was determined by measurement of IgE levels in plasma. A cut-off level of > 70 IU/ml was considered indicative of atopic mothers. Additional variables were recorded such as demographics, household characteristics, parents' education, and environmental exposure to allergens, as well as food consumption and dietary intakes of fatty acids.

The respiratory symptoms that developed in the newborns during the first 18 months after delivery were calculated as episodes per person days, or so-called incidence rate (the number of new cases per population at risk in a given time period). An incidence rate ratio was calculated as the relative incidence rates of respiratory symptoms in the children of the DHA-supplemented mothers compared to those of placebo-treated mothers. Until 18 months, significantly decreased incidence rate ratios were observed for the following respiratory symptoms in children from atopic mothers who had received DHA during pregnancy: a 35% decrease in "phlegm with congestion and/or nasal discharge", a 48% decrease in "fever with phlegm with congestion and/or nasal discharge", and a 57% decrease in the incidence rate of "wheezing with fever". However, in the children of non-atopic mothers, the incidence of some respiratory symptoms was aggravated by DHA supplementation: a 26% increase in the incidence rate ratio of "coughing with wheezing and/or breathing difficulty", a 13% increase in "phlegm with congestion and/or nasal discharge", and a 26% increase in "fever with phlegm with congestion and/or nasal discharge".

After adjustment of the statistical model for the sex of the child, low birth weight and maternal education, the risk for displaying respiratory symptoms was always higher in children from mothers who received placebo instead of DHA. Children from atopic mothers that had received DHA had significantly lower incidence rate ratios than those of non-atopic mothers of "phlegm with nasal congestion or nasal discharge" (22% lower), and "fever with phlegm with congestion and/or nasal discharge" (47% lower). In summary, supplementation of atopic women with DHA during five weeks in the second trimester of pregnancy markedly lowered the incidence rate of several respiratory symptoms in their infants over the first one and a half years. In non-atopic women this effect was not observed and what was observed was a modest increase in incidence rate ratios for some respiratory symptoms.

The authors point out that it was not possible to distinguish between respiratory symptoms of viral infections and allergic reactions. The same authors previously published that pre-natal supplementation with 400 mg DHA reduced symptoms of colds, such as cough, phlegm, and wheezing in one month old newborns, less time ill in three month olds, and a shorter duration of fever, nasal secretions, difficulty breathing, and rash in six month olds. More precise measurements need to be made if this discrimination of allergies from viral colds in babies is required; for example a combination of the family history of atopy, cord blood IgE, and skin assessment may be a suitable way to identify babies at high risk of allergy. To be able to attribute the observed beneficial effects of DHA to the modulation of allergic respiratory symptoms, this discrimination appears important since previous research has not been able to demonstrate a role for LC omega-3s in the allergic manifestations or lower respiratory tract infections in breast-fed infants. A further methodological limitation was that the questionnaire that was used for scoring symptoms had not been fully validated for Mexican mothers.

Using cord blood samples from the same study, the supplementation of pregnant mothers with DHA was shown to change the methylation of specific DNA-sequences in monocytes collected at the time of delivery. That such epigenetic modifications could occur upon changes in the mother-to-fetus supply of an essential fatty acid has long been sus-

The present study suggests that mothers with a history of atopy and low basal intake of omega-3 could consume a modest daily dose of 400 mg DHA in the second trimester of pregnancy to reduce the frequency of respiratory symptoms in their children during the first 18 months of life.

pected. Undoubtedly new research will continue to focus on delineating the precise requirements that the developing fetus has for omega-3 fatty acids in relation to specific modulation of regulatory DNA sequences and the development of the immune system. Since atopy is heritable, it is conceivable that epigenetic modulation activated by specific nutrients may contribute to the modulation of atopy, and if understood can be used to lower symptoms associated with allergies. Such studies may ultimately contribute to our understanding why the offspring of non-atopic mothers did not receive a more marked benefit from supplemental DHA with respect to respiratory symptoms.

Practical and cost-effective interventions that can reduce the incidence of respiratory symptoms in large groups of newborns are of course of significant relevance to public health. Irrespective of the precise mechanism, the present study indicates that mothers with a history of atopy and low basal intake of omega-3 could consume a modest daily dose of 400 mg DHA in the second trimester of pregnancy to markedly reduce the frequency of respiratory symptoms in their children during the first 18 months of life. That appears to be the case in a region of Mexico, and it is hoped that the results hold for other parts of the world.

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BRAIN AND CNS

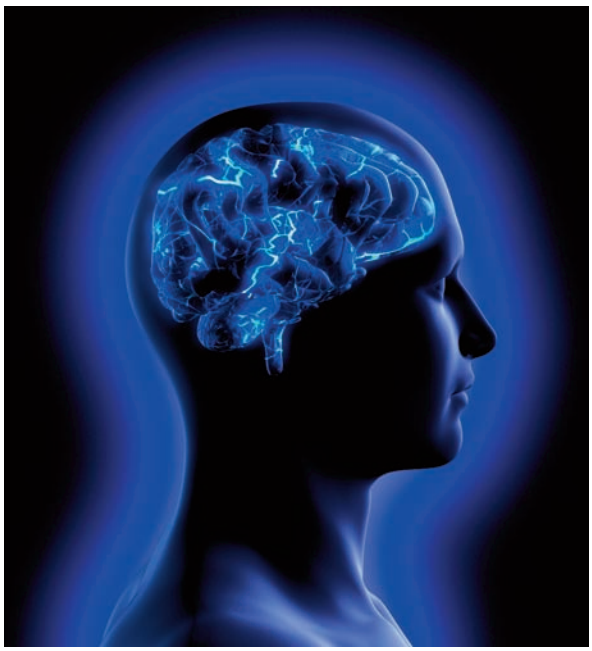
A Moderate Dose of Fish Oil: Beneficial for Drug-Resistant Partial-Onset Epilepsy

Epilepsy is a disorder of the central nervous system (CNS) in which individuals experience epileptic seizures, displays of transient deregulated neural activity in one or both hemispheres. Epileptic activity displays as changes in sensory awareness and involuntary motor activation of specific body parts, which can occur with consciousness preserved, or with loss of consciousness. Seizures can range from mild and unnoticeable to others to generalized seizures that can last several minutes or longer. The different forms and grades of severity of epilepsy may be linked to specific areas of the central nervous system that are damaged, but in many cases the precise location of nervous system dysregulation is unknown.

Drug-resistant epilepsy is defined as the failure to respond to two or more antiepileptic drugs at a therapeutic dose. Further improvements in overcoming currently untreatable epilepsy are the subject of much research.

The prevalence of epilepsy is on the order of one out of every 150 people, but one in every 26 people may be affected with epilepsy at some point in their life. One type of epilepsy involves the temporal lobe, with a sub-type called medial temporal lobe epilepsy being a more common form. Here, sclerosis (*i.e.* a structural change) of the hippocampus appears to constitute an important defect. Seizures are frequently caused by such localized brain damage as a result of injury, hypoxia, exposure to certain chemical substances, or infection during pregnancy, but can also be caused by a brain tumor. Brain injury due to an ischaemic stroke can bring about epilepsy. Genetic predisposition contributes to the development of epilepsy.

Epilepsy is treatable, permitting those affected to live a relatively normal life. Treatment includes the use of antiepileptic drugs that aim to restore alterations in inhibitory neurotransmitter activity in the brain. However, in approximately a third of epilepsy patients, currently available antiepileptic drugs are ineffective. So-called drug-resistant epilepsy is defined as the failure to respond to two or more antiepileptic drugs at a therapeutic dose. Epilepsy associated with tumors is often poorly controlled with drugs and a better option may be surgical treatment. Neuromodulation (*e.g.* deep brain stimulation) holds promises for the treatment of several neurological disorders, including epilepsy. Further improvements in overcoming currently untreatable epilepsy are the subject of much research. Another important treatment consideration is that many available anticonvulsants are associated with a substantial burden of side effects and risk of teratogenicity in the case of pregnancy.



DHA is a structural component of the brain and supports neurotransmission in the central nervous system. In animal models of epilepsy, the provision of DHA has been shown to improve neuronal activity dysregulated in epilepsy, and to delay the onset of epileptic episodes. Animal studies have shown that DHA is important for hippocampal neurogenesis, and it can limit neuronal hyper-excitability in the hippocampus.

Christopher DeGiorgio and collegues in the Departments of Neurology, Cardiology and Neurobiology at the University of California Los Angeles School of Medicine in California, USA, now report on the results of an intervention that studied the effect of fish oil on drug-resistant epilepsy. Twenty-four participants with drug-resistant epilepsy took part in a randomized double-blind cross-over trial that aimed to examine the anti-epileptic effect of two doses of EPA/DHA-containing fish oil. The participants had partial onset epilepsy, which means that the epilepsy displayed starts in one hemisphere (and does not include generalized seizures that originate in both hemispheres). The subjects included both men and women and were aged 18 to 56 years. Study participants were

randomized to six different treatment sequences, with the entire trial lasting 42 weeks. After completing a treatment sequence each subject had thus completed a cross-over trial in which he/she received a low-dose fish oil, a high-dose fish-oil and a placebo oil, in random order. Each treatment period lasted 10 weeks, with wash-out periods lasting 6 weeks between each treatment period. In this study, participants were continued on their anticonvulsant medication regimens.

The study addressed whether people with drug-resistant partial-onset epilepsy received any benefit from supplementation with the long-chain omega-3s EPA and DHA during 10 weeks in an intervention trial with a cross-over format that tested two different dosing regimens.

The low-dose fish oil contained 1080 mg omega-3 fatty acids per day in 3 capsules taken daily, and included 216 mg EPA and 144 mg DHA. The high-dose fish oil contained twice these amounts (six capsules). Six daily capsules of corn oil were used as placebo, and three corn oil capsules were used as placebo to complement the low-dose fish oil intake. Characteristics of the study participants were well characterized at baseline and monitored throughout the study, at the beginning and end of each treatment period. None of the patients had other illnesses, or had consumed fish oils, aspirin, or warfarin 30 days prior to the trial. The cross-over study design was chosen because a parallel intervention is not suitable for trials on epilepsy with a relatively low number of participants and the heterogeneous nature of epilepsy that gives rise to large between-group differences in seizure frequency at baseline.

The primary endpoint of the study was the percentage change in the frequency of seizures for the three different treatments, testing the null-hypothesis that fish oil would not affect the endpoint compared to placebo. The total seizure frequency was defined as the total number of simple partial seizures (fully conscious changes in sensations or movements), complex partial seizures (diverse sensory changes and involuntary movements with partial or complete loss of consciousness) and generalized tonic/clonic seizures (loss of consciousness, with generalized spasms and involuntary muscle activity). Changes in seizure rates were expressed as the percentage of the rate in either one of the two fish oil dosing treatment periods compared to the seizure rate observed in the placebo treatment period, regardless of treatment order.

Low-dose fish oil reduced the average seizure frequency by 33.6% compared to placebo (from 18.3 seizures per month to 12.2). Two of the subjects were free of seizures during low dose fish oil treatment. Surprisingly, the high dose of fish oil did not change the frequency of seizures compared to placebo. A comparison between the low and high dose revealed that a 31% reduction in seizure frequency was attained during the low-dose fish oil treatment. No significant changes in the severity of seizures were found using a Chalfont Seizure Severity Scale. Low dose fish oil resulted in a non-significant increase in high-frequency heart rate variability compared to placebo ($p=0.09$).

The study highlights that dose-ranging studies are fundamental to fully characterize the efficacy of EPA/DHA and fish oil. Loss of efficacy in epilepsy at higher doses of DHA has also been shown in the rat, where increased latencies in experimental seizures induced by treatment with DHA are lost at higher DHA doses. That an optimal dosage regimen is hard to define and implement is perhaps reflected in a study where dietary supplementation with a relatively high consumption of DHA or EPA for four weeks was not found to reduce seizure latency in four different murine epilepsy models.

An increase in high-frequency heart-rate variability (HRV) has been associated with a decreased risk for fatal arrhythmias and sudden death. Epilepsy is also associated with an increased risk of death. The potential effect of the low-dose fish oil on high frequency HRV may point at a concurrent cardio-protective effect in the tested epilepsy patients. In this respect, it is interesting to note that a U-shaped relationship between the level of DHA in red blood cells, as well as between the consumption of long-chain omega-3s, and the risk of atrial fibrillation has been recently reported. Finding the optimal dose of EPA/DHA, instead of merely "higher is better", is therefore an important concept to bear in mind while trying to develop clinically meaningful and insightful use of omega-3s.

Supplementation with a daily dose of approximately 350 mg EPA/DHA during 10 weeks reduced the number of seizures in people with drug-resistant partial-onset epilepsy. The effect was lost at a higher dose.

The cross-over study design has shown that a relatively low dose of EPA/DHA in fish oil capsules offers promising improvements in the frequency of seizures in people with epilepsy, at least in those with partial-onset epilepsy. The

present study constitutes a significant advance and provides important impetus to carry out larger trials to assess the use of EPA/DHA treatment as a new means to target drug-resistant epilepsy. It is important to note that fish oil was not tested as monotherapy for seizures in this study, and was added to ongoing treatment with anticonvulsant medications. Future studies are required to assess the efficacy of long-chain omega-3s as monotherapy for seizure disorders.

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CLINICAL CONDITIONS

Higher Intake of Fish and Long-Chain Omega-3s Linked to Reduced Hearing Loss in Women

The auditory system is an important sensory system, permitting the rapid perception of sounds that reach us, helping us orient ourselves within the surroundings. Through coordination with proprioceptor and the visual systems, the auditory system also helps in fine-tuning the self-awareness and mental image of our body by hearing the relative distances of our body parts when they produce noise. Given the importance of our auditory system, the consequences of hearing loss are significant to daily life, and encompass a wide range of physical and social effects; e.g. compromised social interactions, impairments in learning, loneliness, depression, loss of confidence, loss of income due to working disabilities, fatigue and headaches.

Disabling hearing loss is defined as a hearing threshold greater than 40 decibel (dB) of sound pressure level in the better ear (>30 dB in children). Normal hearing is when auditory thresholds are below 25 dB across a range of frequencies that we can normally perceive. The prevalence of hearing loss globally is startling, with ~5% of the world population experiencing a measurable level of hearing loss. The prevalence of hearing loss increases with age and around a third of elderly people (>65 years) have a significant loss in hearing ability. Prevalence of disabling hearing loss is reportedly highest in South Asia, Asia Pacific and Sub-Saharan Africa. In South Asia, disabling hearing loss is 2.4% in children (up to 15 years), 9.5% in men, and 7.0% in women. In Europe/North-America it affects 4.9% of adult men and 4.4% in women. The lowest prevalence is found in the Middle-East and North Africa (4.1% of men and 2.9% women). Clearly, hearing loss is a disability of major proportions.

The prevalence of hearing loss globally is startling, with approximately 5% of the world population experiencing a measurable level of hearing loss.

Most cases of hearing loss are acquired. Exposure to loud and/or persistent noise over certain noxious levels can cause irreversible damage of auditory tissue structures. The loss of auditory hair cells, the sensory structures that ultimately capture sound transduced through air and bone structures into the cochlea, and damage to the spiral ganglia neurites that innervate these hair cells appears central to irreparable hearing loss. Noise exposure is one important cause of hearing loss, but exposure to ototoxic substances, such as some antibiotics, can also contribute.

Sharon Curhan and colleagues at the Channing Division of Network Medicine, Brigham & Women's Hospital, Boston, MA in collaboration with the Vanderbilt University School of Medicine (Nashville, TN) and the Harvard School of Public Health Medicine in Boston, MA, USA, now report on the results of a prospective cohort study that determined the relationships between fish consumption, long-chain omega-3 intake, and the risk of hearing loss. The study forms part of the Nurses´ Health Study, one of the world's largest currently ongoing prospective cohort studies. The Nurses' Health Study II is a cohort study initiated in 1989 as a joint activity by the Channing Division of Network Medicine and the Harvard School of Public Health. A group of over 116,000 female nurses, who enrolled at an age between 27 and 42 years, are interviewed every two years with validated questionnaires to gather information on their diet, lifestyle variables, and health. Medical records provide information on disease incidence, supplementary questionnaires are used to gather additional information, and tissue samples are collected from subsets of participants. The hearing loss study falls within the scope of better understanding complex chronic diseases using a variety of approaches taken by the Channing Division of Network Medicine.

The present study aims at better understanding if certain dietary habits affect hearing loss incidence. In the Nurses' Health Study II specific information on dietary intake is assessed every four years by means of a semi-quantitative food-frequency questionaire. Baseline characteristics refer to 1991, the year when assessment was first made. Fish and shellfish consumption was determined by asking participants for the frequency of consumption of three classes of fish (canned tuna, light-meat fish, dark-meat fish), as well as shellfish. Total fish

The study aimed at determining if fish consumption and long-chain omega-3 PUFA intake affect hearing loss incidence in a very large group of adult women followed during an 18 year period.

intake was obtained by summing up all fish and shellfish intake. The content of specific fatty acids was calculated using the reported food item intake multiplied by the specific content per serving reported by the US Department of Agriculture and a Harvard University food-composition database. The use of the employed food-frequency questionnaire had been shown previously to allow the ranking of study subjects by fish and fatty acid intake.

In the 2009 questionnaire, women were also asked whether they had a hearing problem and when this was first noticed. After exclusion of women that did not respond, regularly took omega-3 and other supplements, provided incomplete or unreliable information on their dietary habits, or had cancer, the results of 65,216 women were available for further analysis. The primary study outcome, self-reported hearing loss, was scored as either no hearing problem, a moderate hearing problem, or a severe hearing problem. The study authors indicate that self-reported hearing loss assessment is a reasonably reliable method to assess hearing loss, and that audiometric measurements could not be made on such a large study population.

Over the 18-year study period, 11,606 cases of hearing loss were reported to have occurred. Using multivariable models that permitted correcting for potential confounders such as the use of some medicines, intake of different nutrients, age, and race, among other factors, it was found that women who consumed one serving of fish a week had a 5 to 6% lower risk of hearing loss compared to women who consumed fish less than once a month (see Table 1 for the age-adjusted relative risk). Consuming fish at least twice a week was associated with an 18 to 20% decrease in reported hearing loss. The type of fish that the women consumed did not affect the observed risk reduction.

When the researchers looked at the relationship between the cumulative average intake of fatty acids and hearing loss, increased consumption of both total omega-3 PUFA and long-chain omega-3 PUFA was associated with a decreased risk of hearing loss. Linolenic acid consumption was unrelated to hearing loss incidence. The group of women with the highest consumption of LC omega-3 PUFA (median of 0.39 gram per day) had a 22% decreased risk of hearing loss, compared to women with the lowest median intake (60 mil-

Table 1. Relative risk of hearing loss in women observed in the Nurses' Health Study II, expressed as risk ratios relative to the lowest number of servings of fish (left), or the lowest intake of LC omega-3 PUFAs (by quintiles of cumulative average fatty acid intake).

Fish consumption (servings)	Risk Ratio (95% CI)		Median LC omega-3 intake (mg per day)	Risk Ratio (95% CI)
<1 per month	1.00		60	1.00
1-3 per month	1.02 (*0.95-1.10*)		100	0.96 (*0.91-1.02*)
1 per week	0.95 (*0.89-1.01*)		150	0.88 (*0.84-0.93*)
2-4 per week	0.82 (*0.75-0.89*)		250	0.88 (*0.83-0.93*)
≥ 5 per week	0.82 (*0.70-0.97*)		390	0.78 (*0.74-0.83*)

Age-adjusted risk ratios are indicated. CI: confidence interval

ligram a day). No relationship between omega-6 intake or age and hearing loss was found in these women.

In summary, this large prospective study provides support for the existence of a negative association between LC-omega-3 PUFA (and total omega-3) intake as well as fish consumption,

and hearing loss in North-American adult women. Fats of Life has previously reported on studies that also found associations between increased omega-3 intake and reduced age-related hearing loss. Changes in hearing loss in the present study were measured through self-reported indications of hearing capability. Such measurements are not objective readouts of auditory function and do not measure auditory thresholds. Information on subjective awareness on auditory capacity is nevertheless valuable as it refers to the significance people attach to their auditory perception. Limitations in the use of self-reported hearing loss questionnaires have been acknowledged.

The results obtained correspond to relatively low doses of LC omega-3s, within the daily dosing ranges that are recommended for healthy adults. It is important to stress that this study does not prove a causal relationship between fish consumption or LC-omega-3 intake, and a lowered risk of hearing loss. It may happen that North American women who eat more fish or have a higher LC omega-3 intake also experience less exposure to one or more primary causes of hearing loss, such as over-exposure to noise and ototoxic substances. Well-designed intervention studies will need to be undertaken to prove a causal relationship between prevention of hearing loss and increased LC omega-3 intake or fish and shellfish consumption.

Reduced blood flow in the inner ear is believed to be an important contributor to cochlear tissue injury, for example, after noise-induced hearing loss. There is still little direct evidence that LC omega-3 PUFAs may reduce hearing loss by supporting adequate blood flow in the cochlea. In Meniere's disease, the progressive hearing loss may be caused by intermittent ischemic injury of the inner ear, and a protective role for LC omega-3 PUFA in inner ear homeostasis has been postulated. Also, interest in understanding the role of the immune system in inner ear functioning is budding. The role of LC omega-3 PUFA in cochlear and hair cell functioning has certainly not been studied in much detail, in contrast to the visual system where LC omega-3s are known to play essential structural and functional roles.

Fish consumption in North American women during the same time period and with similar age of the women studied here is quite low. Of women in the age group 36-49 years, 15% do not consume any seafood, and 23% consume just one or two servings a month. Since the prevalence of hearing loss is disquieting, the findings of this prospective study are important. They suggest that certain dietary habits such as fish consumption and LC omega-3 intake may be related to hearing loss incidence. How important we may be able to appreciate better approximately 15 years from now, when the youngest nurses that enrolled in this large and ongoing study will have reached the age of 65 years. As the prevalence of debilitating hearing loss will have ascended to approximately a third of the women, it will be very interesting to determine if a delay in progressive hearing loss in the same women studied at present remains associated with higher fish consumption and LC omega-3 PUFA intake.

North American adult women with the highest consumption of LC omega-3 PUFA (median of 0.39 gram per day) had a 22% decreased risk of hearing loss, compared to women with the lowest median intake (60 milligram a day).

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FOL

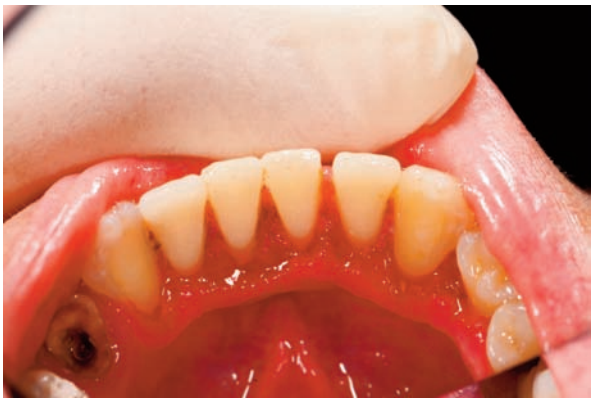
DHA and Low-Dose Aspirin, a Promising Treatment for Periodontitis

One of the most common afflictions in people is gingivitis, a mild inflammation of the gums. Uncontrolled gingivitis can lead to periodontitis, inflammation of the tissue structures underlying the gums, affecting the integrity and anchoring of the teeth. Severe periodontitis was recently reported to affect 11% of the world population and ranks among the 10 most common human diseases. Periodontitis not only affects oral health, but is now strongly suspected to also augment the risk for some systemic diseases that have an inflammatory component, such as cardiovascular disease.

The main cause of periodontitis is poor oral hygiene. Severe forms of periodontitis rank among the ten most common human diseases globally. In recent years, a radically different approach for treating periodontitis is emerging whereby the host is stimulated to control the oral infection and the immune-mediated tissue damage.

The main cause of periodontitis is poor oral hygiene, allowing the growth of harmful bacteria in plaques at the border of the gums. The infection triggers a sustained inflammation of the gums, leading to loss of adherence of the gums to the teeth with the formation of a deepening sulcus (space between the gums and teeth). Sustained inflammation can lead to further retraction of the gums and microbial colonization of periodontal pockets, with loss of supporting structures, exposure of the tooth roots, and damage to the teeth. Other factors such as poor nutrition, smoking, stress and some diseases can contribute to the development of periodontitis. Intervention in periodontitis involves specialized cleaning procedures and surgical intervention, as well as the use of antibiotics, and is generally focused at keeping sub-gingival and periodontal infection at bay. Surgical intervention techniques are also applied to remodel the damaged tissue to try restoring tissue shape. Periodontitis can be halted with proper oral care, but as lost tissue is considered to have limited, if any, capacity to renew, the aim of maintenance regimens is to stop further progression of tissue damage.

In recent years, a different approach is emerging whereby the host is stimulated to control the oral infection and the im-



mune-mediated tissue damage. Experimental research a few years ago showed that in a rabbit model of periodontitis, the EPA-derived lipid mediator resolvin E1 (RvE1), applied locally, was capable of lowering the inflammatory response, halting further tissue damage, and inducing the restoration of lost tissue including bone. RvE1 is formed from EPA under conditions of inflammation where the type 2 cyclooxygenase enzyme is acetylated by aspirin. Whereas there is no clinical evidence from experimental trials that aspirin alone, or supplementation with LC omega-3s alone, affect the incidence of periodontitis, two recent randomized controlled studies have highlighted the effectiveness of the combination of aspirin and DHA to improve the outcome of a regenerative surgical therapy, and a scaling and root planing procedure. The combination of long-chain omega-3 fatty acids EPA and/or DHA together with aspirin is now being advanced as a paradigm shift and low-cost intervention in periodontal therapy.

Now, Naqvi and colleagues at the Beth Israel Deaconess Medical Center, the Harvard School of Public Health, both in Boston, MA, and the Forsyth Institute in Cambridge, MA, USA, have addressed the possibility that the combination of DHA and aspirin, without any concommitant surgical or non-surgical intervention, is able to produce protective effects in moderate periodontitis in adults. Thereto, 55 men and women, aged 40 years and above, with moderate periodontitis were recruited. They had at least four teeth with pocket probing depths greater or equal to 5mm, and had at least 20 natural teeth. Pocket depth is the depth of the sulcus formed between the gums and the teeth, measured by a dentist using a millimetric periodontal probe. The participants were randomized into two groups in a parallel double-blinded design. All participants received a daily 81 milligram dose of aspirin (2-acetylsalicylic acid). This daily amount of aspirin is considered a "low dose". One intervention group received four daily capsules each containing 509 mg DHA (daily dose approximately 2 g DHA). The control group received four identical capules containing a mixture of corn oil and soybean oil.

The intervention period lasted for three months, after which the participants returned for periodontal assessment. The primary outcome assessment was per-pocket change in pocket depth in periodontal pockets with a baseline depth ≥5 mm. The gingival index (a measurement of the level of inflammation of the gums), and the plaque index (a measurement of the level of plaque and soft matter adherent to the teeth surfaces and margin of the gums), were also determined. Additional information on local and systemic inflammation was obtained from measurements of high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and IL-1β in crevicular fluid (the inflammatory fluid accumulating in periodontal pockets), and the plasma levels of hsCRP, IL-6, VCAM and N-terminal telopeptides. Forty-six participants completed the study. The red blood cell membrane levels of long-chain omega-3 PUFA were measured as well.

A group of adults with moderate periodontitis received 2 grams DHA and a low-dose aspirin every day during three months. The effects on periodontal pocket depth and several other aspects of periodontal disease and inflammation were determined.

Average red blood cell membrane DHA levels increased from 3.6% to 6.2% in the participants who received 2 g DHA daily, but remained unchanged in the control group. The study showed that over the three month period, the mean pocket depth of pockets with a baseline depth ≥5 mm decreased significantly more in the people who received DHA and aspirin than in participants who only took aspirin. A clinically significant reduction of pocket depths of 2 mm was more likely to occur in the DHA group than in the control

group, and DHA supplementation also significantly reduced the number of probing sites with pocket depths ≥5 mm. DHA furthermore reduced the gingival index, but had no effect on the plaque index.

A reduction in inflammation, as suggested by the reduced gingival index, was confirmed by the significantly reduced crevicular levels of hsCRP and IL-1β, compared to the control group. No significant differences were found in the systemic levels of inflammatory cytokines between both groups. The authors took great effort to rule out any loss of blinding during the research, and the involved dentists were inter-validated for their ability to make periodontal assessments in a reproducible manner.

In summary, this carefully executed intervention study provides us with the first clinical experimental evidence that supplemental DHA together with low-dose aspirin may be a suitable approach to halt and reverse moderate periodontitis in adults, in the absence of other periodontal interventions. The trial had a relatively small sample size, and larger trials will provide greater power to demonstrate and substantiate the promising findings. The observed effect of DHA and low-dose aspirin in this study should not be extrapolated to the suitability of other long-chain PUFA such as EPA. On the other hand, EPA may be expected to function as well, since it has been originally shown that the aspirin-triggered EPA derivative RVE1 is efficacious in a rabbit model of periodontitis. Undoubtedly, we will see further progress in the treatment of periodontitis based on the principle that stimulating the host anti-inflammatory and inflammation resolving response may be a beneficial way to improve oral health.

Treatment with DHA and low-dose aspirin resulted in a significant reduction in pocket depth of pockets with a baseline depth ≥5mm, as well as a reduction in localized inflammation of the gums and periodontal pockets.

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What is Periodontitis – European Federation of Periodontology: http://www.efp.org/public/more-on-periodontitis.html FOL

GUEST ARTICLE

The 2013 Omega-3 Fatty Acid-Prostate Cancer Debacle

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On July 11, 2013 a paper was published online by Brasky et al. in the *J Natl Cancer Institute* entitled, "Plasma Phospholipid Fatty Acids and Prostate Cancer Risk in the SELECT Trial."(1) This paper was widely publicized across all media platforms, even finding its way onto the network evening news programs.

What did the study actually show? In this study, plasma phospholipid omega-3 levels were measured in 834 men who eventually developed prostate cancer (the time between plasma sampling and diagnosis is not available from the abstract), and 1393 men who did not. Using standard statistical methods, they found that men in the highest quartile of omega-3 had a 43% to 71% increased risk for prostate cancer (depending on severity). This is the same conclusion that the same group reached in 2011 in a study in another cohort entitled, "Serum Phospholipid Fatty Acids and Prostate Cancer Risk: Results from the Prostate Cancer Prevention Trial" (2). So with two studies reaching the same conclusion, it is important to seriously consider its findings.

Here is the conclusion in the abstract from Brasky et al (1): "This study confirms previous reports of increased prostate cancer risk among men with *high* blood concentrations of LC ω-3PUFA. The consistency of these findings suggests that these fatty acids are involved in prostate *tumorigenesis*. Recommendations to increase LCω-3PUFA intake should consider its potential risks." (italics introduced by the author).

What are the problems here? *First, blood levels were far from "high."* The reported EPA+DHA level in the plasma phospholipids in this study was 3.62% in the no-cancer control group, 3.67% in the low grade cancer group, and 3.74% in the high-grade group. These differences between cases and controls are very small and would have no meaning clinically as they are within the normal variation. Based on experiments in our lab, the lowest quartile would correspond to an Omega-3 Index of <3.16% and the highest to an Index of >4.77%. These values are obviously low, and virtually none of the subjects were in "danger" of having an Omega-3 Index of >8%. In Framingham[a], the mean Omega-3 Index of participants who were *not* taking fish oil supplements was 5.2% and for those taking supplements, it was 7.5% (3). Both of these numbers are considerably higher than the values reported by Brasky et al., even in their highest quartile. Thus, it is extremely unlikely that these patients were taking fish oil supplements. Indeed, the SELECT study (in which all these patients were participants) was a randomized trial of vitamin E and selenium supplements for the prevention of prostate cancer. In the study protocol, it is stipulated that if the subjects wanted to take a multi-vitamin, the study would provide it; nothing is said about fish oil supplements, but it is hard to imagine their use was widespread in this trial.

So to conclude that regular consumption of two oily fish meals a week or taking fish oil supplements (both of which would result in an Index above the observed range) would increase risk for prostate cancer is extrapolating far beyond the data. This study did not test the question of whether *giving* fish oil supplements (or eating more oily fish) increased prostate cancer risk; it looked only at blood levels of omega-3 which are determined by intake, other dietary factors, metabolism and genetics. The endless repetition of "supplements are dangerous" in the news media is not based on any data from this study.

Associations do not imply causation. The second problem with the Brasky conclusion was the use of the word "tumorigenesis" – tumor causation. This is the most basic of logical errors that both scientists and science writers should be well aware of. Even granting that the associations they reported are real, the findings of this study do not mean that EPA and DHA play any role in the *development* of prostate cancer. For example, it is possible that some component of whatever fish these patients were consuming was carcinogenic, in which case the serum omega-3 levels were just a marker of fish (i.e., carcinogen) intake. They also failed to consider another potential explanation for their observed association, i.e., reverse causation, which is always a possibility in studies with this design. A much larger proportion of men who ultimately developed cancer (30-40%) had PSA levels >3 at baseline (compared to 7% of the controls). Thus, it is possible that sub-clinical prostate cancer was already developing in the higher risk men. He et al. (4) and Azordegan et al. (5) both provide evidence that, in pre-cancerous tissues, early changes in fatty acid metabolism (e.g., increases in the activity of delta-6-desaturase which is the rate limiting enzyme in the generation of long-chain from short-chain omega-3 fatty acids) could increase tissue (and possibly plasma?) levels of

long-chain n-3 fatty acids. Hence, it is possible that metabolic changes (e.g., desaturase upregulation) associated with the carcinogenic process could have raised omega-3 levels. Differences in fish intake (unmeasured in this study) or in fish oil supplement use (prohibited in this study) may have had nothing to do with the microscopically higher plasma levels.

A Wider Perspective

The authors also failed to present the fuller story taught by the literature. The same team reported in 2010 that the use of fish oil supplements was *not* associated with any increased risk for prostate cancer (6). A 2010 meta-analysis of fish consumption and prostate cancer reported a reduction in late stage or fatal cancer among cohort studies, but no overall relationship between prostate cancer and fish intake (7). Terry et al. in 2001 (8) reported higher fish intake was associated with lower risk for prostate cancer incidence and death, and Leitzmann et al. in 2004 (9) reported similar findings. Higher intakes of canned, preserved fish were reported to be associated with reduced risk for prostate cancer (10). Epstein et al. found that a higher omega-3 fatty acid intake predicted better survival for men who already had prostate cancer (11), and increased fish intake was associated with a 63% reduction in risk for aggressive prostate cancer in a case-control study by Fradet et al (12). The incidence of prostate cancer is much higher than mortality from prostate cancer, and LC n-3 PUFA may play a role in limiting the transition from sub-clinical prostate cancer stages to aggressive forms that are responsible for death from prostate cancer (13). So there is considerable evidence actually FAVORING an increase in fish intake for prostate cancer risk reduction.

Another piece of the picture is to compare prostate cancer rates in Japan vs the US. Here is a quote from the World Foundation of Urology*:

"[Prostate cancer] incidence is really high in North America and Northern Europe (e.g., 63 X 100,000 white men and 102 X 100,000 Afro-Americans in the United States), but very low in Asia (e.g., 10 X 100,000 men in Japan)." *http://www.prostate-cancerprevention.net/index.php?p=prostate-cancer*

Since the Japanese typically eat about 8x more omega-3 fatty acids than Americans do and their blood levels are twice as high, you'd think their prostate cancer risk would be much higher… but the opposite is the case.

There is also a wealth of evidence from randomized clinical trials (RCT) with fish oils and omega-3 concentrates in which the incidence of cancer (rarely sub-setted) is always tracked as a possible adverse event. The table below shows the findings for the eight major studies reported to date, which included over 78,000 patients. In none of these studies was cancer incidence significantly increased by omega-3 fatty acid supplementation.

There will always be mixed findings in studies of "diet" and "disease" since both predictor and outcome entail so many variables, known and unknown. Higher omega-3 levels are associated with lower rates of death from any cause (22, 23), from sudden cardiac arrest (24), and with slower rates of cellular aging (25). It is therefore important to put these findings into perspective (which the authors failed to do). First consider the risk of dying from prostate cancer vs ischemic heart disease (IHD). Based on the National Vital Statistics Report for deaths in the US in males in 2010, (http://www.cdc.gov/nchs/data/dvs/deaths_2010_release.pdf), there were about 28,500 deaths from prostate cancer and 207,500 deaths from IHD: a 7.3x higher rate of death for heart disease. If one assumes (conservatively) that higher fish intake reduces risk for death from heart disease by only 10%, and (liberally) increases risk for death from prostate cancer by 50%, then the chances of dying from CHD are still 4.4x higher than from prostate cancer. This very crude analysis suggests that even in the worst case scenario, the

Table: Reported incident cancer diagnosis (or cancer deaths)

Trial	n	Duration (yrs)	Placebo	N-3
Alpha-Omega (14) (prostate cancer)	4837	3.4	0.8%	1.4%
GISSI-Heart Failure (15) (cancer death)	6975	3.9	3.2%	3.1%
GISSI-Prevenzione (16)	11,320	3.5	2.25%	2.65%
JELIS (17)	18,645	4.6	2.4%	2.6%
SUFOLOM3 (18) (cancer death)	2501	4.2	6.5%	7%
Origin (19)	12,536	6.2	"no difference in the rate of cancer"	
Risk and Prevention (20)	12,513	5	7.2%	7.9%
Omega (21)	3851	1	1.4%	1.7%

benefit of higher omega-3 intakes/levels still far outweighs the risk.

In summary, the work of Brasky et al. does add to the evidence-base for omega-3 fatty acids and prostate cancer, which taken as a whole (not even getting into animal studies, which are typically positive) support a neutral, if not beneficial, effect of fish oil in prostate cancer. The RCT data do not support an effect of omega-3 on cancer risk in general, and a 2012 review of omega-3 and prostate cancer concluded, "Thus, epidemiological studies provide inconsistent results, suggesting an inverse association of LC n-3 PUFA" (26). A recent report from the European Food Safety Authority's Panel on Dietetic Products, Nutrition and Allergy (27) examined the fish oil – prostate cancer question and concluded after extensive review that "there is no evidence for a role of EPA and/or DHA intake in the development of prostate cancer." Another comprehensive meta-analysis of this question by Crowe et al. (28) (on which Brasky was a co-author) concluded more appropriately, "There was no strong evidence that circulating fatty acids are important predictors of prostate cancer risk. It is not clear whether the modest associations of stearic, eicosapentaenoic, and docosapentaenoic acid are causal". This is a far more appropriate conclusion than that expressed in Brasky et al. (1), which is tempered by the limitations of observational cohort designs. Unfortunately, none of these later findings have found their way into the evening news.

[a] Framingham refers to Framingham, Massachusetts, where a group of residents is extremely well characterized with respect to their blood lipid composition and cardiovascular health, as part of longitudinal studies carried out within the Framingham Heart Study.

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