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EDITORIAL

The Essential Fats of Life — Unabated

I am pleased to present you with a new issue of the PUFA Newsletter. Under auspices of the Global Organization of EPA and DHA (GOED) for the first time, a selection of recent publications of new research on omega-3s is highlighted in the form of a set of summaries. Unabated – a way to describe the pace of activities in the field of PUFA research. Overwhelming, some may feel, with close to a hundred original scientific publications appearing each month on omega-3 PUFAs alone. Fats of Life will continue to strive to providing the readership with relevant, and hopefully interesting, information on the most important and innovative research in this field. In addition to the traditional Fats of Life summaries, we also plan to bring you reviews on studies at the frontier of research. New findings provide the cornerstones for potential new treatments and may yield further insight into the way that long chain omega-3 PUFAs work in health and disease. Attention to both the small and giant steps of progress in understanding the contribution that omega-3 PUFAs make to nutrition and therapy is important. Highlighting studies from clinical groups and pioneering researchers across the world, Fats of Life aims to provide a source of scientific information on PUFAs presented in an understandable and educative format.

The current PUFA Newsletter highlights a number of recent studies. A pioneering study has explored how our genetic variability affects the way we respond to long chain omega-3 supplements and pharmaceuticals. A second study brings to attention the potential to provide relief in depressive symptoms in chronic hemodialysis patients. Another study provides indications that restoring neurocognitive processes in malnourished preadolescent children is achievable with a modest daily dose of EPA/DHA. Two fundamental studies offer insight into a new mechanism whereby the central nervous system obtains the DHA it needs to function properly, and further progress into the mechanism whereby PUFAs mediate their actions is helped by reference profiles of omega-3 lipid mediator derivatives in plasma and serum. A retrospective review offers the first insights in the potential efficacy of omega-3 PUFAs in parenteral nutrition for infants with Short Bowel Syndrome. And one study addresses the different ways in which EPA/DHA supplementation and statin drugs work together to bring about additive actions in correcting blood triglyceride imbalances in obesity and insulin-insensitive people.

This PUFA Newsletter additionally features an Invited Opinion by Dr. Aldo Bernasconi summarizing the views expressed at a recent scientific meeting on executing clinical studies on long chain omega-3 PUFAs properly and its implications for clinicians reading the studies. We are also pleased that Joyce Nettleton, former PUFA Newsletter editor, has contributed a Guest Article, reflecting on the developments in the omega-3 field during her tenure.

While essential fatty acids affect us all, we also know that there is a long way to go to bring old and new information into recommendations for nutritional adequacy and clinical practice. We are faced by several challenges in using information; handling the flood of newly published studies, understanding what the new information offers us in relation to what we already know, and on a personal level, setting aside some time to really read. Fats of Life can help you with the first two, by providing a peer-reviewed summary of selected recent studies. The last challenge is for you. May one article resonate with you and pique your curiosity to learn more.

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CARDIOVASCULAR HEALTH

Insight into the Variations in the Individual Response to Supplemental EPA/DHA Intake

The magnitude with which we respond to specific nutrients present in our food is not the same for everyone. Individuals carry significant genetic variability, and also vary in the way genetic information is expressed. Hence our responses to the external world can vary significantly. This variation in response also applies to the absolute needs for long-chain omega-3 fatty acids for health and the effects of their dietary intake on different processes in the body.

This is apparent, for example, when we consider that a substantial percentage of people taking omega-3 supplements do not experience a decrease in blood triglyceride (TG) levels, even though a decrease in blood TG levels is one of the hallmarks of omega-3 action if we look at the entire population combined. A mean decrease in plasma TG levels is measurable within a few weeks of increased omega-3 intake, particularly in those with elevated TG levels. It is notable that about a third of individuals do not respond to long-chain omega-3 supplementation with a measurable decrease in TG level.

A proper understanding of the heterogeneity in responses to omega-3 fatty acids has not received much detailed attention thus far. Understanding why some people do respond to omega-3 and others do much less so is of interest. Why increase your omega-3 PUFA intake if you know you will not receive additional benefit at a specific point in your life over your minimally recommended needs for EPA and DHA? On the other hand, a person might switch to taking EPA/DHA if it were clear that he/she would be a high responder and could decide to stop taking a specific prescribed drug instead, to markedly reduce the risk for a specific disorder. In order to be able to make such decisions in the future with a high degree of precision and confidence, a complete understanding of the factors that contribute to this heterogeneity in individual responses to long chain omega-3 is required and the variability should be quantifiable.

A substantial percentage of people taking omega-3 supplements do not experience a decrease in blood triglyceride (TG) levels. This study identifies new variations in our genome that contribute to the individual differences in responsiveness to EPA/DHA intake, through a genome-wide association study.

While we suspect that genetic variability is important, however, we have still to indicate which genes, gene variants, and epigenetic regulation contribute to the individual response to omega-3 supplementation. Now, a study undertaken by Rudkowska and colleagues at Laval University, Quebec, Canada, has examined this question. They undertook the first genome-wide association study (GWAS) to uncover in an unbiased manner those variations in our genome that contribute to the observed variation in the response to EPA/DHA intake. The researchers studied the reduction in blood TG levels as a measurable outcome for the response to supplemental omega-3 intake.

Previous studies by this group have addressed the contribution of specific known genetic variations to the TG lowering effect after omega-3 fatty acid intake. Those included specific single-nucleotide polymorphisms (SNPs), one-base pair changes in or between genes that affect gene expression and function, which had been described in prior research. By comparing the presence or absence, or degree of a response or distinguishing characteristic (a measurable phenotype), with the probability of having one or several specific genetic variations, a GWAS offers the possibility to find associations in an unbiased fashion, permitting the discovery of genetic variants that contribute to a specifically studied trait, in this case TG lowering as a result of EPA/DHA supplementation. At this point in time, undertaking a GWAS study potentially permits identifying previously unknown genetic variants, and should identify those variants already known to contribute to a specific response.

Rudkowska and colleagues identified from a group of study participants, drawn from a Canadian population of overweight/obese men and women, one sub-group of individuals (n=81) that responded to EPA/DHA supplementation with a decrease in plasma TG level of greater or equal than 0.01 mmol/liter, and a second sub-group (n=60) that did not decrease TG. The subjects were asked to take 5 grams a day of fish oil concentrate containing 1.9-2.2 g EPA and 1.1 g DHA, during 6 weeks. The study carefully controlled for the basal intake of food- and supplement-derived omega-3 intake, and established that there were no differences in baseline omega-3 status between the two groups. Blood samples were taken prior to and at the end of the intervention period, and plasma samples prepared for analysis of a number of blood parameters. From genomic DNA extracted from whole blood the frequency of alleles of SNPs was determined using a bead chip technology, permitting the detection of the presence of more than 4.3 million markers corresponding to short DNA sequences covering the human genome. Of these, almost 2.7 million SNPs were tested for statistically significant associations with the responsiveness to EPA/DHA supplementation.

The frequency whereby specific DNA sequences were occurring in the responder versus the non-responders was reported as odds ratios, defined as the proportion of individuals in the non-responder group having a specific variation over the proportion of individuals with that variation in the responder group. In order to facilitate identifying those positive associations that might be missed when using a very

stringent theoretical likelihood, the researchers employed a statistical threshold that was recently indicated to be more inclusive given that SNPs that are closely located within the genome do not segregate independently.

The construction of a Genetic Risk Score (GRS) was established based on the identified genomic variations. Of interest, the study did not stop here, and the value of the identified SNPs in predicting the responsiveness to omega-3 intake was assessed by determining the identified genomic variations in stored DNA samples of a second intervention study, the FINGEN study. This study had been carried out in the UK a few years earlier and had determined that over 30% of study participants did not respond with a decrease in plasma TG level with the daily intake of 1.8 gram EPA+DHA as a supplement over a 8 week period. In a similar fashion responders were defined as people who displayed a reduction in TG level greater than or equal to 0.01 mmol/liter. DNA was genotyped for the identified variants and the GRS was applied to determine the degree of identification of responders versus non-responders based on the measured allele frequencies.

The results of the first supplementation study show that when the test population was segregated by responder phenotype, the ingestion of EPA/DHA stimulated a marked reduction in plasma TG level (mean decrease of 0,50 mmol/l) in responders. Responders had a higher average baseline TG level (1.53 mmol/l) than non-responders (1.03 mmol/l), although baseline TG levels in both groups could be considered to be within a range considered normal (below 1.7 mmol/l). Average body weight index between responders and non-responders was the same. The TG level in responders after supplementation reached the baseline TG levels of the non-responders, showing that EPA/DHA lowered TG levels when given to people who have higher levels at the start of the intervention. Of interest, the non-responders actually showed a statistically significant increase in plasma TG level (+0.17 mmol/l), indicating that the nature of responsiveness to EPA/DHA in the responder and non-responder groups was different. In responders, but not in non-responders, the total and high-density lipoprotein (HDL)-bound cholesterol levels increased, and fasting insulin levels decreased. It is important to note that baseline as well as supplement-induced increases in plasma phospholipid levels of EPA, DHA, total omega-3 and total omega-6 PUFA were identical between responders and non-responders. Differences in responsiveness are therefore not due to differences in the absorption or tissue distribution of supplemented omega-3 fatty acids.

The genomic analysis demonstrated a difference in allele frequency of 13 SNPs (some are shown in Table 1). Several identifed SNPs found in regions of the genome located between genes, potentially modulating the expression of genes located in the vicinity. Several SNPs are reported within genes, such as *MYB*, *NXPH1*, *NELL1* and *IQCJ-SCHIP*. These polymorphisms were not previously recognized to play a role in the response to long chain omega-3 fatty acids, but have been reported already to play roles in gene regulation, signaling between adjacent cells, lipoprotein metabolism, and the production of ceramide (a bioactive lipid and important signaling molecule). The results suggest that the responsiveness to EPA/DHA supplementation is intricately linked to other lipid metabolic and signalling pathways.

Table 1. Some of the newly identified polymorphisms in the genome-wide association study undertaken to find genetic variations that associate with the responsiveness to lower blood triglyceride levels upon supplementation with EPA and DHA.

Description of polymorphism	SNP	Allele	Allele frequency	
			Responders	Non-responders
Intron variant IQCJ-SCHIP1	rs2621308	A	0.15	0.40
Intergenic - 300 kb upstream of SLIT2	rs2952724	A	0.48	0.18
Intergenic - 300 kb upstream of PHF17	rs1216352	A	0.23	0.51
Intron variant MYB	rs6920829	G	0.05	0.23
Intron variant NXPH1	rs6463808	A	0.09	0.30
Intron variant NELL1	rs752088	G	0.27	0.54

A genetic risk score (GRS) that relates the frequency of the genetic variants to the responder phenotype was developed. The score for each individual falls in a range from -1 to 8 points, attributing points for each allele using 10 of the 13 identified SNPs. A higher score indicates that an individual carries more at risk alleles for a non-responder phenotype, and a lower score, a higher proportion of alleles that increase the probability to respond well to long chain omega-3 supplementation with a decrease in TG levels. The sensitivity of the GRS was 84% (the chance to correctly identify a responder), and the specificity 69% (the chance to correctly identify a non-responder as such). Application of the GRS to the FINGEN replication cohort did not, however, find a very strong predictive power for the newly identified polymorphisms in explaining who was a responder and who was not; while the GRS could identify true responders in 70% of cases, the probability to incorrectly identify a non-responder as such was 75%. Whereas 22% of variation in TG level could be explained by the GRS in the original study, only 2% of the variation could be explained in the validation study, pointing out that additional risk factors still need to be identified that together explain the TG response to omega-3 supplementation in different populations.

This study offers new insight in the genetic variability that determines how individual persons respond with a decrease in triglyceride levels in blood following the intake of EPA/DHA for several weeks. The possibility to measure our individual genetic variability will allow the development of methods for predicting the extent to which each of us responds to specific essential nutrients and particular health conditions.

The GWAS approach by Rudkowska and colleagues shows the power of genome-wide screening approaches to increase our understanding of how the body reacts to specific nutrients, and allows the development of novel predictors of efficacy upon supplementation. Phenotypic variability may also be due to non-genetic factors, for example, long term dietary habits are different between populations and could affect the way genetic information is expressed. It appears that small non-identical conditions in which both studies were carried out with regards to dose, geographic localization, and supplementation period, may affect the predictive value of the GRS in its current form, and reveal that additional risk factors may be identified and incorporated. The distribution of EPA and DHA into specific blood lipids after dietary supplementation also reveals variability in the human population, but it is unusual to observe people that are completely deficient in omega-3 absorption and tissue distribution. Taken together, inter-individual variability in the regulation of some physiological functions by long chain omega-3 PUFA is strongly dependent on genetic influence on how cells respond to omega-3 incorporated in tissues, but non-genetic influences can be expected to contribute as well.

This study indicates an attractive way forward to developing a GRS that incorporates identified genetic polymorphisms to predict an individual person's response to omega-3 intake. In the words of the authors, subjects who decrease their plasma TG levels following omega-3 PUFA supplementation have a different genetic profile compared to individuals who do not respond to the supplementation. The findings in this original study thus set out a path involving follow-up analysis, and possibly new genome-wide studies, to increase the ability and confidence in predicting how we respond individually to dietary and supplemental omega-3 fatty acids.

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FOL 5

Correcting VLDL Kinetic Abnormalities in Obesity and Insulin-Resistance

Obesity is frequently accompanied by dyslipidemia, consisting of changes in triglyceride (TG) and cholesterol levels in blood outside the ranges considered normal. Many obese subjects have significantly elevated concentrations of cholesterol incorporated in the lipoprotein particle LDL (low-density lipoprotein). Another notable change is an elevated level of the lipoprotein VLDL (very-low density lipoprotein), which carries TG and cholesterol produced in the liver to other parts of the body where these lipids can be used for energy and tissue growth. Whereas countless studies have been dedicated to understanding the potential role of cholesterol in metabolic disorder and cardiovascular pathologies, and finding means to control its levels, less attention has been focused on the significance of elevated triglycerides. A reappreciation of a role of elevated TG levels in metabolic syndrome and cardiovascular disease may be emerging. Both the overproduction of VLDL-TG and insufficient clearance are considered to contribute to the development of hypertriglyceridemia in obesity.

The long chain omega-3 PUFA EPA and DHA exert a measurable influence on lipid metabolism in humans, and a reduction in plasma TG level can be observed relatively rapidly (within a few weeks) when the dietary intake of EPA and DHA is augmented, particularly in people with elevated TG levels. In fact, the efficacy with which long chain omega-3 can lower triglycerides is so convincing that multiple pharmaceuticals containing concentrated EPA and DHA have been approved around the world. The effect is dose- and time-dependent (among other factors, such as purity and molecular form of the employed omega-3 PUFA) and average reductions in TG levels in the order of 30-40% in hypertriglyceridemia can be achieved. Fish oil has been shown to lower TG levels by lowering the assembly and secretion of TG-rich VLDL by the liver into the circulation.

Researchers are interested in correcting elevated triglyceride and cholesterol levels in circulating lipoproteins found in dyslipidemia associated with insulin-insensitivity and obesity. This study aims at understanding the mechanism whereby long chain omega-3 PUFAs and a statin exert additive actions in correcting high triglyceride levels associated with the lipoprotein VLDL.

Given the changes in levels of both cholesterol and TG in obesity, researchers are interested in correcting these alterations by combining the cholesterol-lowering activities of statins with the TG-lowering action of increased long chain omega-3 intake. Atorvastatin, a widely prescribed statin, has been shown by itself to decrease the level of lipoproteins containing apoB100, corresponding to lipoproteins that carry cholesterol within the circulation (LDL, IDL and VLDL), by promoting their catabolism. The assessment of statin efficacy has led to the recognition that these compounds can also contribute to lowering plasma TG through an action on VLDL formation, alongside their primary function to reduce endogenous cholesterol biosynthesis. Potential benefits for the treatment of hypertriglyceridemia by combining statins with EPA/DHA have been identified in a large review of studies

that had looked at the interactions of statins with various dietary supplements.

Given the knowledge that both statins and omega-3 PUFAs can lower plasma TG, studies have addressed whether the combination of both has additive effects on plasma TG lowering in the context of obesity. This potentiating action has recently been shown to not be the result of an increased removal of TG-rich lipoprotein particles containing apo C-III. Apo C-III lipoproteins comprise VLDL particles and TG-rich "remnants" (lipoprotein particles which have transferred most of their lipid content to peripheral tissues), which are cleared more slowly from the circulation and levels of which are elevated in obesity and hypertriglyceridemia. Left open to a different interpretation, what mechanism may be responsible for the potentially more efficacious TG-lowering effect when long chain omega-3 supplementation and statin use are combined?

The present study by Ng and colleagues at the University of Western Australia, was undertaken to gain further insight into the kinetics of VLDL when atorvastatin treatment is combined with omega-3 supplementation. The research group is well positioned to execute this detailed analysis in humans,

relying on many years of experience in studying the precise regulation of lipid metabolism through the labelling of specific lipoproteins, allowing the tracking of temporal changes in response to pharmacologic and dietary intervention.

This study is a randomized, double-blind, placebo-controlled intervention trial in obese, normotensive and insulin-resistant middle-age men. After a 2-week run in period, the study subjects were randomly assigned to one of three 6-week treatments; *i)* atorvastatin (400 mg/d), *ii)* atorvastatin plus a high dose (4 g/d) EPA/DHA-ethyl ester (EE) concentrate (45% EPA-EE and 39% DHA-EE), and *iii)* atorvastatin placebo plus corn oil (4 g/d). The compliance for the statin and omega-3 oil was determined by counting of tablets and capsules, respectively. Also the change in plasma lathosterol (the immediate precursor for endogenous cholesterol biosynthesis) and EPA and DHA levels were measured to verify that subjects were following their indicated intervention.

Prior to and after the 6 week intervention period, the subjects were admitted to a metabolic ward. After a 14 hour fast, blood samples were taken to measure plasma TG, cholesterol associated with HDL and LDL, total non-HDL cholesterol, apolipoprotein (apo) A-I, apoB-100, apoB-48, remnant-like particle (RLP)-cholesterol, non-esterified fatty acids, insulin, and glucose. Insulin resistance was calculated from fasting glucose and insulin concentrations using the homeostatic model assessment formula. Specifically, VLDL kinetics in blood were determined by measurement of the changes in B-100 concentration using a deuterium-labeled leucine tracer technique permitting the calculation of production and degradation rates of VLDL, IDL and LDL particles. The administration of deuterated glycerol allowed assessment of endogenous synthesis of TG and the determination of the time course of VLDL-associated triglyceride formation and utilization. Through combining a best-fitted model for the changes in VLDL lipoprotein particles, with the tracing of deuterated glycerol incorporated in the plasma VLDL-associated TG and free plasma glycerol, it was possible to measure transport rates of TG in the VLDL lipoprotein pool, consisting of the VLDL-TG production rate and fractional catabolic rate. The measured concentrations of VLDL particles (as VLDL-apoB-100) and VLDL-TG concentrations, as well the production and degradation rates of the VLDL particle and VLDL-TG were used to determine how atorvastatin and omega-3 supplementation decreased TG levels and affected VLDL kinetics.

The combined treatment with atorvastatin and EPA/DHA led to a 42% reduction in plasma triglyceride levels compared to placebo, and constituted a larger decrease than the 26% decrease associated with atorvastatin alone. The combined treatment led to a significantly greater decrease in the number of Very-Low density lipoproteins (VLDL)-particles and VLDL-associated triglyceride than the treatment with atorvastatin alone.

The combined treatment with atorvastatin and EPA/DHA led to a 42% reduction in plasma TGs ($P<0.05$) compared to placebo, and constituted a larger decrease than the 26% decrease associated with atorvastatin alone ($P<0.05$). Atorvastatin alone or in combination with omega-3 PUFA decreased plasma total and non-HDL cholesterol, LDL-cholesterol, RLP-cholesterol, apoB-100, and apoB-48. There was a significant increase in HDL-cholesterol only in the case where atorvastatin and omega-3 PUFA were given together. It is of importance to note that these observations were made in the context of a significant increase in plasma EPA and DHA concentrations in omega-3 supplemented subjects. Atorvastatin was effective in reducing endogenous cholesterol biosynthesis, as indicated by a reduction in plasma lathosterol concentrations of approximately 75%.

The mechanism whereby supplementation with long chain omega-3 PUFAs and a statin exert additive actions to decrease the circulating TG present in very low-densitity lipoprotein (VLDL) particles in blood is shown to be due to EPA/DHA reducing the formation and secretion of VLDL-TG particles into blood, and the statin increasing the breakdown of these lipoproteins. The combination of both actions brings about a more effective reduction in TG levels in the obese and insulin-insensitive people studied here.

With respect to VLDL, the combined treatment of omega-3 and atorvastatin led to a significantly greater decrease in VLDL-TGs than atorvastatin alone. This additional effect on TG levels was due to a decrease in the VLDL-TG production rate. The VLDL-TG fractional catabolic rate that was increased by atorvastatin was not further increased by the concomitant intake of omega-3. In other words, the more effective lowering of plasma TG by omega-3 PUFAs in the context of taking atorvastatin is likely to be mediated by decreasing the synthesis and secretion of VLDL into the circulation (by the liver). The investigators showed good correlations between the decrease in the concentration of VLDL-TG and in VLDL-apoB-100 after atorvastatin plus omega-3 treatment, as well as between the VLDL-TG production rate and VLDL-apoB100 production rate. This rein-

forces the finding that the TG-lowering action of atorvastatin plus omega-3 PUFA is mostly related to an effect on the number of VLDL particles formed and secreted into the circulation.

There is budding interest in evaluating the potential causal effects of elevated plasma TG levels on different aspects of metabolic syndrome such as obesity and loss of insulin sensitivity, as well as in cardiovascular disease. A growing appreciation that the action of statins reaches beyond their effects on endogenous cholesterol biosynthesis is also generating attention to further explore their modulatory role on TG distribution and catabolism. The present study highlights the distinct mechanisms whereby atorvastatin and omega-3 PUFAs can reduce plasma TG levels. This mechanistic study suggests that a combined use of statins and omega-3 PUFA exert a greater TG lowering effect in the context of metabolic syndrome. It is tempting to speculate that individuals with a higher omega-3 status will respond better to statin treatment for lowering plasma TG, a potentially useful approach in correcting dyslipidemia in obese and insulin-insensitive people.

The conclusion of this study is that in obese and insulin-resistant middle-age men, hypertriglyceridemia can be lowered through a combined treatment of atorvastatin and omega-3 PUFAs, whereby the statin promotes the lowering of plasma TG by stimulating the removal of VLDL from the circulation while omega-3 PUFA supplementation slows down the formation of TG-rich VLDL and its secretion into the circulation.

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MATERNAL AND INFANT HEALTH

Towards Faster Weaning from Parenteral Nutrition in Neonates with Short Bowel Syndrome

Short Bowel Syndrome (SBS) is a complication of low gestational age pre-term infants (<37 wks gestational age) who develop necrotizing enterocolitis, as a result of the surgical removal of a significant part of the inflamed and damaged intestinal tissue. SBS can also result from the need to resect a portion of the small intestine in neonates in several other disorders involving malformations, obstructions, or damage to the intestinal tract. Congenital cases of short small intestinal length occur, but are relatively rare. Loss of a large part of the small intestine significantly compromises nutrient absorption, and can lead to fluid imbalances. Pediatric SBS is associated with high morbidity and mortality, while care for pediatric SBS patients is extremely costly.

Since the 1960s, the treatment of SBS is supported by providing parenteral nutrition (PN) to deliver nutrients intravenously until the intestine hopefully regains function, and the intake of nutrients by the enteral route can be recovered. The use of PN is therefore transitory with the aim that infants can be weaned from the parenteral infusion within a period of several months. The gut has functional plasticity and can regain length after surgical resection but the outcome of SBS has not been easy to predict. The use of PN is traditionally associated with additional problems that can contribute to mortality, namely PN-related liver injury leading to cholestasis, and infections secondary to having an intravenous catheter in place. Mortality increases with duration of PN and can reach very high rates. The use of PN in pediatric SBS is viewed as a race between the recovery of intestinal function and the mortality associated with PN. Therefore, an important objective is to achieve weaning from PN as early as feasible. Weaned patients may still remain on rehydration by intravenous administration.

The inclusion of fish oil in parenteral emulsion increases the amount of EPA and DHA provided to critical care infants to help the body lower hepatic damage associated with the use of parenteral nutrition, significantly reducing mortality. A summary of the work carried out at Children's Hospital in Boston and presented at 2012 GOED Exchange by Dr. Mark Puder can be found here.

Studies have been undertaken to better understand which factors determine and may predict survival and weaning from PN in pediatric SBS patients. Some key contributing factors include the length of remaining bowel as percentage of normal bowel length for a specific infant's gestational age, as well as the development of cholestasis as a result of PN. It still remains difficult to predict individual patient outcomes, but some progress may be in the making. The aim of the study highlighted here is to review how changes in the employment of so-called hepatoprotective strategies and multidisciplinary care may have changed the outcomes of PN use in pediatric SBS, and to define the predictive factors in this clinical setting.

In the present study Fallon and colleagues have undertaken a retrospective review of medical records related to pediatric SBS at the Boston Children's Hospital during the period 2004 through mid-2012. This period is characterized by the implementation of novel strategies found to lower the risk of developing liver injury in infants receiving PN and prevent the development of cholestasis, as well as improve multidisciplinary treatment of hospitalized infants. Cholestasis results from a decreased ability of the liver to excrete bile acids via the bile ducts as a consequence of liver parenchymal injury. In addition, with a view to reducing mortality it is essential to avoid the further transition of the injured liver to fibrosis. With hepatoprotective strategies it is understood that the specific composition of the emulsion used for the PN is changed in such a manner that the hepatotoxic effects are eliminated, and specific nutrients are provided that support liver function and tissue protection. This has been achieved in recent years in the author's clinic through the employment of lipid emulsions characterized by a relative lowering of the content of vegetable oil-based lipids (particularly linoleic acid) and inclusion of fish-oil based lipids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The present study looks at the use of PN in pediatric SBS and predictors of weaning from PN, covering a time period when medical attendance of pediatric SBS at this hospital has undergone some notable changes compared to older studies.

The present study reviews the possible changes in variables that may predict time to wean from parenteral nutrition in hospitalized infants with Short Bowel Syndrome during the period that inclusion of fish oil in parenteral emulsions started to be used in neonatal care at the Boston Children's Hospital (since approx. 2003).

Demographic and clinical data were collected from medical records in the period of 2004-2009. Neonates had less than or equal to 100 cm of small intestine at no more than 30 days

corrected gestational age, were diagnosed with a surgical gastrointestinal disease, and were PN-dependent for at least 2 weeks. Data on four study end-points were collected from the medical records of 63 neonates who met the inclusion criteria;

i) wean from PN without reinitiation for at least 1 year, *ii)* PN dependence (the infant did not wean from PN), *iii)* transplant, or *iv)* death before the end of the study (mid June 2012).

The investigators next calculated the probability that weaning from PN occurred, per each 10 cm increment of small intestinal length. The time to wean from PN for neonates with at least 50 cm of remaining intestine was compared with those having less than 50 cm of remaining small intestine by calculating the cumulative probabilities to wean from PN. Thereafter the predictive value for various parameters that might determine the probability to wean from PN was determined.

Of the 63 neonates who met the study criteria, 40 weaned from PN (63%) and 11 remained PN-dependent at the end of the study (June 2012). Four neonates received a transplant and 8 died (12.7%) while on PN. Fifty-one (81%) of the patients had received a fish oil-containing lipid emulsion as PN, the rest an emulsion based on vegetable-oil based lipids. The median residual small intestinal length differed between neonates that weaned from PN (55.0 cm) and those that remained PN dependent (26.0 cm, P=0.006), were given a transplant (34.9 cm) and died (27.8 cm). Neonates who had 50% of the predicted small intestinal length had a 95% chance to wean from PN. For this group of infants the median duration of PN until wean was 4.6 months, whereas for infants with less than 50% of predicted length this period was about 4 times as long (17.8 months). The median PN duration for infants who weaned from PN was 6.5 months.

Small intestinal length in SBS was the primary predictor of wean. Additionally, the number of surgical interventions, the number of infections and gestational age were associated with the success or failure to wean. In a multivariate analysis, only small intestinal length (positive association) and the intestinal lengthening procedure (which aims to provide an increased length of tissue that can potentially restore functional intestine; negatively associated) predicted the success of wean. These two factors as well as having the entirety of care executed within the author's institution were predictors for the duration of time to wean.

Implementation of fish-oil containing parenteral nutrition at the Children's Hospital in Boston started from approximately 2003 onwards under a special compassionate care arrangement with a manufacturer of EPA/DHA-containing emulsions. A retrospective review of pediatric SBS over the period of 1977 to 2003, before the commencements of use of fish oil-containing parenteral emulsions, had shown that in a different children's hospital, mortality was 27.5%, while 63.8% of SBS infants weaned from PN (Table 1). Risk factors that contributed significantly to mortality were cholestasis and small bowel length below 10% of expected length. The current review is timely since it reports on SBS cases in a subsequent eight-year time period. Although the collected data on pediatric SBS are still limited, it appears reasonable to view that in the present study mortality may be reduced. The authors point out that hepatoprotective intervention and a multidisciplinary care for SBS patients are helping to achieve this. No evidence is available yet to indicate that the percent-

Table 1. Comparison of reported mortality and weaning from parenteral nutrition in infants with Short Bowel Syndrome in two retrospective medical record reviews covering different time periods characterized by the absence and introduction of long chain omega-3 PUFA in parenteral nutrition.

Period (years)	# of SBS infants reviewed	% Infants receiving PN w/ EPA/DHA	% Infants weaning from PN	Mortality (%)
1977-2003	80	0	63.8	27.5
2004-2012	63	81	63	12.7

age of infants that wean from PN is altered substantially, but the median duration of PN until wean may be shortened compared to studies covering the use of PN in infants without inclusion of long chain omega-3 PUFAs.

The retrospective study by Fallon and colleagues is highlighting the potential for progress in the treatment of pediatric SBS patients by potentially shortening the time on post-operative PN, and lowering mortality. The introduction of long chain omega-3 PUFA in parenteral emulsions is likely to help protect the liver during the time the gut is given a chance to restore and rebuild additional capacity to regain its function. The modulation of cholestasis as an outcome of the hepatoprotective strategy was not addressed in this study. Not all patients received PN containing omega-3 PUFAs, but the authors reported that there was no difference in the use of fish oil-containing PN emulsions between patients who died and those who did not. It is also a challenge to make adequate comparisons between results obtained from different clinical centers, and hopefully the variables associated with different hospital settings in the US and internationally can be addressed in multicenter evaluations in the future. Given the still limited numbers of patients who have been reviewed it will be of interest to see future studies that further document the potentially life-saving new approaches for SBS infants.

Factors that predicted weaning from parenteral nutrition in infants with Short Bowel Syndrome were found to be the length of intestine that infants had left (positive association), and undergoing a surgical procedure that aims at lengthening the remaining bowel (negative association). The review suggests that the overall mortality rate may be reduced compared to reviews covering older periods, although the number of cases reviewed is still small.

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IMMUNE FUNCTION

Reference Profiles for PUFA-derived Lipid Mediators in Human Plasma and Serum

The long chain polyunsaturated fatty acids (PUFA) arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are used by the body to produce lipid mediators, which are derivatives with a variety of functions including regulating inflammatory responses, while protecting tissues against inflammatory insult and infection. AA, EPA and DHA are substrates for enzymatic reactions that incorporate oxygen at specific locations in the fatty acid molecule with a well-defined stereospecificity (spatial orientation), underlying the formation of lipid mediators called eicosanoids when derived from AA and EPA (with 20 carbons) and docosanoids when formed from DHA (having 22 carbons). These lipid mediator families encompass the EPA-derived E-series resolvins, the DHA-derived D-series resolvins, protectins and maresins, and more recently docosapentaenoic acid (omega-3)-derived resolvins, protectins and maresins, which have been found to exert extremely potent anti-inflammatory and inflammation resolving actions in the body.

EPA and DHA are used by tissues to form Specialized Pro-resolving Lipid Mediators (SPMs), which are extremely potent regulators of inflammation and help the body to handle infections, protect tissues from inflammatory damage, and resolve inflammation.

A few of these newly described substances have been studied in more detail, and have been recognized to constitute novel autacoids, local hormones which are generated in organs and by peripheral blood cells to locally activate G protein-coupled receptor-mediated cellular and tissue responses, followed by metabolic degradation. They have been collectively called Specialized Pro-resolving Lipid Mediators (SPMs) by the laboratory that discovered them. An expanding field of research is currently uncovering new mechanisms of their actions that may help us expand our understanding of the essential role of PUFA in health.

It is important to determine to what extent the levels in body fluids reflect the situation within tissues in normal physiology as well as in disease. For human studies particularly, there is a need to improve and establish suitable methodologies for the measurement of PUFA-derived lipid mediators in blood and other biological fluids given the obvious difficulties to measure directly within tissues. Their transient nature due to rapid formation and fast metabolic degradation precludes the accumulation of high levels within tissues. The analytical chemistry required for the detection of SPM *in vivo* demands extremely high instrument sensitivity and specificity, as well as adequate sample isolation, storage, and extraction steps prior to analysis. The continued improvements in analytical methodologies and advances in the development of robust sample preparation methods allow researchers to progressively overcome previous limitations. As the field develops, this will also give dietitians and clinicians the means to monitor lipid mediator status in patients, for example in relation to supplementation with EPA and DHA through the diet.

Suitable analytical methodologies to measure SPMs in blood are currently being developed. Since the levels of these lipid mediators are very low in the circulation, highly sensitive methods are required. Once these methods are proven to be robust, the relationship between EPA and DHA status of a person and the formation of PUFA-derived lipid mediators can be accurately measured.

The present study by Colas and colleagues from the laboratory of Charles Serhan at the Brigham and Women's Hospital Harvard Medical School in Boston, MA, USA, sets a reference for the developing field of omega-3-derived lipid mediator research by improved methodology for the detection of a range of lipid mediators in human plasma, serum and lymphoid organs. Noticeably, this study employs the use of reference materials to provide an indication as to baseline values for a range of SPMs and related PUFA-derived lipid mediators in human plasma and serum.

This study employs reference plasma and serum samples in order to provide baseline values for the concentrations in blood of a range of lipid mediators derived from PUFA. These values will be helpful for the developing field.

The methods described in this study build further on the previously developed strategies from this pioneering laboratory, which encompass the isolation of novel lipid mediator autacoids, synthesis of authentic standards, confirmation of postulated chemical structures, and detection of lipid mediators in cellular systems and *in vivo* tissues and fluids. In this study the authors placed further effort on the automation of sample preparation, improved control over sample stability, and robust identification of lipid mediators by liquid chromatography-tandem mass spectrometry (LC-MS/MS; an analytical methodology of choice in this field of research). The implementation of a more sensitive mass spectrometer, which pushes the detection limits for omega-3 PUFA-derived lipid mediators in plasma samples from the previous limit of detection of ~10 picogram to ~0.1 picogram, greatly expands the possibility to measure the levels of substances that previously were present below (undetectable) or close to the limit of detection.

The characterization of reference materials is a strong point of this study and provides values for future studies that focus on measuring the levels of PUFA-derived mediators and metabolites in human plasma and serum. A reference human plasma (SRM 1950) prepared from pooled plasma from 100 healthy volunteers was acquired from the US National Institutes of Standards and Technology (NIST). Human serum was acquired from two commercial sources and corresponded to pooled human sera each from 100 healthy donors. Individual plasma and sera from individuals was also prepared freshly in the laboratory from venous blood samples obtained

from healthy volunteers (n=10) before and 4 hours after taking 1.4 gram of encapsulated fish oil containing 650 mg EPA and 250 mg DHA as triglycerides, together with 81 mg aspirin given 2 hours after omega-3 supplementation. A low dose of aspirin is known to activate the formation of specific SPMs that have increased metabolic stability (so called aspirin-triggered resolvins). Serum was prepared from venous blood permitted to coagulate and undergo full clot formation during 24 hours at room temperature.

Detailed methodological procedures are provided in the study. In summary, serum and plasma samples are carefully handled at cold temperatures, spiked with internal standards, and after precipitation of protein extracted by solid-phase extraction to isolate the lipid mediators. Substances are separated by HPLC and detected by tandem mass spectrometry with detection settings optimized for each analyte. Multiple reaction monitoring, a technique to sequentially and selectively weigh each eluting analyte, permits quantitation of specific lipid mediators. The detection of 6 diagnostic ions and recording of full mass spectra were used to confirm the identity of each detected analyte, SPM and pathway marker. Endogenous SPMs were shown to be relatively stable when serum was stored at -20°C for 5 days under the sample storage conditions used by the laboratory. Analytes were quantitated based on calibration with synthetic standards for each lipid mediator and the use of class-representative deuterated internal standards. Forty-three relevant PUFA-derived lipid mediators were reported in the study, including twenty-one DHA-derived mediators (covering the D-series resolvins, protectins and maresins, six EPA-derived mediators (including E-series resolvins), and sixteen AA-derived eicosanoids (including the lipoxins, as well as several leukotrienes, prostaglandins and thromboxane).

Employing the optimized targeted lipidomics approach, the researchers first studied pooled human serum. Nearly all target compounds were detectable in levels ranging from a few picogram/ml to 2 nanogram/ml. Important SPMs such as DHA-derived resolvins D1 (RvD1), RvD2, RvD3, protectin D1, and maresin 1, EPA-derived resolvin E1 (RvE1), and the AA-derived lipoxin A_4 were present in levels between 5 and 120 picogram/ml.

Analysis of the reference human plasma samples provided a very different picture with measurable levels of only RvD1, RvD5 and RvD6, 4S,14S-dihydroxy-DHA, RvE2, AA-derived leukotriene B_4 (LTB_4) and a number of other leukotriene pathway markers, as well as several prostanoids (prostaglandin (PG) D_2, PGE_2 and $PGF_2\alpha$). The concentrations of the lipid mediators that were detected in plasma were approximately ten- and a hundred-fold lower than in serum samples. Twenty-four lipid mediators that were measurable in human serum were undetectable (absent) in plasma. The results are important since they point out that serum contains many PUFA-derived lipid mediators that are not detectable in plasma, and these are most probably formed during the process of blood cell activation and blood clot formation. The neutrophil-activating lipid mediator LTB_4 was the only measured lipid mediator present in plasma (at very low levels of 3.4 picogram/ml) but undetectable in serum. A range of SPMs could not be detected in the reference plasma were detectable in the freshly prepared plasma samples. These were present in very low levels, in the range of 0.1 to 9 picogram/ml. RvE1, which was absent in the reference pooled plasma sample, was present in freshly prepared plasma. This indicates that reference tissue fluids can be suitable as PUFA-derived lipid mediator standards, but only under conditions that are carefully controlled to preserve stability.

Many lipid mediators formed from AA, EPA and DHA, including SPMs, can now be reliably detected in human plasma and serum. Their levels change substantially 4 hours after supplementation with EPA and DHA. This study shows that control over sample preparation and storage is critical for obtaining meaningful results.

Toward structure-function: Upon supplementation of human volunteers with EPA/DHA and aspirin treatment the levels of RvD1, RvD2, 17-epi-protectin D1, RvE2 and RvE3 rapidly increased. Specific individual lipid mediators appeared to de-

This is the first study demonstrating functional metabolomics with enhanced phagocytosis from SPM following ingestion of an omega-3 capsule.

crease, pointing at a dynamic regulation of peripheral blood lipid mediator profiles upon intervention. The functional relevance of the increase in the sum of the plasma levels of RvD1, RvD2, RvE2, RvE3 and 17-epi-protectin D1 showed a fine relationship with the phagocytic activity of human whole blood. In this respect, the stimulation of phagocytosis may constitute a useful readout of the inflammation resolution-promoting role of EPA and DHA. Basal phagocytotic activity in the limited number of healthy volunteers was significantly increased after just 4 hours of a single EPA/DHA supplementation together with low dose aspirin.

A few studies have measured the levels of specific SPMs in the circulation. Mas and colleagues recently demonstrated the presence of RvD1 and RvD2 in plasma in concentration ranges about 10 fold higher than reported here, after 3 weeks of supplementation with EPA and DHA. In a metabolomic analysis by Psychogios and collaborators of pooled human plasma, both RvD1 and RvE1 were detected in concentrations approximately 3 and 20 fold higher, respectively. In addition to the SPMs investigated in detail in the present study, a range of lipid mediators derived from long chain PUFA have been described in the literature and are actively studied by various research groups interested in determining how profiles change upon dietary supplementation. It is also of interest to keep in mind that there are lipid mediators that have not yet been discovered given the continued description of new biologically active PUFA derivatives, and recent examples illustrate this point. The present study is highlighted as it sets one important milestone, and we will undoubtedly see continued and expanding interest in long chain PUFA-derived lipid mediators and their functions.

The progress in developing suitable analytical methodologies to measure omega-3 PUFA-derived biologically active derivatives may one day help physicians guide patients with dietary recommendations and the use of novel lipid mediator-based therapeutic approaches.

This study has shown that advances in methodology permit the improved detection of a large panel of PUFA-derived pro-resolving lipid mediators in human plasma, serum and tissue that can be obtained. The preparation of serum with inherent cell and platelet activation, coagulation and possibly clot retraction, may lead to the formation of many lipid mediators that are not present in plasma. On the other hand the study points out that the use of reference materials requires extreme control over the storage conditions and sample handling that govern stability of PUFA-derived lipid mediators. Elucidation of the mechanism of action of EPA, DHA, and other PUFAs will remain one front of exciting research - driven by the continuous development of rigorous identification, sample handling, and functional profiling methodologies. In the future the development of such appropiate methodologies will allow doctors guide patients with dietary guidance and the use of upcoming lipid mediator-based therapeutic approaches.

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BRAIN AND CNS

Facilitating Transport of DHA into the Brain

The brain is rich in docosahexaenoic acid (DHA). This fatty acid is a structural element for brain tissue, plays a number of important roles in neural development and plasticity, and protects the brain from inflammatory insult. The brain is relatively separated from the rest of the body by the presence of a blood-brain barrier, which restricts the uncontrolled access of substances, and possibly infectious material, present in blood, and thus remains relatively isolated from immune reactions occurring in the rest of the body. Oxygen and those nutrients that the brain needs for its function cross the blood-brain barrier through specialized transport mechanisms. Some of these may require energy (active transport by specialized protein carriers), or involve modified diffusion (facilitated diffusion), or involve small vesicles that move across the endothelial cells that line the small blood vessels in the brain (vesicular transcytosis). But how does this privileged organ obtain the DHA it needs? DHA is strongly retained by tissues and also accumulates in specific tissues with high demands for DHA according to a concept termed biomagnification. Biomagnification of DHA implies an active form of accretion by tissues that need substantial levels of DHA - higher than could be delivered by mere diffusion. For example, the placenta employs active transport mechanisms to deliver this fatty acid to the growing fetus.

The brain avidly acquires DHA from the circulation and retains it strongly. How the transport process of DHA across the blood-brain barrier really works has not been understood in much detail. In the present study a protein has been identified that transports a specific lipid structure called a lyso-phospholipid, containing one DHA molecule, as its preferred chemical form for transport.

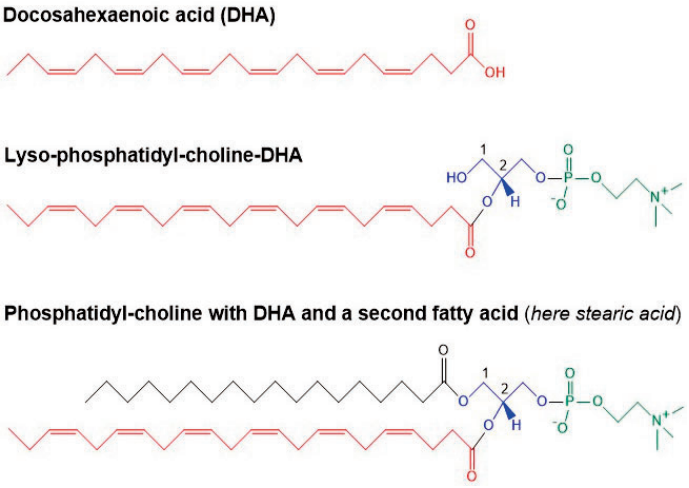
How the brain might be acquiring DHA in an active fashion to satisfy the requirements for DHA is still incompletely understood. Previous research has indicated that plasma unesterified DHA may contribute to the delivery of DHA to the brain, with DHA rapidly esterified into brain lipids, predominantly within phospholipids. Also, a specific chemical form of DHA circulating in blood had been recognized that supports delivery to brain tissue. In this lipid species, DHA is part of a lysophospholipid, which is a phospholipid with one fatty acid instead of the two molecules of fatty acids found in most cell membrane phospholipids (*Figure 1*). Lysophospholipid-DHA species are found in human plasma bound to albumin, with the DHA distributed about equally in the first and second carbon position of the glycerol backbone. Lysophosphatidyl-choline-DHA had previously been shown to deliver DHA more effectively to the brain than unesterified DHA in the rat, but the specific mechanism whereby this DHA-containing lipid would permit enhanced delivery of DHA to brain tissue has remained undiscovered.

Lysophospholipids are generated by the action of lipases, enzymes that cleave off one fatty acid molecule of a phospholipid. For example, the lipase phospholipase A2 in the liver can generate lysophospholipids followed by their secretion into blood as bound to albumin. Lysophospholipids can also be formed by lipases carried by lipoproteins, lipid particles that distribute various types of lipids in the body. Lysophosphatidyl-choline-DHA is one minor species of several types of lysophospholipids that circulate in blood.

A recent study by Nguyen and colleagues from the Duke-NUS Graduate Medical School in Singapore describes the identification of a protein called Mfsd2a as an endogenous transporter of DHA into the brain. First, the Mfsd2a protein was shown to be expressed in the endothelial cells that contribute to forming the blood-brain barrier. Knocking-out of the gene coding for Mfsd2a in mice leads to a smaller brain size, and also an increase in anxiety, for example the willingness of the animals to venture out into an illuminated area. Mice also displayed marked deficits in learning and memory. A lipidomic analysis of brain tissue by which all phospholipid and lysophospholipid species were measured and quantitated revealed that brains from Mfsd2a-deficient mice displayed lower levels of those phospholipids that normally contain DHA. The total level of DHA in brain was decreased by approximately 60%. Along with the defect in incorporating normal levels of DHA into brain tissue, mice that had no Mfsd2a protein had a significantly lower number of neurons in the cerebellar and hippocampal regions of the brain.

When the Mfsd2a protein was expressed in a cell type that normally does not contain the protein, it rendered the cells capable of incorporating DHA. Using this experimental cell model for DHA uptake studies, the researchers showed

Figure 1. What is a lysophospholipid? Shown here are the chemical structures of DHA (top), a lysophospholipid containing a DHA molecule (middle), and a phospholipid with one DHA molecule and one other fatty acid (bottom).



The lysophospholipid that the Nguyen study found to be transported into the brain by the Mfsd2a protein is lysophosphatidyl-choline-DHA (middle). This lipid consists of a glycerol group (blue; common to all phospholipids and lysophospholipids), a phospho-choline group (green; common to one class of phospholipids and lysophospholipids), and DHA (red), which can potentially be attached at either position 1 or 2 of the glycerol group. The second fatty acid (black color) in phospholipids can be one of several fatty acids, usually a saturated or mono-unsaturated fatty acid.

that DHA as part of lysophosphatidyl-choline was effectively incorporated into cellular lipids. However, Mfsd2a did not facilitate the transport of free unesterified DHA. Further delineation of the structural requirements required for transport by Mfsd2a established that the lysophospholipid optimally needs the presence of a phosphocholine headgroup and one long chain fatty acid molecule, which can be DHA. A closer look at the concentration range in which lysophosphatidylcholine (LPC) containing DHA (LPC-DHA) was transported into cells revealed that no saturation was observed up to high concentrations, suggesting that the transport rate will increase in correspondence with the concentration of LPC-DHA present in blood and passing along the blood-brain barrier.

The efficiency of transport was higher when DHA was the fatty acid contained within the LPC molecule, compared to other fatty acids such as oleic acid or palmitic acid. This specific recognition and transport of DHA was shown to be sensitive to the presence of sodium ions, a characteristic often observed in proteins that facilitate the transport of specific molecules across cellular membranes. The Mfsd2a transporter recognized the LPC-DHA molecule in different forms that may circulate in blood; whether bound to albumin, present within a micellar structure or solubilized as single molecules, the protein facilitated transport into cells. The researchers next showed that when injected directly into the circulation, the uptake of LPC with radiolabelled DHA (LPC-^{14}C-DHA) was reduced by ~90% in mice that did not have Mfsd2a. This indicates that this lipid species is a functional substrate for DHA transport from the circulation to the brain (in mice).

The study on Mfsd2a has been carried out in mice, so it remains to be established that the Mfsd2a transporter is a functional DHA transporter also in humans. The Mfsd2a protein is conserved across various organisms, including fish and mammals, so there is a good chance that Mfsd2a is of significant importance in contributing to the transport of DHA into the human central nervous system. Naturally, this remains to be confirmed. The Mfsd2a transporter has also been found to be important in the formation of the blood-brain barrier, indicating that DHA is also needed for establishment of an intact yet highly selective barrier that the brain needs to function well. The researchers have proposed that the name Mfsd2a is changed to sodium-dependent lysophosphatidyl-choline symporter 1 (NLS1). The demonstration that a structural requirement for DHA transport to the brain is that the fatty acid is part of a specific lysophospholipid suggests that strategies that are focused on selectively increasing DHA delivery to the brain, for example to support the prevention and treatment of neurological disease in which insufficiency of DHA has been implicated, could consider means of increasing this form of circulating DHA. The identification of Mfsd2a (NLS1) as the first facilitative transporter of DHA-containing phospholipids has opened a new door to advance our understanding of how the body distributes an essential long chain omega-3 PUFAs in relationship to the demands that

A protein that facilitates the transport of DHA from blood into the brain has been identified for the first time, in mice. The protein is called Mfsd2a or NLS1. It transports DHA as part of a larger lipid called a lysophospholipid. Since the gene sequence for the protein is present in many organisms, including humans, it is expected that Mfsd2a also transports lysophoshatidyl choline-DHA into the central nervous system of humans. This remains to be confirmed.

specific tissues have for DHA under different conditions of health and availability.

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MENTAL HEALTH AND COGNITION

EPA/DHA — Perspectives for Neuropsychological Improvement in Malnourished Pre-adolescent Children

Malnutrition, defined by insufficient energy and protein intake, seriously affects cognitive functions. Nutritional deficiencies early in a child's development have marked consequences for cognitive development and overall growth at later ages of development. Malnutrition is a preventable malady but is tightly linked to low socio-economic status, hampering implementation of corrective strategies. Along with insufficient energy and protein intake, generalized or specific micronutrient deficiencies can occur. Omega-3 deficiencies can also result from malnutrition, as a result of *i)* the utilization of dietary linolenic acid to generate energy, *ii)* deficient transformation of linolenic acid to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) due to deficiencies in other micronutrients that act as cofactors for long chain omega-3 PUFA biosynthesis, and *iii)* reduced intake of EPA and DHA directly from foods of animal origin (particularly from rich sources such as fish). Severe malnutrition is known to lead to a decrease in DHA in plasma

Neuropsychological functions continue to develop after infancy. There is growing interest in understanding the relative contribution of essential fatty acids to cognitive development and plasticity in neuropsychological functioning in school age and preadolescent children.

lipids. Little information is available with respect to the specific contribution that correction of long chain omega-3 PUFA intake can make to cognitive function in the context of malnutrition and underprivileged socioeconomic status.

A number of studies aiming to understand the role of long chain omega-3 PUFAs in cognitive development have focused on the perinatal period and the first few years of life. Fewer studies have investigated the role of omega-3 PUFAs in cognitive development in school age and preadolescent children. During preadolescence cognitive abilities continue to develop with increases in verbal working memory, goal-directed behavior, selective attention, strategic planning and organizational skills, but there is a need to better understand the role of specific micronutrients in this period. There is growing interest in obtaining a better picture particularly with regard to the extent of the plasticity and reversal of neurocognitive deficits that occur in preadolescent children and adolescence. Total blood levels of DHA, and EPA plus DHA, in 7-9 year olds are positively associated with reading skills (DHA) and working memory (DHA, EPA, EPA plus DHA, and total omega-3 levels). Supplementation with DHA can improve reading, working memory and externalizing behavior in apparently healthy children with below average reading performance.

In rodents a connection between a deficiency of DHA in brain and cognitive defects is well documented. It is clear that neuropsychological functions continue to develop after infancy, but there is a paucity of information on the relative contribution of essential fatty acids during this period. In the context of malnutrition where a range of macro- and micro-nutrients are affected by deficient intake, the relative role of omega-3 on cognitive functions in preadolescence is not well documented.

This study by Portillo-Reyes and colleagues, from the department of Psychology at the Autonomous University of Ciudad Juarez, Mexico, has focused on pre-adolescent schoolchildren of low socio-economic status, with a mild to moderate level of malnourishment. The researchers investigated whether supplementation with a modest daily dose of EPA/DHA would improve cognitive functions. Of significance, the researchers made an assessment of a large panel of nutrition-associated changes covering different aspects of cognition. Low socioeconomic status was defined according to population and housing census indices from the Mexican National Institute for Statistics and Geography (INEGI). Nutritional status was deduced from anthropometric measurements (age, weight, and height) from which height/age, and weight/height indices were used for determination of the level of malnourishment.

Fifty-five children of age 8 to 12 years (school grades 3 and 4 elementary) with a mild to moderate level of malnourishment were identified (corresponding to 85-95% in height/age and 70-90% weight/height indices). The children were randomly assigned to a treatment or placebo group after informed consent from primary caregivers. Excluded were children who had any neurological or hormonal diseases, or who had taken an omega-3 or vitamin supplement in the past 6 months. The study was a double-blind, placebo-controlled study. Children in the treatment group received a daily dose of three gelatin capsules of fish oil (containing together 270 mg EPA and 180 mg DHA), administered one in the morning, one at midday and one in the evening. The children in the placebo group received 3 capsules of soybean oil. The intervention lasted for 3 months. During the study 5 children dropped out from the placebo group, leaving 20 children in the placebo group and 30 children in the treatment group to complete the study. Compliance was determined by counting of capsules. Baseline omega-3 status in blood was not determined, but the parents of the children were interviewed on the dietary habits of the children, with special attention to the intake of food with a high content of omega-3.

The present study has addressed the possibility that supplementation with EPA/DHA can improve neuro-psychological functioning in preadolescent children with mild to moderate malnutrition. A panel of different aspects of cognition was assessed.

Changes in neuropsychological functions were evaluated through the application of a wide battery of different tests, 19 in total, which collectively measure processing speed, visuomotor coordination, attention, memory, language, and executive function. In addition, absenteeism and changes in school performance were recorded. An equal distribution of the children in the two groups with respect to demographic variables was verified. Analyses of variance measurements were made to determine the distribution of means of the various neuropsychological test variables among placebo and treatment groups before and after the intervention period. In addition to determining statistical significance of measured differences, effect sizes were determined in order to obtain measures of

clinical significance. Individual responses of the test variables were classified as no improvement, medium-size improvement, and large-size improvement. The improvement frequency in each group was then compared for placebo and omega-3 supplemented children.

No statistically significant differences were found between the children of the two groups with respect to age, IQ, absenteeism, and school performance. Neither were statistically significant differences found in the mothers of the children regarding age, IQ, academic level, or economic status. Eight percent of children ate fish-containing meals at least twice a week, 39% once a week, 19% once every two weeks, and 34% once a month. Over 60% of the children consumed canned tuna and sardines as the only source of fish. Consumption of omega-3 enriched foods was low (10% of children reportedly drank omega-3 enriched milk).

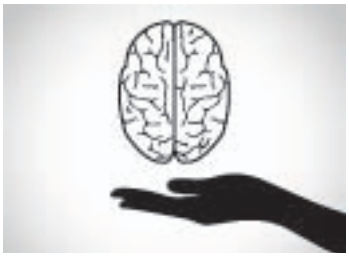
A three month supplementation with a modest daily dose of 450 mg EPA/DHA was shown to bring about meaningful improvements in cognitive functioning in preadolescent children with mild to moderate malnutrition.

After three months children supplemented with EPA/DHA had a statistically significant ($P<0.05$) improvement in the following neurocognitive variables: Symbol Search, which is a measure of processing speed (matching symbols appearing in different groups), Embedded Figures (finding a figure as quickly and accurately as possible within a more complex figure) and Visual Closure (completing figures that are interrupted), which are tests to determine visuoperceptive integration, Block Design, which is a test for visuoconstructive integration (requires arranging red and white colored blocks into a specific design), Stroop Color and Stroop Color Word, which are tests that require the fast and accurate naming of colors and reading of colored words, and Matrix Reasoning, a test that requires choosing one of several given solutions to complete a picture matrix. The Stroop and Matrix Reasoning tests, respectively, measure reasoning and inhibitory aspects of the so-called executive functions of the brain, which are involved in the control and regulation of cognitive processes.

Analysis of group effect sizes showed that the children that had taken the EPA/DHA supplement for 3 months showed a moderate to big improvement in 12 of the 19 test variables (Cohen's *d* value above 0.5). In the placebo group effect size improvements were observed in 4 variables, which were shared among the 12 variables that improved in the EPA/DHA group, suggesting that these improvements are unrelated to the daily supplementation with EPA/DHA. The 8 variables that improved in effect size were related to processing speed, visuoperceptual integration, visuocontructive integration, verbal memory, and executive functions. There was also an increase in the number of children with a moderate to large improved effect size for academic performance.

When looking only at the children in the study with a clinically relevant large effect size (a Cohen *d* value greater than 0.8), for 8 neurocognitive variables, more than 80% of the children were found to have received EPA/DHA. In 14 tests more than 60% of the children with large improvements had received EPA/DHA. These improvements were related to processing speed, visuoperceptive integration, visuoconstructive integration, attention, verbal memory, visual memory, and several aspects of executive function (reasoning, working memory, and inhibition). In 4 tests with a clinically relevant large improvement, there was no difference in the percentage of children who had received EPA/DHA or placebo.

The study results point to a measurable improvement in several neuropsychological functions, suggesting that clinically relevant recovery in cognitive functions is achievable in preadolescent children in the context of malnutrition by supplementation with a modest daily omega-3 intake during 3 months. Even in the context of a deficit in multiple macro- and micro-nutrients, which can underlie malnutrition, it appears that restoration of long chain omega-3 intake can provide measurable improvements in cognitive functions. A previous study with 6-10 year old marginally nourished schoolchildren that looked at supplementation with DHA (100 mg) and linolenic acid, but no EPA, provided together with a mixture of micronutrients, did find a benefit in cognitive performance outcome but the effect could not be attributed to the supplementation with omega-3s. There are a number of studies in children that have shown improvements in cognitive processes, such as learning and attention, with daily doses of EPA plus DHA that are higher (250-500 mg), in children that have low omega-3 intake and tissue levels, but not specifically studied on a background of malnutrition (see references *worth noting* below*)*. The present study was relatively small in number, and omega-3 status was not measured although dietary intake of long chain omega-3 was determined to be relatively low. Future studies may be adding valuable supportive evidence for the potential benefits of EPA/DHA for children in poor and underprivileged communities with limitations in access to essential nutrients.



This study provides important indications that in the context of mild to moderate malnutrition, preadolescent school children from families of low socio-economic status can receive significant benefits from increased daily EPA/DHA intake, leading to measurable improvements in cognitive functions and performance at school.

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Addressing Depression in Maintenance Hemodialysis Patients

In end-stage renal disease, over 90% of kidney function has been lost. Hemodialysis has long been a solution to prolong the life of end-stage renal disease patients. Depressive symptoms and distress are very common in chronic hemodialysis patients with end-stage renal disease, with an estimated prevalence rate of 20 to 70%. Longitudinal studies have shown that the level of depression in hemodialysis patients is variable over time, and is affected by a multitude of personal, interpersonal and nutritional factors. Episodes of depression can negatively influence nutritional status and immune function, and can affect compliance of dialysis treatments. Potentially, the pathologies underlying renal failure, including kidney damage due to diabetes, hypertension, and some disorders affecting the glomeruli, may also be further aggravated. The somatic aspects of depression resemble uremia, an increase in circulating urea that is no longer cleared optimally by the damaged kidneys and by necessity is removed *via* dialysis. Systemic markers of inflammation are measurable in hemodialysis patients, and increased levels of circulating pro-inflammatory cytokines and acute phase proteins have been shown in depressed hemodialysis patients. Although depression, inflammation, and declining health may contribute to a significantly increased mortality observed in patients with end-stage renal disease on hemodialysis treatment, the precise causal relationships are difficult to establish. What is clear is that an opportunity and need to treat depression in end-stage renal disease patents has been identified to potentially offer a better quality of life and potentially therapeutic benefits to a demanding problem with high social and medical costs.

Depressive symptoms are very common in chronic hemodialysis patients with prevalence rates up to 70%. An opportunity exists to treat depression and offer a better quality of life for hemodialysis patients.

Long chain omega-3 fatty acids EPA and DHA play central roles in the regulation of inflammation with research continuing to identify mechanisms. Increasing their intake from dietary sources or through supplementation supports anti-inflammatory actions under conditions involving acute inflammatory insult, and reduced risk of adverse clinical outcomes in a range of chronic inflammatory disorders, including hemodialysis patients. Several observational studies have shown that depressive symptoms are associated with low dietary intake of omega-3 PUFAs and low tissue levels. Supplementation with omega-3 PUFA, and EPA in particular, has shown considerable success in alleviating depression. The potential benefits for EPA/DHA in treating depressive symptoms in hemodialysis patients, and the association with lowering inflammation, have not been assessed.

An accurate assessment of depression is critically important in order to measure and establish the relationships between external factors, deteriorating health and depression. A clinical psychiatric evaluation of depression is not the same as a self-assessment of depression; the somatic and cognitive aspects of depression are tested in different ways, and although clinical depression may overlap with the recognition of depressive symptoms, a clinical diagnosis is made using different methodologies. Symptoms of depression are also not to be confused with distress, which is common in hemodialysis patients. The Beck Depression Inventory (BDI) is a test for depressive symptoms that employs self-rated scales for symptom frequency and severity. The BDI is therefore useful for the assessment of depressive symptoms, and can be validated and aligned with the clinical assessment of major depressive disorder. Structured clinical interviews are used in clinical research to validate major depressive disorder, such as SCID and MINI tests. The use of the BDI is therefore limited to assessing depressive symptoms.

Increasing the intake of long chain omega-3 PUFA, particularly EPA, can alleviate depressive symptoms. The potential benefits of EPA and DHA to treat depressive symptoms in maintenance hemodialysis patients has not been assessed yet.

This study by Gharekhani and colleagues (Tabriz Medical University, Iran) addresses for the first time the question of whether supplementation with EPA and DHA has anti-depressive effects in chronic hemodialysis patients. In addition, the study measures potential changes in circulating cytokines and C-reactive protein in order to determine if changes in systemic markers of inflammation might be associated with omega-3 supplementation and depression.

Adult patients on maintenance hemodialysis were recruited from two teaching hospitals. Stringent exclusion criteria were applied in order to obtain patients (n=54) who had a BDI score higher than 16 (depressed), and among other criteria, were not in a terminal stage, did not suffer from other psychiatric disorders or inflammatory or infectious disease, and had not taken omega-3 supplements in the last 3 months. Pa-

tients were randomly assigned to either an omega-3 supplementation or placebo group. The omega-3 supplementation consisted of a daily dose of 1080 mg EPA and 720 mg DHA in the form of 6 capsules. Placebo consisted of the same volume of paraffin oil in the same capsule format. The intervention trial was single blinded and lasted for 4 months. Compliance was assessed by capsule counts. Dietary habits were controlled to remain stable during the study, but blood omega-3 fatty acid measurements were not carried out in this study. At the start and end of the study serum samples were prepared and stored for analyses of C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and IL-10, as well as ferritin and parathyroid hormone. Depression was measured with a BDI questionnaire, which had been validated for use in the Iranian population. A BDI score of ≥ 16 was used to estimate the lower limit of diagnosis of depression (the test outcome can range from 0 to 63).

The two groups did not significantly differ from each other at the start of the trial with respect to age (~57 yrs), gender, dialysis history, the pathologies underlying the development of renal failure, or medication use. Patients in the omega-3 supplementation group had a slightly better dialysis adequacy (a measure of the quality of dialysis related to urea removal efficiency, length of dialysis session and ultrafiltration volume) than patients from the placebo group, but the difference was not statistically significant (*P* value .057). Mean and median BDI scores were the same between the placebo and omega-3 groups. Baseline parathyroid hormone was elevated in all subjects, pointing at secondary hyperparathyroidism as a consequence of permanent kidney damage, and ferritin levels were elevated, with a wide range of values over 200 pg/ml.

After 4 months of supplementation, there was no change in BDI score in the placebo group (from 21 (4.7) to 20.3 (7.6); mean (SD)). In the omega-3 supplemented patients there was a highly significant decrease in BDI score (from 23.5 (7.5) to 13.4 (5.7); mean (SD), *P* value 0.001), which effectively classifies the patients that had received EPA/DHA as no longer having depressive symptoms.

The results of the study indicate that four months of supplementation with a daily dose of 1.8 g EPA/DHA significantly lowered depressive symptoms in chronic hemodialysis patients.

In the placebo group the parathyroid hormone increased, possibly reflecting progressive hyperparathyroidism associated with renal dysfunction and hypocalcemia. Also ferritin levels increased in the placebo group, likely associated with inflammation and liver damage. In the omega-3 supplemented group these two parameters did not significantly increase from baseline, although baseline levels of ferritin were higher in the omega-3 group. Omega-3 supplementation induced a minor decrease in serum IL-6, but small differences in baseline levels between both groups are notable as well. Given the small yet apparent differences in some biochemical parameters between both groups, the evaluation of statistically signifcant differences between the omega-3 and control groups at the end of the intervention could be reinterpreted if the authors would correct for the observed baseline values of measured variables in each group.

In subsequent regression analyses that aimed at determining the strength of association between different variables, the authors found a significant correlation between the BDI score and the supplementation with omega-3, but no other variables correlated with depressive symptoms. The use of omega-3 PUFAs did not correlate significantly with the levels of circulating cytokines. The level of C-reactive protein was possibly negatively associated with omega-3 use, but on the border of statistical significance. Omega-3 did, however, strongly associate negatively with ferritin level, suggesting that inflammatory tissue damage to the liver may be lower in the omega-3 supplemented patients.

The study by Gharekhani offers a noticeable result that may be of practical use for the treatment of depressive symptoms

in adults on maintenance hemodialysis with end-stage renal disease. The study has a few weaknesses recognized by the authors themselves, first, that dietary intake and omega-3 status has not been measured, and second, the study has a relatively small sample size. The outcome is in line with other studies that have indicated that supplementation with long chain omega-3 PUFAs can be of benefit in the treatment of symptoms of depression and potentially as adjunct to anti-depressive medication. A relationship between omega-3 supplementation and effects on systemic measures of inflammation could not be established. Other studies have reached similar and mixed conclusions collectively pointing out that it is difficult to establish a relationship between omega-3 dosing regimens and inflammatory cytokines. The effect on ferritin may suggest that supplementation with EPA/DHA has a tissue protective effect in hemodialysis patients.

Hemodialysis introduces a distinctive variable and challenge to an omega-3 supplementation study where distribution of the absorbed EPA and DHA occurs via the circulation in the form of more complex lipids such as phospholipids and triglycerides, as well as free fatty acids and lysophospholipid bound to serum proteins such as albumin. Hemodialysis is performed several times a week, but it is not known if this procedure affects circulating omega-3 levels, potentially through removal of lysophospholipid and free fatty acids in equilibrium with serum protein binding sites. The results of this study suggest that daily supplementation with fish oil overcomes the potentially negative effects of hemodialysis on omega-3 status. A study that specifically addresses the effects of hemodialysis on omega-3 status and clearance is required. The near significant difference in baseline hemodialysis adequacy noted in this study reminds us that a study with a higher number of patients may also address the question if small changes in dialysis adequacy would skew the response to a better anti-depressant effect of EPA/DHA in these patients.

This intervention used paraffin oil as the placebo. Although there was no further information provided with regard to the grade of this food-grade white mineral oil, there were no measurable adverse effects reported. Ultra-refined paraffin oil is used in Iran and other countries as an oral laxative for treatment of constipation in children, at much higher doses than used in the present study, and appears safe to use.

The present study suggests that supplementation with EPA/DHA may offer a relatively simple means to decrease depressive symptoms in maintenance hemodialysis patients.

The researchers have published three additional publications on this study population reporting the effects of EPA/DHA supplementation on quality of life, nutritional status, and anemia, which may be of interest to the reader (*references below*). The authors themselves recognize the need to repeat the study with higher numbers of patients and measuring the changes in omega-3 status before and during intervention.

The results suggest that supplementation with EPA/DHA may offer a relatively simple and non-toxic means to decrease depressive symptoms in maintenance hemodialysis patients. Additional studies that replicate the findings offered in this study are needed in order to substantiate this outcome.

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GUEST ARTICLE

Omega-3 PUFAs: Who Could Have Predicted?

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Past Editor, PUFA Newsletter

How far omega-3 (n-3) PUFA research has come! The 1936 medical observations of Israel Rabinowitch describing the rarity of arteriosclerosis among the Inuit of the eastern Canadian Arctic and similarly among the northwest Greenlanders led to the report of high levels of long-chain omega-3 PUFAs n-3 LC-PUFAs) in Inuit plasma lipids by Bang and Dyerberg. Finally, Dyerberg and colleagues suggested that high levels of n-3 LC-PUFAs were linked to the prevention of thrombosis and CVD. The involvement of these PUFAs in reducing the risk of cardiovascular disease is now widely recognized. An overview of the diversity of research that has been published over the last 10 years follows, which strongly suggests the continued development of our understanding of omega-3 actions in human health.

The physiological effects of n-3 LC-PUFAs—lower resting heart rate, blood pressure and plasma triglycerides; improved endothelial function; lower inflammatory markers; enhanced cardiac function; and the molecular effects on membrane structure and function, modification of ion channels, signaling proteins and gene expression—all point to more favorable cardiovascular outcomes. In spite of recent studies suggesting that supplemental n-3 LC-PUFAs in heart disease patients with state-of-the-art medical care provide no further reduction in cardiovascular mortality and morbidity, others reported as much as a 35% lower risk of CVD mortality in healthy older adults with the highest levels of plasma phospholipid n-3 LC-PUFAs. The n-3 LC-PUFAs may be most effective in deterring CVD in healthy adults, in those at high risk of sudden cardiac death and in secondary prevention. How they function in specific types of CVD remains to be clearly elucidated, but the evidence supports the conclusion of Wu and Mozaffarian that "moderate dietary intake of fatty fish should be one cornerstone of a heart-healthy diet."

In maternal and infant health, DHA is now recognized as "conditionally essential," while alpha-linolenic acid, its plant-based precursor, is insufficiently converted to DHA in humans to meet the needs of the developing fetus and infant. We now have international recommendations for pregnant and lactating women to consume at least 200 mg of DHA daily. Given the importance of DHA in the structure and function of the developing brain and the low intakes of n-3 LC-PUFAs in many Western countries, especially among vegetarians, clear recognition of DHA as a vital nutrient for women in the child-bearing years is an important step forward.

However, it has been difficult to demonstrate clear benefits of maternal DHA or n-3 LC-PUFA supplementation on childhood outcomes. Most large controlled trials have not found statistically significant or meaningful differences in the growth or birthweight of term infants whose mothers consumed n-3 LC-PUFA supplements in pregnancy, but there are exceptions. In contrast, preterm infants may experience improved growth and global neurodevelopmental scores. Similarly, evidence of improved cognitive and visual development in the infants of supplemented mothers remains inconclusive. Some have reported significant developmental benefits with maternal n-3 LC-PUFA supplementation, but questions about the suitability of global tests of cognition or individual neurodevelopmental tests at particular ages have been inadequately addressed.

The most widely reported benefits of DHA supplementation in pregnancy are greater gestational age, fewer very early preterm births (<33 weeks' gestation) and a lower rate of preterm birth. However, the evidence is insufficient to warrant their deliberate use to avoid preterm deliveries.

A potentially promising area where the increased consumption of n-3 LC-PUFAs or fatty fish in pregnancy may be beneficial is reducing the risk of allergies, particularly in infants at high risk for them. Data remain inconsistent, with some studies reporting no reduction in the risk of allergic disease in children whose mothers consumed n-3 LC-PUFAs during pregnancy or even a higher risk associated with higher LC-PUFAs in cord serum phospholipids.

Eating fish 2 to 3 times a week during pregnancy was associated with a lower risk of asthma in the offspring, a significantly lower risk of eczema at one year of age and less atopic wheeze and house dust mite sensitization at age six. Regular fish consumption in infancy was also associated with significantly less allergic disease up to 12 years of age and with a lower risk of eczema at age 2 years. Relatively consistent data indicate that maternal n-3 LC-PUFA supplementation during pregnancy is linked to less atopic eczema in early childhood.

One of the most riveting developments in n-3 LC-PUFA research has been the progressive identification, characterization and functional investigation of EPA- and DHA-derived mediators that stop inflammation and promote resolution. These include resolvins and their cousins, protectins and maresins. Rapid advances in this field have expanded the view of inflammation to include the cessation of inflammatory responses and the promotion of proresolving activities. This category of substances, now called specialized proresolving mediators, suggests promising clinical applications to more rapidly stop inflammation, hasten healing and return to homeostasis.

Although not yet commercially available for human treatments, resolvins led to significant clinical improvements in animal models of periodontitis, asthma and lung injury. Interestingly, an observational study from Japan reported that periodontal patients with low intakes of DHA had significantly more periodontal disease events compared with those having high DHA consumption. Similarly, DHA-derived neuroprotectins reportedly conferred neuroprotection in experimental stroke and retinal pigment epithelial cells, and reduced infarct volume in permanent ischemic stroke. Neuroprotectin D1 was also shown to promote corneal nerve regeneration and increase nerve sensitivity following surgery in animals, suggesting an adjunct treatment in corneal surgery.

Resolvins and protectins reduce inflammation, promote cell survival and limit tissue damage in an increasing range of pathologies including infectious disease, acute lung injury, neuropathic pain after nerve trauma, inflammatory pain, acute kidney injury, possibly multiple sclerosis and other inflammatory clinical conditions. Several clinical trials of these substances have been or are currently being conducted, a necessary prelude to their therapeutic approval.

In experimental traumatic brain, spinal cord and nerve injury, Adina Michael-Titus and others have clearly demonstrated the therapeutic potential of DHA, EPA and their derivatives. The provision of DHA immediately after brain or spinal cord injury in animals was associated with significantly reduced lesion size, neuronal and oligodendrocyte loss and apoptotic cell death. As a result, the treated animals experienced improved functional outcomes, increased neuronal cell survival and reduced macrophage and inflammatory responses. Dietary provision of these fatty acids also reversed the decline of several nuclear receptors and increased neurogenesis associated with age-related memory loss. In cell culture studies n-3 LC-PUFAs promoted neurite growth in the sensory neurons of young and aged animals. Depletion of brain DHA prior to injury exacerbated the damage following brain trauma, impeded recovery and contributed to poorer sensorimotor outcomes. These and other studies have laid the foundations for clinical trials in humans. At least two clinical trials have been approved to study DHA or n-3 LC-PUFAs in individuals with sports-related concussion and three in individuals with spinal cord injuries. Two favorable case reports described the use of large doses of n-3 LC-PUFAs along with other treatments in the partial recovery of a patient who suffered traumatic brain injury and in the survivor of a mine explosion. These reports have been quite dramatic.

Another topic moving forward quickly, if not yet decisively, is the effect of DHA and n-3 LC-PUFAs in age-related cognitive decline and Alzheimer's disease (AD). The pathology of AD begins decades before the occurrence of clinical symptoms, and signs of neurodegeneration have been reported prior to the development of abnormal beta-amyloid proteins in cognitively normal adults. An expanding literature has described many interactions between DHA or n-3 LC-PUFAs in AD patients, but dietary studies reported no improvements once the condition is established. However, higher blood levels of EPA and DHA were linked to the preservation of brain and hippocampal volumes and changes in gray and white matter, decreases in which are associated with the early stages of cognitive decline.

Observational studies have largely reported an inverse relationship between fish consumption or higher serum concentrations of DHA and impaired cognition, while interventions with n-3 LC-PUFAs in healthy older adults showed little or no benefit. Some studies with n-3 LC-PUFA supplementation in patients with mildly impaired cognition reported improvements in short-term and working memory, but others found no effects. This literature was reviewed in detail recently. More extensive literature suggests that these fatty acids are likely involved in many aspects of cognitive function and related pathologies.

Finally, the translation of research knowledge into clinical and public health applications is proceeding rapidly. N-3 LC-PUFAs are involved in cell differentiation and proliferation in stem cells, adipocytes and mesenchymal stromal cells with implications for guiding tissue development more favorably. In CVD, n-3 LC-PUFAs significantly lower high triglyceride levels and reduce arterial stiffness. Their use in surgical patients, either preoperatively or in postoperative care is associated with reduced inflammation and improved recovery, although not all surgical applications have reported beneficial effects. Data on the presurgical treatment with n-3 LC-PUFAs of cardiac patients at risk of atrial fibrillation are mixed, although benefits have been reported. N-3 LC-PUFAs appear to reduce the occurrence and severity of postoperative depressive symptoms in certain patients and cognitive decline in others.

In neuropsychiatry, n-3 LC-PUFAs are finding more widespread applications and are being investigated for their potential to reduce the risk of childhood behavioral difficulties and adult suicide, although the interpretation of conflicting data is difficult. Studies often use widely varying doses for different treatment periods and include several interventions. In other research, animal studies have suggested beneficial outcomes related to n-3 LC-PUFA treatments in bone, intestinal tract, corneal surgery, in dry eye syndrome and chronic kidney disease. These PUFAs have also shown promise in total parenteral nutrition, palliative cancer treatment, slowing the progression of prostate cancer, nonalcoholic liver disease, periodontitis and chronic headache pain. There is every reason to think this list will soon be many times longer. FOL

INVITED OPINION

Clinical Studies on the Effect of Omega-3 Fatty Acids on Cardiovascular Outcomes

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There is a long history of clinical research showing that long-chain omega-3 fatty acids – the kind of fatty acids found at relatively high levels in fish oils - have a cardio-protective effect. This research includes several large randomized control trials (RCTs). These trials, in which participants are randomly selected to receive either a treatment or a placebo, have long been considered to be the gold standard for clinical research.

One surprising aspect of this research is that while the earliest trials showed that consumption of fatty fish or fish oils have a clear benefit for cardiovascular health, later studies have found or reported either a smaller effect, or no effect at all.

The first large RCT on this subject was the Diet and Reinfarction Trial (DART), whose conclusions were published in 1998 [1]. The researchers recruited 2033 men who had recovered from a myocardial infarction, and randomized them to receive advice on three possible dietary changes: to increase consumption of fatty fish, to increase the ratio of polyunsaturated to saturated fat, or to increase cereal fat consumption. The study found that consumption of two weekly servings of fatty fish not only reduced the risk of death by ischemic heart disease (IHD), but also reduced all-cause mortality by 29%.

The following large RCT, the GISSI-Prevenzione trial [2], confirmed these results. In a group of 11,324 patients who had survived a recent heart attack, fish oil consumption reduced the risk of all-cause mortality, sudden death, and coronary death. The findings of these two large studies not only agreed with each other, but also with several prospective cohort studies, including Harris et al [3], which found that long-chain omega-3 consumption was associated with decreased cardiovascular risk. The accumulated evidence led to dietary recommendations, issued by multiple countries and organizations (including the World Health Organization and the American Heart Association), to eat heart-healthy fish or to increase omega-3 intake for their cardio-protective properties.

Later studies, on the other hand, have failed to find the same effects ([4-6], for example), finding either evidence of a much smaller protective effect, or no effect at all. This surprising development has caused a great deal of confusion, increased by articles in the popular press and opinion pieces that exaggerate the significance of these findings as being contrary to established science and policy, and therefore more newsworthy. As a result, there is some debate about whether fish oils reduce cardiovascular risk, and even about whether the dietary recommendations to eat more fish are appropriate.

These are valid concerns. The findings of the research are apparently contradictory, and one possible explanation for this could be that, somehow, all of the early research is flawed. A second possibility is that, in the intervening years, some changes in the healthcare environment or behavior have made it more difficult to conduct effective clinical trials about the cardiovascular benefits of long-chain omega-3 fatty acids.

Recently, two groups of researchers, working independently, reached the conclusion that the second explanation is more likely. The results from the earlier research and subsequent dietary recommendations have had the effect of increasing the intake of omega-3s, and later studies now have study participants with a higher baseline omega-3 intake than those of the earlier trials. The participants in the newer studies already have some benefit from the cardio-protective effects of fish oils, and the study treatment only adds a smaller benefit compared to the older trials. In a way, omega-3 fatty acids are a victim of their own success as cardio-protective compounds.

In addition, in the last couple of decades the standard of medical care for cardiovascular health has changed significantly, with the introduction or widespread usage of a number of medications, including aspirin, statins and beta-blockers. Participants in newer trials have better cardiovascular care and prevention, and this makes it much more difficult to isolate the protective effect of a single factor, like fish consumption or long-chain omega-3 intake.

The first group was led by Dr. Michael James, of the Royal Adelaide Hospital in Adelaide, Australia. Their thoughtful review [7] was recently published by the *British Journal of Nutrition*, and makes the point that changes in both omega-3 intake and the standard of cardiovascular care call into question whether RCTs are the most appropriate study design. A typical clinical trial is used to study the safety and effectiveness of a candidate drug, where only the treated group receives the studied compound. In contrast, omega-3 fatty acids are nutrients that will be consumed by both the treated and the placebo groups of any clinical study, which reduces the differences between cases and controls. This makes detecting a possible beneficial effect much harder.

The problem is compounded by the fact that, for a number of cardiovascular outcomes, the benefits of taking additional omega-3s strongly depend on the patients' baseline intake of EPA and DHA [8]. This is particularly true of arrhythmia and hypertension: while a patient with an habitual low intake

would see large protective benefits from eating a couple of portions of fish per week, somebody with a higher intake would see a smaller additional benefit.

This creates a problem for newer clinical studies. Because omega-3 fatty acids are generally recognized to have a cardio-protective effect, their intake has increased rapidly since 2000 [9]. More recent studies not only have to deal with the fact that the subjects in the treated and placebo groups will both be taking omega-3s, but also with the fact that since everyone's usual intake is much higher than it was two decades ago, the effect of treatment will be smaller. This makes detecting an effect all the more difficult. Newer studies need to compensate for this difficulty by enrolling a much larger number of participants, by using larger dosages (fish oils are considered safe) or by enrolling only subjects with very low habitual intake and requiring that participants maintain that low intake for the duration of the study.

The second group is a panel of experts convened by GOED, the Global Organization for EPA and DHA Omega-3s. This panel, formed by a group of seven experts with different backgrounds, conducted a workshop on the Design of Clinical Studies of Omega-3 Fatty Acids with Cardiovascular Outcomes at the recent biannual meeting of ISSFAL, the International Society for the Study of Fatty Acids and Lipids, a leading organization of top scientists dedicated to the study of the health effects of dietary fats, oils and lipids. The proceedings of this workshop are being prepared for publication.

While the workshop's objective was to provide practical advice to researchers on how to design omega-3 clinical trials, the panel spent considerable time in discussing the reasons why newer trials fail to find the same cardiovascular benefits identified by the older studies. Some of the resulting conclusions and recommendations can be used as a guide to evaluate the results of cardiovascular studies involving omega-3 fatty acids:

1 *Did the study include a sufficient number of participants?* Because of increases in omega-3 intake, and because of improvements in the standard of care for cardiovascular prevention, the expected effect of an omega-3 intervention will be smaller than if the study had been conducted twenty years ago. Newer clinical trials need to consider this in their design, and need to use a correspondingly larger number of participants.

2 *Did the study only include people with low omega-3 intake?* Alternatively, did the study record and use the participants' omega-3 status, both at baseline and after treatment? Higher baseline intakes would make detecting an effect much harder.

3 *Was the dosage sufficient?* In general, higher doses have a more protective effect. Many studies use a low dose, which makes them more likely to return a neutral conclusion. There is no need for that. Fish oils are both easily accessible and safe.

4 *Was there concomitant medication use?* Likely yes, particularly in prevention studies involving people who already had a cardiac event. But the use of modern medications makes the effect of omega-3s harder to detect, so studies need to record medication usage, and consider it explicitly during their data analysis.

To conclude, there is an apparent contradiction between the promising results of the earlier clinical trials and the neutral findings of newer studies. This has created some controversy about whether current guidelines to eat more fish or increase the intake of long-chain omega-3 fatty acids are appropriate, which has led to calls to revert existing public health policies. We believe this would be a serious mistake. The majority of the scientific evidence shows that omega-3 fatty acids have a cardio-protective effect, and the fact that recent studies show a reduced or neutral benefit is, more likely than not explained by an increased consumption of omega-3s and by increases and changes in the usage of medications for cardiovascular disease protection (aspirin, statins, beta-blockers, ACE inhibitors, angiotensin II-receptor blockers). This does not mean that omega-3 fatty acids have no role in cardiovascular protection. Quite the contrary. Ischemic heart disease and stroke remain the two leading causes of death worldwide [10]. Unlike modern drugs, fish oils and EPA/DHA-containing dietary supplements are fundamental for early prevention — before any cardiac event or elevation of the levels of risk factors — they have a role in the reduction of the risk of both hypertriglyceridemia and hypertension, and are universally considered safe and free of side effects. They remain a safe and important tool in the prevention of cardiovascular disease.

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