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Rapid Response with DHA Might Halt Damage in Spinal Cord Injuries

One of the newer applications of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) is in surgery and trauma, specifically spinal cord injury. In a guest commentary in this issue of the *PUFA Newsletter*, Adina Michael-Titus discusses the ability of these PUFAs, particularly docosahexaenoic acid (DHA), to promote neuronal cell survival, protect their function and limit much of the secondary damage that occurs in spinal cord injury. Patients who suffer such injuries are often immobilized for life, but the hope raised by Michael-Titus' and colleagues' studies is that nerve cell function, motor ability and spinal cord integrity might be substantially improved if DHA is administered immediately after injury. A detailed report of Michael-Titus' recent work appears in "Clinical Conditions."

The cardiovascular benefits of n-3 LC-PUFAs in reducing the risk of sudden cardiac death are well known, but the details of how they affect atrial fibrillation, the most common arrhythmia, are less clear. A new report provides evidence that n-3 LC-PUFAs prolong the refractory period following atrial fibrillation long enough to prevent the fibrillation cycle from becoming established. These PUFAs also improve arterial elasticity, which improves blood flow.

Against the encouraging reports from many clinical investigations came the heartless conclusion of the US National Heart Lung and Blood Institute on the effective-

ness of n-3 LC-PUFAs on arrhythmias. Although the Institute acknowledged that the weight of evidence favors the reduction in risk of sudden death from n-3 LC-PUFAs, it chose to call for more research rather than inform or inspire public policy. Public health deserves a bolder policy from its scientific institutes.



Other reports in this issue describe corneal protection in dry eye syndrome with eye drops containing alpha-linolenic acid, improved motor function in 7-year-old children whose mothers had higher levels of DHA at birth, and evidence that usual DHA intakes in Canadian women may be insufficient for optimal visual acuity in their infants. Solid links between n-3 LC-PUFAs and various immune-based conditions remain difficult to establish clearly, although these fatty acids continue to be linked to various mental functions.

May this issue provide reading pleasure and inspire discussion.

Joyce A. Nettleton
Editor, *PUFA Newsletter* and *Fats of Life*
joyce@fatsoflife.com



■ GUEST ARTICLE

The Therapeutic Potential of Omega-3 Fatty Acids in Neurological Injury

Adina T. Michael-Titus, D.Sc.

Neuroscience Centre, Institute of Cell and Molecular Science, Barts and the London School of Medicine and Dentistry, U.K.

Research focused on the activity of omega-3 polyunsaturated fatty acids (n-3 PUFAs) in the adult nervous system and in neurology and psychiatry has gathered pace in the last decade. But one area with significant unmet needs is neurological trauma. These comments review studies supporting the therapeutic use of n-3 PUFAs in neurotrauma, emphasizing spinal cord injury, and highlight some issues to resolve before clinical application.

Spinal cord injury: pathogenesis and evolution

Neurological trauma is heterogeneous in its presentation and its consequences are often devastating for the patient, as illustrated by the example of spinal cord injury (SCI). SCI affects mainly young patients, as most injuries occur between the early teenage years and before the age of 40. The current management of SCI is mainly based on surgical interventions that aim to stabilise and decompress the spinal cord and on rehabilitative strategies. Although these lead to some improvement in outcome, there is still a clear need for agents that would make a significant difference to the patient's quality of life and help protect and repair the spinal cord. The neurological damage created by SCI is the consequence of two processes: a) primary injury, which occurs at the site of impact, and b) secondary injury, which is created by the propagation of destructive processes over a larger area of the cord.¹ The primary injury leads to extensive and irreversible loss of neuronal and glial cells. The secondary injury phase is also accompanied by massive and rapidly spreading cell loss. It is practically impossible to alter the primary injury, but it may be possible to decrease the impact of the secondary injury using neuroprotective pharmacological approaches.²

The pathogenesis underlying secondary injury includes excitotoxicity associated with massive glutamate release, increased inflammation and increased oxidative stress. Apart from cell loss, secondary injury also leads to axonal dysfunction and degeneration, and the formation of large cavities and cysts in the spinal cord. Within

the preserved tissue, a glial scar forms gradually, which acts as a powerful barrier against axonal regrowth. As a consequence, severed ascending and descending tracts cannot grow and reconnect with their targets. Therefore, an ideal therapeutic strategy for SCI should include a neuroprotective component and promote regeneration either through a stimulant effect and/or by counteracting the effects of an inhibitory environment.

Neuroprotective effects of omega-3 fatty acids in spinal cord injury

Several studies document a marked neuroprotective effect of n-3 PUFAs against excitotoxic processes.³⁻⁵ These fatty acids, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), exert anti-inflammatory effects and can inhibit cyclooxygenase activity and the formation of proinflammatory eicosanoids and cytokines.⁶ Furthermore, n-3 PUFAs have an antioxidant effect, as *in vitro* and *in vivo* evidence suggests that DHA increases the activity of glutathione peroxidase and superoxide dismutase. As excitotoxicity, inflammation and oxidative stress are part of the pathogenesis of secondary injury, we hypothesized that n-3 PUFAs might be beneficial in treating SCI. Further evidence came from a study by Lang-Lazdunski and collaborators³ who showed that the administration of the n-3 PUFA, α -linolenic acid (ALA), decreased neuronal loss and improved neurological outcome in transient spinal cord ischemia in rats.

In our first studies on spinal cord trauma using spinal cord hemisection in the rat, ALA or DHA was administered as an intravenous bolus 30 minutes after SCI.⁷ Both fatty acids induced significant neuroprotection: they reduced neuronal and oligodendrocyte loss and decreased apoptotic cell death. Furthermore, post-injury administration of ALA and DHA significantly improved the neurological outcome. The study also provided evidence that n-3 PUFAs protected against the destruction of myelin. Interestingly, an equimolar dose of the n-6 fatty acid arachidonic acid (ARA) administered after injury induced totally opposite effects: it exacerbated the tissue damage and considerably worsened the functional outcome.

These observations were subsequently confirmed and extended in a more severe model of SCI in the rat, induced by compression.⁸ This study tested not only the effect of DHA administered acutely after injury, but also DHA added to the diet (400 mg/kg/day) for 6 weeks after injury. The results confirmed the neuroprotective potential of n-3 PUFAs and showed a dramatic neuronal and glial rescue and greatly improved motor performance in the animals exposed to DHA

after injury. Further, there was an additional benefit of chronic exposure to high levels of DHA after injury. The additional neuroprotective effect of the DHA-enriched diet was not apparent until the second week after injury.

The study of DHA in SCI compression also clarified some of the mechanisms underlying the neuroprotection: DHA reduced SCI-induced oxidative stress and inflammatory responses. In parallel with reduced cell death was marked axonal protection. It is widely accepted that the functional outcome after SCI could depend on the survival of a small number of axons, i.e., sparing just 10-15% could improve the outcome. In this respect, it was important to demonstrate the effect of DHA on parameters that reflect axonal damage. The n-3 PUFAs reduced signs of axonal dysfunction, such as the accumulation of the β -amyloid precursor protein or the reduction in non-phosphorylated neurofilament-positive axons.

However, one equally important aspect not directly addressed in these studies is whether long-chain (LC) n-3 PUFAs could support regeneration. In vitro evidence suggests that n-3 LC-PUFAs have trophic effects on neurites in hippocampal and cortical cells.⁹ Effects seen in cultured embryonic cells have been confirmed in adult neurons and even in aged animals.¹⁰ Interestingly, in a model of nerve injury, facial nerve transection in mice, DHA supplementation (300 mg/kg/day for 14 days) following injury significantly affected the response of neurons and microglia to injury. DHA supplementation induced a pro-regenerative response (e.g., increased number of calcitonin gene-related peptide and galanin-immunoreactive sprouting fibres) and this was correlated with better recovery of function.

These observations, particularly those in rodent models of SCI, suggest that the administration of n-3 LC-PUFAs, such as DHA, after SCI could confer significant neuroprotection. The time window for giving an acute bolus is compatible with intervention by emergency services and could become a first line intervention in neurologically injured patients. Furthermore, the observations so far suggest that n-3 PUFAs could also be integrated into the chronic management of SCI. Interestingly, there is already evidence that use of parenteral preparations enriched in n-3 LC-PUFAs in critically ill patients leads to a much improved clinical outcome.¹¹ Importantly, the damaging effect induced by ARA suggests that using preparations enriched in soybean lipids (a source of n-6 PUFAs), which is still common practice in some clinical settings, may lead to detrimental neurotrauma consequences.

The translational potential: remaining issues

The results obtained with DHA in models of SCI and nerve lesions demonstrate unequivocally that DHA has a significant intrinsic neuroprotective effect after acute neurological injury and has pro-regenerative potential. The evidence of neuroprotection at the spinal cord level is corroborated by observations at the supraspinal level, suggesting neuroprotection after traumatic brain injury.¹² Considering the well-documented safety and tolerability of these compounds, treatment with n-3 LC-PUFAs holds great promise in the field of traumatic spinal cord and brain injury. It remains to be established if EPA has comparable potency or whether EPA and DHA co-administered would act synergistically. More work is also required to clarify doses and durations of administration.

One of the major questions is what are the critical targets involved in these effects? DHA has a multitude of targets, ranging from ion channels to nuclear receptors. These could explain both the acute and delayed effects. For example, DHA could block depolarization-induced increased glutamate efflux and the activation of glutamate receptors that lead to excitotoxicity. These effects may result partly from the inhibition of voltage-sensitive Na^+ and Ca^{2+} channels¹³ and possibly through the activation of the two-domain background K^+ channels, such as the TREK-1 channel. DHA is a ligand of transcription factors, e.g. the retinoid X receptors,¹⁴ which could lead to coordinated modulation of the expression of many genes involved in neuroprotection. Finally, chronic exposure to DHA after injury could affect the composition and dynamics of phospholipids in cell membranes (e.g. phosphatidylserine) with direct consequences for cell survival.¹⁵ Last, but not least, it is now well established that n-3 LC-PUFAs give rise to active metabolites. These, especially neuroprotectin D1 derived from DHA, may be key mediators of the antioxidant and anti-inflammatory effects of DHA.¹⁶ Future studies will establish if these derivatives have an even higher intrinsic efficacy than DHA.

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■ CARDIOVASCULAR HEALTH

Arrhythmias

National Heart Lung and Blood Institute Reports on Omega-3s and Arrhythmias

In 2005, a workshop of experts in cardiology and lipid metabolism convened by the National Heart, Lung and Blood Institute (NHLBI) of the National Institutes of Health, Bethesda, USA, examined the evidence that omega-3 polyunsaturated fatty acids (n-3 PUFAs) protect against cardiovascular disease and prevent arrhythmias. Their report appeared in September 2007. Highlights of this report and an invited commentary on the workshop by participant Alexander Leaf follow. Readers are likely aware of Leaf's extensive contributions to the study of arrhythmias and his clinical trial in patients with implanted defibrillators.

A workshop convened by NHLBI in the U.S. examined the evidence that n-3 LC-PUFAs prevent arrhythmias. Some 80% to 90% of all sudden cardiac deaths stem from ventricular arrhythmias.

The NHLBI report focused on arrhythmias, the major underlying cause of sudden cardiac death, which accounts for about 37% of all cardiovascular disease deaths. Some 80% to 90% of all sudden cardiac deaths stem from ventricular arrhythmias. Workshop participants

evaluated the evidence from epidemiological and clinical studies in humans, animal and cell culture studies and various mechanisms through which n-3 PUFAs might exert their effects on arrhythmias. These fatty acids clearly affect the electrophysiological properties

of the heart, but they have both pro- and anti-arrhythmic effects. The report noted that observational studies and randomized clinical trials have demonstrated, with the exception of the DART-2 trial, significantly reduced risk of sudden cardiac death with the consumption of fish or n-3 long-chain (LC) PUFAs.

When it comes to the effects of n-3 LC-PUFAs in patients with implanted cardioverter defibrillators (ICDs), results are murkier. There have been three clinical trials in these patients and results are conflicting, with pro-arrhythmic effects reported in a subgroup in one study. Even though patients with ICDs are not typical cardiovascular patients and participants in these studies were clinically heterogeneous, these studies have clouded the evidence supporting an anti-arrhythmic effect of n-3 LC-PUFAs in reducing heart disease mortality. The importance of the clinical status of the patient was emphasized in a recent discussion of fish oils and atrial fibrillation. With this type of arrhythmia, there had been only 3 studies published at the time of the NHLBI report, each in diverse populations. Results were conflicting and thus inconclusive. More data have since been published. Likewise, data were too sparse to draw conclusions about the influence of alpha-linolenic acid, the medium chain n-3 PUFA found in plants, and sudden cardiac death.

Although "the weight of evidence from epidemiological and clinical trials supports the hypothesis that the n-3 LC-PUFAs found in fish reduce the risk of sudden death," the NHLBI authors concluded that more evidence was needed before public policy guidelines could be developed. Public health deserves a bolder public policy.

Studies in animals have been more consistent and support the benefits of n-3 LC-PUFAs in the prevention of arrhythmias. These experiments are much easier to control, but have less certain applicability to humans. Cell studies in isolated or cultured myocytes have examined changes in membrane composition and ion channels for sodium, potassium and calcium,

often producing complex results. Responses to n-3 LC-PUFAs differed with the dose, species from which the myocytes were obtained, previous exposure to n-3 LC-PUFAs and duration of the exposure. The many effects of n-3 PUFAs in cultured myocytes include reduced sodium currents; reduced calcium influx into the cell; downregulation of the genes affecting inflammation, cell growth and cardiac matrix remodeling; reduced occurrence of early afterdepolarizations; and improve the integrity and structure of the myocyte

membrane. Since the NHLBI review, it was reported that fish oil-fed dogs were significantly more resistant to induced atrial fibrillation and that resistance was associated with significantly reduced expression of 2 connexins.

Although the NHLBI report observed that “the weight of evidence from epidemiological and clinical trials supports the hypothesis that the long-chain n-3 fatty acids found in fish reduce risk of sudden cardiac death,” and that these fatty acids “indisputably” affect fundamental elements of cardiac electrical activity, they concluded that more evidence was needed before public policy guidelines could be developed. Does their caution derive from a clinical subset of patients whose risks may be completely different from those of the general population? If so, is it wise to withhold advice from the large majority of individuals who might benefit from increased intake of n-3 LC-PUFAs because a relatively small subset might not benefit? Public health deserves a bolder public policy.

London B, Albert C, Anderson ME, Giles WR, Van Wagoner DR, Balk E, Billman GE, Chung M, Lands W, Leaf A, McAnulty J, Martens JR, Costello RB, Lathrop DA. Omega-3 fatty acids and cardiac arrhythmias: prior studies and recommendations for future research: a report from the National Heart, Lung and Blood Institute and Office of Dietary Supplements Omega-3 Fatty Acids and Their Role in Cardiac Arrhythmogenesis Workshop. Circulation 2007;116:e320-e335.

Acute Exposure to Omega-3 PUFAs Prevents Harmful Atrial Remodeling

Atrial fibrillation, uncontrolled trembling in the heart's upper chambers, is the most common arrhythmia and increases the risk of stroke 5-fold.

Atrial fibrillation affects 1.5% to 2.0% of the population in the developed world. After 40 years of age, lifetime risk of atrial fibrillation is 26% for men and 23% for women. Heartbeats normally originate with electrical impulses generated in the atria. When this activity becomes disorganized, the pattern of heartbeats becomes arrhythmic.

Atrial fibrillation may occur without symptoms, but may lead to palpitations, chest pain or congestive heart failure. Having the condition increases the risk of stroke



Figure 1. Structure of the heart showing active blood flow in the atria. Image reproduced with the permission of the Heart Rhythm Society.

5-fold. If sustained, the condition alters the electrophysiological and structural characteristics of the atrial muscle so that it is more susceptible to the initiation and maintenance of arrhythmia, a result called atrial remodeling. Atrial fibrillation shortens the time the atrium takes to recover from each electrical impulse (refractory period), thereby feeding back to the process of fibrillation. Oxidative stress contributes to atrial fibrillation pathology and increases the risk of atrial fibrillation.

The ability of long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFAs) to reduce asynchronous activity in cultured heart cells was reported in 2000 and an association between fish consumption and lower incidence of atrial fibrillation was first observed 4 years later in adults 65 years old or more. Since these reports, others have failed to find an association between fish consumption and atrial fibrillation. However, a randomized controlled trial of supplementation with n-3 LC-PUFAs prior to coronary artery bypass graft surgery reported a 54% reduction in postoperative atrial fibrillation in patients consuming the n-3 LC-PUFAs, plus a significantly shorter hospital stay. As many aspects of the heart's electrophysiology are modified by n-3 LC-PUFAs, these discrepancies call for resolution.

In this study, Dr. Daise da Cunha and colleagues at Ohio State University, Columbus, USA, investigated the effect of n-3 LC-PUFAs on the electrophysiological adaptation of the atria to rapid atrial pacing induced by electrical stimulation. The study was conducted in anesthetized dogs using a pacing stimulus of 400 beats/min. Measurements of the effective refractory period—the interval when electrical impulses are not conducted through the atrium—were obtained at baseline and for 6 hours, with atrial tachyarrhythmias (rapid, irregular beats) induced for 10 seconds at 5 minute intervals at the end of each study. Exposure to n-3 LC-PUFAs (eicosapentaenoic acid and docosahexaenoic acid) was by intravenous infusion over the first 65 minutes of the study. Two controls were conducted, one with n-6 PUFA infusion, the other with saline.

The effective refractory period was unaffected by the n-3 LC-PUFA infusion over a 6-hour time frame during the normal heartbeat cycle. After rapid atrial pacing was induced, the effective refractory period remained

Acute exposure to n-3 LC-PUFAs, but not n-6 PUFAs, prolonged the atrial refractory period following rapid electrical stimulation, thereby breaking the cycle of atrial fibrillation.

unaltered in the n-3 LC-PUFA group for the first 2 hours; by 3 hours, it was significantly prolonged compared with both control groups. In the controls, there was significant shortening of the effective refractory period within 30 minutes of stimulation. There were no time dependent changes in the electrocardiograms of any group and atrial fibrillation was not induced in any group at the end of the study.

The study showed that acute exposure to n-3 LC-PUFAs, but not n-6 PUFAs, prolonged the atrial recovery period following rapid electrical stimulation, thereby breaking the cycle of atrial fibrillation. In the normal resting heart, the n-3 LC-PUFAs had no effect on the usual effective recovery period, suggesting that these fatty acids do not affect cardiac electrophysiology in the unstressed state. This finding was verified by the lack of changes in the electrocardiograms.

In vitro and long-term feeding studies have shown that n-3 LC-PUFAs alter calcium and potassium currents in ventricular myocytes, whereas in this study, acute exposure to n-3 LC-PUFAs was without effect on the electrocardiogram in either unstimulated or stimulated conditions. Ion currents were not measured. Short-term exposure may not provide sufficient time for these fatty acids to be incorporated into the muscle tissue, although uptake of docosahexaenoic acid into rat heart in less than 3 hours has been reported. Long-term exposure to n-3 LC-PUFAs through a 12-week feeding period in rabbits was associated with reduced susceptibility to atrial fibrillation induced by increased atrial pressure. Several mechanisms that lengthen the effective refractory period may be occurring simultaneously, including those involving oxidative stress or inflammation. Mechanistic explanations underlying the prolonged refractory period await further investigation.

In an accompanying editorial, Opthof and Den Ruijter note that n-3 LC-PUFAs completely prevented the reduced refractory period that usually accompanies rapid atrial pacing. This observation suggests the potential for n-3 LC-PUFAs to prevent a first occurrence of atrial fibrillation or the progression of a single episode into a chronic condition. These authors caution, however, that there is "no such thing as one type of atrial fibrillation," the electrical stimulation used in this study may not mimic what happens in human physiology and the circumstances under which n-3 LC-PUFAs may be

effective are unknown. The da Cunha study and the potential for n-3 LC-PUFAs to have both anti- and pro-arrhythmic effects make it imperative that the conflicting observations with n-3 LC-PUFAs in atrial fibrillation be resolved, different patient groups be studied and the mechanisms involved be elucidated.

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Vascular Function

Marine Omega-3 PUFAs Improve Arterial Elasticity in 2 Months

With aging and vascular disease, arterial elasticity diminishes, bringing with it increased risk of hypertension, stroke and cardiovascular mortality. The consumption of omega-3 polyunsaturated fatty acids (n-3 PUFAs), both long-chain docosahexaenoic acid (DHA) and medium-chain alpha-linolenic acid, has modest blood pressure-lowering effects, but there is limited information about their effects on arterial elasticity. In a preliminary study, 1.8 g eicosapentaenoic acid (EPA) daily for one year was associated with a slight decrease in pulse wave velocity compared with a significant increase in

Aging and vascular disease diminish arterial elasticity, bringing with it increased risk of hypertension, stroke and cardiovascular mortality.

this measure in the control group. Pulse wave velocity uses ultrasound waves to assess the speed of blood in determining the elasticity of the artery walls. More rigid blood vessels hasten the pulse wave, while a slower velocity reflects greater arterial elasticity. In another study of more than 500 elderly men with

hyperlipidemia who consumed 2.4 g n-3 LC-PUFAs daily for 3 years, arterial elasticity measured increased significantly, i.e., pulse wave velocity decreased compared with the control participants.

In the study reported here, Dr. S. Wang and colleagues of Xi'an Jiaotong University in China, used pulse wave velocity to assess arterial elasticity in 43 overweight Chinese patients with hypertension who consumed fish oil or placebo supplements for 8 weeks. Daily n-3 LC-PUFA supplementation provided 900 mg/day of n-3 LC-PUFAs from salmon oil, containing 540 mg EPA and 360 mg DHA. Participants had a body mass index

of ≥ 23 and systolic blood pressure ≥ 140 with diastolic pressure ≥ 90 . Blood pressure control medications were suspended for 2 weeks prior to the study. Measures of pulse wave velocity, plasma lipids and blood pressure were obtained at baseline and after 56 days.

After 8 weeks of fish oil supplementation, there was a significant 21% increase in arterial elasticity, but no changes in the placebo group. Heart rate, systolic and diastolic blood pressures, plasma lipids and soluble vascular adhesion molecule-1 did not change significantly in either group during the study. As expected, n-3 PUFAs in serum fatty acids increased significantly only in the fish oil group, mainly at the expense of monounsaturated fatty acids.

There was a significant 21% increase in arterial elasticity with the consumption of 900 mg/day of n-3 LC-PUFAs in these overweight patients. Blood pressure did not change.

The primary outcome in this study was a significant increase in arterial elasticity with the consumption of 900 mg/day of n-3 LC-PUFAs for 8 weeks. This change occurred despite the lack of change in blood pressure. Large artery elasticity has a genetic

component, but is also blood pressure dependent, with decreased elasticity occurring at high blood pressure. However, hypertension triggers arterial hypertrophy, which reduces arterial stiffness. It is noteworthy that a significant reduction in arterial stiffness occurred in a relatively short time, 8 weeks, with a dose of n-3 LC-PUFAs that has been linked to reduced cardiovascular mortality in survivors of myocardial infarction. These results strengthen previous findings of improved arterial compliance with the consumption of n-3 LC-PUFAs, suggesting another way in which these fatty acids protect heart health.

Wang S, Ma AQ, Song SW, Quan QH, Zhao XF, Zheng XH. Fish oil supplementation improves large arterial elasticity in overweight hypertensive patients. Eur J Clin Nutr 2007; doi: 10.1038/sj.ejcn.1602886.

High Dose EPA Reduces Arterial Stiffness That Accompanies High-Fat Meals

Various studies have reported that consumption of fish or fish oils may affect vascular function in ways that increase forearm blood flow and arterial elasticity, while reducing blood pressure. However, there is some disagreement about the effects on vascular relaxation of docosahexaenoic acid (DHA), a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA), in spite of its association with improved endothelial function.

Diminished arterial function is believed to contribute to atherosclerosis and diabetes. Consumption of fish or fish oils may improve vascular function and increase blood flow.

High-fat meals can impair endothelial function and flow-mediated dilation within 3 to 4 hours, the time when peak postprandial lipemia—the rise in blood fats follow-

ing a meal—occurs. Different fatty acids also affect flow-mediated dilation, at least in individuals with type 2 diabetes, with saturated fatty acids reducing it and n-3 PUFAs increasing it. However, others have reported increased forearm blood flow and flow-mediated dilation in healthy volunteers following a fatty meal, changes that were not associated with impaired endothelial nitric oxide release. Increases in flow-mediated dilation may offset reductions in blood flow, helping to maintain healthy vascular reactivity. Diminished endothelial function is believed to contribute to atherosclerosis and diabetes. In this report, the effect of eicosapentaenoic acid (EPA), an n-3 LC-PUFA, on postprandial vascular function, including oxidative stress, nitric oxide production, was examined in 21 healthy, non-smoking, young men, aged 18 to 35 years. Seventeen men completed the study.

After an overnight fast, participants were given a high-fat test meal containing 51 g of fat mainly from high-oleic sunflower oil that included either 5 g of EPA (and about 700 mg DHA) or 7 g of additional oleic acid. Measurements of blood pressure, digital volume pulse to assess arterial stiffness and vascular tone, and plasma isoprostanes, triglycerides and fatty acids were obtained at baseline, 3 and 6 hours after consumption of the test meal. Participants consumed a second high-fat meal containing 44 g fat (26 g saturated fat) after 4 hours to enhance any lipemia-induced change in arterial function and mimic typical eating behavior.

Men consuming the EPA-enriched meals exhibited a steep increase in plasma EPA that peaked 5 hours after the test meals (Figure 1). There was a small, but statistically significant, increase in DHA as well. Neither fatty acid concentration was altered by consumption of the placebo high-oleic acid meal. Arterial stiffness calculated from the digital volume pulse measurements was significantly reduced 6 hours after the EPA test meal, but not after the placebo meal. Vascular tone decreased at 6 hours in both groups, suggesting vasodilation, but did not differ between the two meals. Plasma 8-isoprostane $F_{2\alpha}$ concentration, a marker of lipid peroxidation, increased after 6 hours in the EPA group, but the two groups did not differ significantly. Lack of group

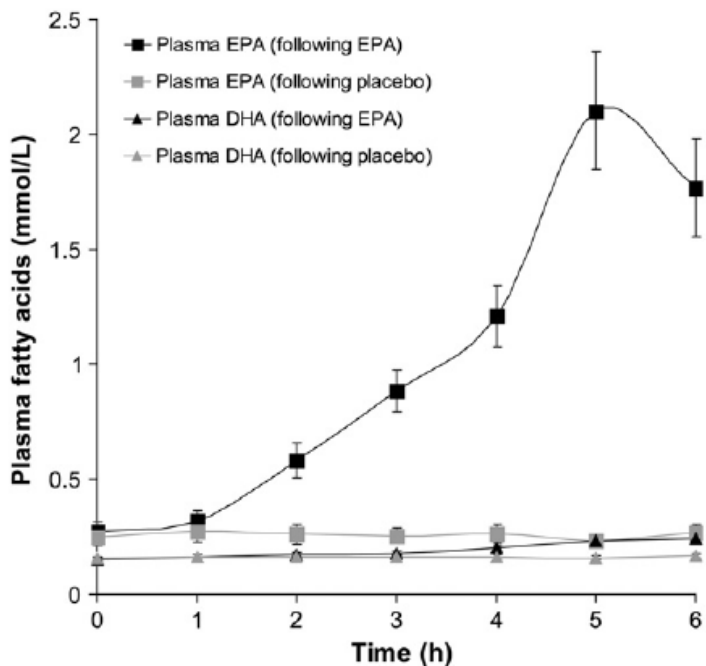


Figure 1. Plasma (n-3) PUFA concentrations in healthy men following consumptions of EPA and placebo test meals on 2 separate occasions. Values are means \pm SE, n=16 (missing data due to sample loss). Treatment effect plasma EPA ($P < 0.0001$) and DHA ($P < 0.005$), treatment x time plasma EPA ($P < 0.0001$) and DHA ($P < 0.0001$), 2-way repeated measures ANOVA. Image: *J Nutr* 2008; 138:287-291. (c) 2008 American Society for Nutrition.

difference is likely attributable to the small number of participants. Total nitrate and nitrite, metabolites of nitric oxide, decreased in both groups compared with baseline values, but changes did not differ between the groups. There were no significant differences in blood pressure and heart rate either.

The reduced arterial stiffness observed with increased consumption of a high dose of n-3 LC-PUFAs—5 g of EPA—supports previous reports of improved endothelial function with n-3 LC-PUFAs (vasodilation, brachial artery dilation) in healthy individuals and patients with type 2 diabetes and hyperlipidemia. The marked increase in plasma 8-isoprostane $F_{2\alpha}$ suggests increased lipid peroxidation not specific to EPA. Overall, the study suggests that consumption of EPA contributes to improved endothelial function during postprandial lipemia that may offset the potentially harmful effects of high-fat meals associated with impaired vascular activity.

Hall WL, Sanders KA, Sanders TAB, Chowienczyk PJ. A high-fat meal enriched with eicosapentaenoic acid reduces postprandial arterial stiffness measured by digital volume pulse analysis in healthy men. *J Nutr* 2008;138:287-291.

■ MATERNAL AND INFANT HEALTH

Perinatal DHA Associated with Better Motor Function at Age 7

Optimum development of the central nervous system in pre- and postnatal life requires sufficient amounts of long-chain polyunsaturated fatty acids (LC-PUFAs) of both the omega-6 (n-6) and omega-3 (n-3) families. Arachidonic acid (ARA) and docosahexaenoic acid (DHA) comprise the majority of LC-PUFAs in the brain and eye and are important for brain structure and function. Provision of LC-PUFAs to pregnant women, especially n-3 LC-PUFAs, has been associated with higher scores in mental processing at age 4, higher scores for eye and hand coordination at age 2½ years, better neurologic status at 18 months of age and higher visual acuity scores at age 4 months. In the largest study of

Provision of long-chain PUFAs to pregnant women, especially those of the omega-3 family, is associated with higher scores in mental processing, better eye and hand coordination, higher visual acuity and better neurologic status in the offspring.

its kind, children in the Avon Longitudinal Study whose mothers who ate more than 340 g (12 oz.) of fish/week had significantly higher IQ scores at age 8, fine motor skills at 42 months of age and higher social development scores at 42 months compared with children whose mothers ate less than 340 g of fish or none at all.

In the study described here, a cohort of 750 Caucasian children in the Netherlands was followed from birth as part of a long-term study on the relationships between maternal essential fatty acid status, pregnancy outcomes and children's mental development. The investigators assessed the children's neurological, cognitive, visual and motor functions when they were 7 years old. By then, 306 children were available for examination. Motor function was assessed using the Maastricht Motor Test, which measures quantitative and qualitative performance in 20 tasks. For example, a child standing on one leg was measured quantitatively for the time she holds the position and qualitatively for movement during the position. All scores were recorded on a 3-point scale and the scores were added to give a total quantity score. Fatty acid profiles were measured in plasma phospholipids obtained from umbilical cord blood at birth and venipuncture at age 7 in 261 children.

All children except one born with a birth trauma had neurological function in the normal range. Motor scores were obtained for 290 of the 306 children and scores

did not differ between children breast-fed or given infant formula without LC-PUFAs.

When the investigators examined the motor test scores, nearly all quantitative or qualitative assessments were significantly higher in girls than boys. Girls edged out the boys in the total motor score as well (119 vs 111, $P < 0.0001$).

To see if umbilical or plasma fatty acid levels at age 7 were related to the motor scores, the study authors performed backward stepwise multiple regression analyses. All analyses were corrected for sex, age at measurement and cognitive performance, as these variables were significantly related to the motor scores. For total motor test scores at age 7, DHA at birth was significantly and positively related to movement outcomes, whereas arachidonic acid was not. Neither DHA nor arachidonic acid measured in the children at age 7 was related to total motor test scores.

For the motor quality scores, umbilical DHA was again positively associated with higher scores, but arachidonic acid was of borderline significance for an inverse effect. At age 7, neither DHA nor arachidonic acid in plasma phospholipids was associated with qualitative motor scores. These LC-PUFAs were unrelated to any motor quantitative scores for either umbilical plasma values or those obtained at age 7.

DHA at birth, but not at age 7, was associated with better total and qualitative motor performance. Arachidonic acid was not associated with motor scores.

The key observation in this prospective study was that DHA at birth, but not at age 7, was positively associated with better total and qualitative motor performance. Thus, prenatal supply of DHA may be important in childhood motor function, although this study could not establish causality. Others have reported a significant relationship between cognitive and motor performance in children aged 5 to 6 years after the influence of attention had been controlled. A relationship between qualitative, but not quantitative, motor performance and the chance of developing attention-deficit hyperactivity disorder has also been noted. Umbilical DHA has been associated with neurodevelopmental outcomes at 18 months of age. That the effect of prenatal DHA supply might bear on developmental outcomes years later underscores the importance of sufficient maternal n-3 LC-PUFA consumption during pregnancy.

Bakker EC, Hornstra G, Blanco CE, Vles JS. Relationship

between long-chain polyunsaturated fatty acids at birth and motor function at 7 years of age. Eur J Clin Nutr 2007; doi:10.1038/sj.ejcn.1602971

Usual Maternal DHA Intakes May Increase Chance of Lower Visual Acuity in Infants

Optimum fetal and infant neurodevelopment requires adequate amounts of long-chain polyunsaturated fatty acids (LC-PUFAs), especially those of the omega-3 (n-3) family. Both arachidonic acid and docosahexaenoic acid (DHA) are required. The availability of arachidonic acid appears to be adequate, whereas the adequacy of DHA depends on maternal stores and the consumption of preformed DHA. Conversion of the parent 18-carbon n-3 PUFA, alpha-linolenic acid, to DHA in humans is very low, $< 0.1\%$. Thus, the fetus depends on the adequacy of the mother's diet to furnish the necessary DHA. The most recent expert recommendation for maternal DHA consumption during pregnancy and lactation is 200 mg of DHA/day.

The fetus depends on the adequacy of the mother's diet to furnish the necessary DHA for neurodevelopment. Women in North America seldom consume 200 mg of DHA/day. How many might be deficient?

Women in North America seldom consume 200 mg of DHA daily and estimates of their mean intake range from 40 to 120 mg/day in Canada and the United States. Individual intakes range from 20 to > 500 mg/day. Although precise estimates of how

much DHA is needed during pregnancy are unknown, it would be expected from these data that a certain proportion, possibly the majority, of pregnant women consume insufficient DHA to meet their infants' needs.

To learn more about the proportion of pregnant women who might be deficient in DHA, Sheila Innis and Russell Friesen at the University of British Columbia, Vancouver, Canada, conducted a trial among 135 pregnant women who were recruited at 14 to 16 weeks' gestation. Participants were randomized to receive either 400 mg/day of DHA or a corn-soybean oil placebo providing 530 mg linoleic acid and 80 mg alpha-linolenic acid per day. Women consumed the capsules from enrolment through delivery. The investigators designed the study to determine the distribution of development scores for infants whose mothers consumed adequate DHA. This distribution served as a reference for the comparison of development scores from infants whose mothers did not receive supplemental DHA. As a reflection of early development, the investigators selected visual acuity, assessed at 60 days of age using the Teller Acuity Cards

(Illus.). This test is a sensitive and robust measure of differences in visual acuity attributable to DHA status.

The investigators assessed maternal DHA status by measuring the fatty acids in the ethanolamine phosphoglyceride fraction of the participants' red blood cells. This determination was selected to minimize the variability in fatty acids associated with changes in plasma lipoproteins, gestation and diet. Red cell ethanolamine phosphoglycerides also have higher concentrations of DHA than other phospholipids.

Mothers participating in the study were 73% white, 76% university-educated and 33 years old on average at enrolment. All infants were born at 37 to 42 weeks' gestation. Fifty of 67 infants in the placebo group and 42 of 68 in the DHA group were exclusively breast-fed at 60 days of age. All formula-fed infants received term formula containing DHA and arachidonic acid. Maternal fatty acid intakes assessed at enrolment and 36 weeks' gestation did not differ between the DHA and placebo groups, but DHA consumption ranged from 10 to 760 mg/day.

From 16 to 36 weeks' gestation, maternal red blood cell DHA increased significantly in the supplemented women, but not in the placebo group. At 36 weeks, DHA concentrations were 32% higher in the supplemented group (11.7 vs. 8.9 g/100 g fatty acids, respectively). Supplemented women also had significantly lower docosapentaenoic acid of both the n-6 and n-3 families, whereas placebo women had higher concentrations of n-6 docosapentaenoic acid at 36 weeks compared with values at 16 weeks. Increased levels of this fatty acid are usually associated with low levels of DHA. Arachidonic acid concentrations diminished in both groups over the 20-week gestation period, with levels in the supplemented group being significantly lower than in the placebo group.



Visual acuity measurement with Teller Acuity Cards. Image courtesy of Grendahl Eye Associates.

Mean visual acuity scores for infants of placebo and DHA-supplemented mothers were 2.4 vs 2.6 cycles/degree, a difference that was not statistically significant. Average scores for girls were greater than for boys (2.6 vs 2.3), but these differences, too, did not reach statistical significance. When the scores were

analyzed separately for girls and boys, the trend for higher scores in the supplemented infants was observed in girls and boys, but the differences missed statistical significance. In multivariate analysis, infants in the placebo group were 3 times more likely to have lower visual acuity scores—scores below the mean—than infants of mothers consuming greater amounts of DHA (Odds Ratio: 3.4). The investigators noted that only infant sex and intervention group were related to visual acuity. Maternal smoking, alcohol consumption and duration of breast-feeding were unrelated to visual acuity scores.

In evaluating the relationships between maternal ethanolamine phospholipid fatty acids and infant visual acuity, the only significant association was observed for the concentration of n-6 docosatetraenoic acid (22:4n-6) and reduced visual acuity scores in both girls and boys. DHA and n-6 docosapentaenoic acid were not significantly associated with visual acuity.

Infants in the placebo group were 3 times more likely to have lower visual acuity scores than infants of mothers consuming greater amounts of DHA.

Although DHA is necessary to support central nervous system development, how much is needed and what constitutes inadequate maternal DHA status are not known with precision. What is known is that DHA intakes tend to be low in countries with

low fish consumption compared with countries having higher fish consumption. In this study, infants of mothers not consuming supplemental DHA were 3 times more likely to have visual acuity below the mean than infants of mothers who consumed 400 mg of DHA/day. Others have reported that term infants who received DHA from breast milk compared with those fed formula without DHA had significantly better visual acuity. Mothers not consuming extra DHA had significantly higher concentrations of n-6 docosapentaenoic acid in their red blood cell ethanolamine phosphoglycerides, a finding that is characteristic of DHA deficiency.

By comparing visual acuities in infants of women consuming their usual diet with those whose mothers consumed 400 mg DHA/day and thus were unlikely to have inadequate DHA intakes, the investigators demonstrated that current DHA intakes are associated with a 3-fold greater risk of lower infant visual acuity scores. This observation implies that current DHA consumption among these pregnant women—median intake was 110 mg/day—is insufficient for optimum infant development.

Innis SM, Friesen RW. Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. Am J Clin Nutr 2008;87:548-557.

■ IMMUNE FUNCTION

Omega-3 PUFAs Linked to Reduced Immune Regulatory Protein in Atopic Children

The occurrence of allergic diseases and atopy—allergic hypersensitivity responses in the body not in direct contact with the allergen—has been increasing worldwide without obvious cause. Atopy usually appears as eczema, conjunctivitis, rhinitis and asthma and is defined by the presence of elevated serum levels of immunoglobulin E (IgE) and positive responses to skin prick tests. Besides family history, many factors have been associated with higher risk of atopy, including: pollen, hygiene, IgE sensitization, breast-feeding, rural environment,

Many factors are associated with higher risk of atopy, but the key is whether any of the observed associations are causative, and if so, for which types of atopy. Studies with fish oil remain inconclusive.

African-American heritage, airborne allergens, high intake of polyunsaturated fatty acids (PUFAs) and others. Factors reportedly associated with lower incidence of atopy include day care attendance, maternal fish consumption in pregnancy, preterm birth, higher cholesterol levels

in infancy and childhood and early exposure to infections. Other factors have been reported, but the key is whether any of the observed associations are causative and if so, for which types of atopy. Here, the answers are unclear.

Fish consumption in early childhood and increased maternal intake of long-chain omega-3 PUFAs (n-3 LC-PUFAs) in pregnancy have been associated with reduced atopy and some allergic conditions, but others reported that modification of dietary n-3 and n-6 PUFAs was unhelpful in preventing atopy and asthma. In complex conditions with multiple etiologic factors, such as allergic diseases, modifying single factors might be expected to have a limited impact. However, the many modifications in immune function associated with n-3 LC-PUFAs in clinical conditions and cultured immune cells continue to drive the search for a better understanding of how these fatty acids might be involved in the development and treatment of allergic conditions.

In the study reported here, researchers in Norway examined the relationships among atopy and cord plasma IgE

levels, LC-PUFAs and a protein known as CD23. This receptor protein is found on mature B cells (from bone marrow) and activated macrophages (immune cells). It is involved in controlling IgE synthesis and mediating many IgE-related immune responses. But how it might be involved in atopy, a condition characterized by increased IgE synthesis, is uncertain.

CD23 has a low affinity for IgE and being soluble, it can circulate in the blood and interact with other cells. For example, CD23 activates monocytes and triggers the production of several inflammatory mediators from T cells. It also participates in inflammatory responses associated with intestinal responses to food allergies by releasing chemokines in human intestinal epithelial cells. However, mice lacking the CD23 protein had increased IgE production, whereas those making excessive amounts had strongly suppressed IgE responses. What accounts for these paradoxical effects? It turns out that endogenous protease enzymes cleave CD23 into different fragments that either stimulate or inhibit IgE synthesis in human B cells.

In patients with allergic asthma, blocking CD23 was associated with significantly lower IgE concentrations and only mild adverse events. In another study of asthmatic patients, those who took an antibody to block CD23 binding used less asthma medications and had fewer asthma exacerbations. Similar symptomatic improvements were reported in asthmatic patients consuming n-3 LC-PUFAs who exhibited reduced airway narrowing, use of medications and inflammatory mediators in exercise-induced bronchoconstriction. In contrast, several studies in children reported that cord blood levels of soluble CD23 were unrelated to atopy at age 3 years. Evidence for a moderating effect of high plasma n-3 LC-PUFAs in childhood asthma appears weak.

Kristine Byberg and colleagues at the University of Bergen, Norway, wanted to explore the relationship between CD23 and plasma n-3 LC-PUFAs in allergic children. From a cohort of children born at the Stavanger University Hospital from 1994 to 1995 who had cord blood samples available, they recruited 35 children with a positive skin prick test at age 3 years. These children were matched with 35 non-atopic controls born on the same day. All atopic children had a history of atopic dermatitis as well. Cord blood plasma samples were analyzed for LC-PUFAs, soluble CD23 and IgE.

The most striking observation was the inverse association between total plasma n-3 PUFAs and the concentration of soluble CD23. Significant inverse correlations were observed with DHA, EPA and DPA (n-3), but not with alpha-linolenic acid. There was no association

between soluble CD23 and any n-6 PUFA. Individual LC-PUFAs were not significantly different between atopic and non-atopic children, but a weak association between reduced eicosapentaenoic acid (EPA) and atopy ($P=0.056$) was observed. The sum of EPA and alpha-linolenic acid was significantly lower in atopic children compared with controls (7.8, CI=6.1-9.9 vs 9.6, CI=7.3-14.2, $P=0.03$). In girls ($n=18$), atopic children had significantly lower EPA concentrations than non-atopic girls. No n-6 LC-PUFAs were related to the presence of atopy.

Higher levels of n-3 LC-PUFAs were associated with lower concentrations of CD23, but IgE levels were unrelated to the n-3 LC-PUFAs. In girls, atopic children had significantly lower EPA concentrations than non-atopic girls.

Plasma IgE concentrations were unrelated to n-6 or n-3 PUFAs in all children or in atopic and controls analyzed separately. IgE concentrations were inversely associated with eicosanoic acid and levels of this fatty acid were significantly

lower in children with detectable IgE levels. The implication of this observation is unknown.

The significance of the inverse association between n-3 LC-PUFAs and soluble CD23 is unclear, given the lack of association between n-3 LC-PUFAs and IgE levels. Although it remains plausible that n-3 LC-PUFAs might reduce the synthesis of IgE through reduced levels of CD23, the prevalence of asthma, rhinitis and eczema at age 6 were reportedly unrelated to soluble CD23 or serum IgE levels. The weak association between higher levels of EPA in cord blood and reduced occurrence of atopy provides some support for an effect of n-3 LC-PUFAs, but it will require more data and consideration of confounding factors to substantiate such an effect.

Byberg K, Oymar K, Aksnes L. Fatty acids in cord blood plasma, the relation to soluble CD23 and subsequent atopy. Prostaglandins Leukot Essent Fatty Acids 2008;78:61-65.

Could Fish Consumption in Pregnancy Affect Respiratory Illness in Early Childhood?

Prenatal exposure to environmental toxins, cigarette smoke, alcohol and allergens is associated with impaired lung function, wheezing illness and immune deficits. Ambient air pollution in early life has also been linked to wheezing in infants. Immunomodulatory and anti-inflammatory effects of maternal consumption of fish or fish oil have been reported in some, but not all, studies and may underlie the reduced symptom sever-

ity observed in infants at high risk of allergic diseases. In spite of reductions in inflammatory mediators, the prevention of childhood allergies with control of house dust mites or increased consumption of omega-3 fatty acids (n-3 PUFAs) has not been demonstrated. Thus, the effect of these fatty acids, while suggestive, is disputed.

Maternal exposure to fine particles in pregnancy was linked to greater childhood coughing. But children of mothers who ate fish experienced less time coughing.

The novel epidemiological study described here examined the relationships between maternal prenatal exposure to fine particles ($\leq 2.5 \mu\text{m}$ in size) and respiratory symptoms in the offspring in the first 2

years of life, taking into consideration breastfeeding, maternal fish consumption and other confounding factors. Participants included 465 non-smoking women in Krakow, Poland, who gave birth to a single child between 29 and 43 weeks of gestation. Fish consumption was estimated from food frequency questionnaire data obtained in the second and third trimesters of pregnancy. Samples of home air particulates were collected by personal monitors worn by each participant for 2 consecutive days during the second trimester. Samples from the monitor's filter were analyzed for particles of $\leq 2.5 \mu\text{m}$ and the data converted to particulate mass/cubic meter. The median particulate mass was $35 \mu\text{g}/\text{m}^3$, with concentrations ranging from 10 to $295 \mu\text{g}/\text{m}^3$. To put these numbers in perspective, the US Environmental Protection Agency's (EPA) guideline for ambient air quality over a 24-hour period is now $35.0 \mu\text{g}/\text{m}^3$, reduced from the standard of $65.0 \mu\text{g}/\text{m}^3$ in effect at the time of this study. Twenty-four percent of participating mothers had a history of atopy.

Data about breastfeeding and the children's health and respiratory symptoms were obtained by in-home interviews every 3 months for 2 years. Children's respiratory events were classified as cough with or without cold, wheezing or whistling in the chest irrespective of respiratory infection and difficult (puffy) breathing.

In this population sample, about 13% of mothers did not eat fish. Those who did had a median intake of 150 g/week, with higher intakes in the third trimester compared with the second (176 vs 139 g/week). The most common children's respiratory symptom was cough (420 children over the 2-yr study), followed by difficult breathing (201), then wheezing (125). The mean duration of coughing was greater in the second year of life than the first (23 vs 19 days), while difficult breathing

lasted longer in the first year (13 vs 11 days). The duration of all symptoms was greater in children of mothers exposed to particulate levels above the median (Figure 1), but these differences were not statistically significant.

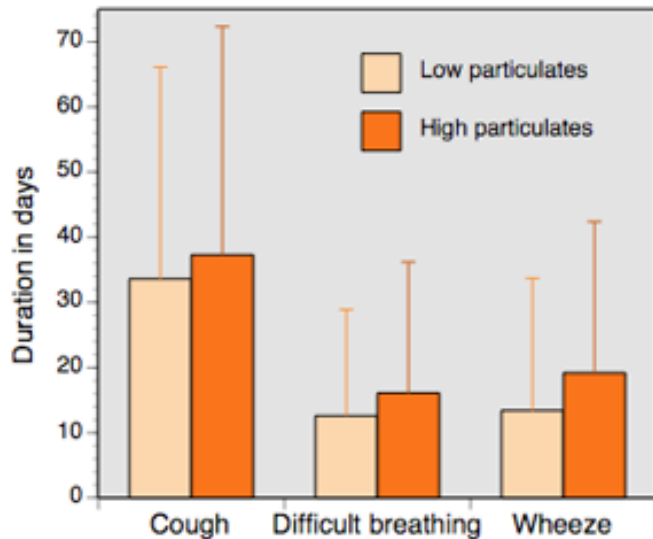


Figure 1. Mean duration of children's respiratory symptoms over 2 years relative to maternal exposure to fine particles in the second trimester.

The risk of developing each of the respiratory symptoms was calculated in relation to maternal exposure to fine particles in the second trimester adjusted for fish consumption, child's sex, maternal education, parity gestational age, maternal atopy, breastfeeding, postnatal environmental tobacco smoke and household molds. Curiously, exposure to fine particles well below the former EPA guideline of 65 µg/m³, but met EPA's current air quality standard, yet was associated with a 2.5-fold increased risk of cough.

The greatest risk factor for all respiratory symptoms was exposure to molds, which was associated with an 11-fold greater adjusted risk of having more cough days, a nearly 3-fold increase in wheeze and a 2-fold greater difficulty in breathing. The adjusted risk of more cough days was significantly associated with maternal exposure to fine particles (RR=2.5), but was reduced in mothers whose fish consumption was above the median (RR=0.85) (Figure 2). Interaction between fish consumption and particulates was not significant for reduced risk of cough. Fish consumption was also associated with significantly lower risk of difficult breathing and wheeze, but here the interaction between fish consumption and particulates was significant.

Other studies have reported a reduced risk of wheeze and atopy in children who consumed fish. Lower risk of childhood asthma was also associated with maternal

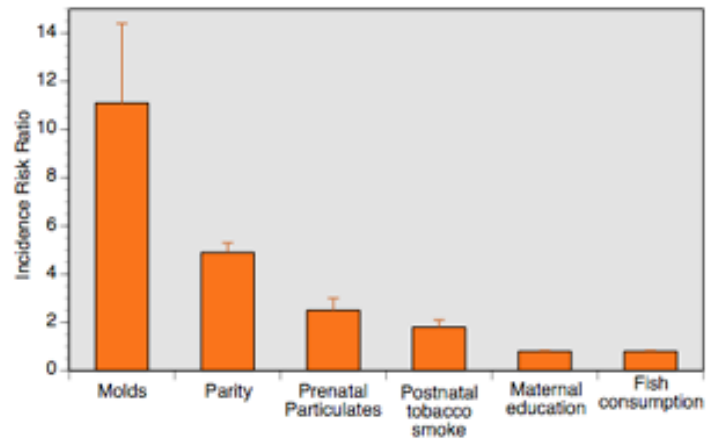


Figure 2. Incidence Risk Ratios + SE for number of days of cough symptoms in the first 2 years of life in relation to selected exposure variables.

fish consumption in pregnancy. What is novel about this study is that maternal fish consumption in pregnancy was associated with lower risk of children's respiratory symptoms when the mothers were exposed to fine particles during pregnancy. One caution about this observation, as the authors noted, is that the study could not distinguish between pre- and postnatal exposure to fine particles. Pregnancy exposures may have been a surrogate for current environmental exposure. However, the study joins others in suggesting that maternal fish consumption may benefit the child's respiratory system.

Jedrychowski W, Flak E, Mroz E, Pac A, Jacek R, Sochacka-Tatara E, Spengler J, Raub V, Perera F. Modulating effects of maternal fish consumption on the occurrence of respiratory symptoms in early infancy attributed to prenatal exposure to fine particles. Ann Nutr Metab 2008;52:8-16.

MENTAL HEALTH

EPA Eases Anxiety, DHA Cools Anger in Substance Abusers

Various studies have implicated low fish consumption or reduced blood levels of long-chain omega-3 fatty acids (n-3 LC-PUFAs) in the occurrence of various psychiatric disorders, alcoholism and among substance abusers. It has been known since 1996 that n-3 PUFAs, especially eicosapentaenoic acid (EPA), are reduced in major depression. A recent post-mortem analysis reported significantly lower levels of cortex docosahexaenoic acid (DHA) in patients with major depressive disorder. Clinical trials using n-3 LC-PUFAs or purified EPA or DHA to treat a variety of mental disorders have generally, but not always, reported favorable outcomes. It is unclear whether both EPA and docosahexaenoic acid (DHA) are effective and if so, what a suitable dose range is for each fatty acid. For example, some studies

Low fish consumption or reduced blood levels of n-3 LC-PUFAs are implicated in various psychiatric disorders, alcoholism and substance abuse. A pilot study reported improvements in anxiety among substance abusers who consumed n-3 LC-PUFAs. EPA has a similar effect in this report.

in depressed patients have used high doses (>6 g/day), while others reported that 1 g/day was effective, but doses of 2 to 4 g/day were less so. In a pilot study among substance abusers, supplementation with 3 g/day of EPA+DHA for 3 months was associated with reduced anxiety scores. In one report, both n-3 and n-6 PUFAs were sig-

nificantly lower in substance abusers who relapsed compared with those who did not.

This study reports the associations between changes in anger and anxiety scores and serum levels of EPA and DHA in 22 substance abusers who consumed about 3 g/day of n-3 LC-PUFAs or soybean oil for 3 months. The n-3 LC-PUFA capsules provided 2.2 g/day of EPA, 500 mg/day DHA, and 50 mg of n-3 docosapentaenoic acid and alpha-linolenic acid. Participants were enrolled in an outpatient substance abuse program in Brooklyn, New York, and were free of major psychiatric and physical illnesses and had liver function tests no greater than 1 SD above maximum normal values.

Participants completed a dietary history questionnaire and a modified version of the Profiles of Mood States questionnaire 1, 2 and 3 months following the start of the study and gave blood samples at the beginning and end of the study. Fatty acids were determined in plasma. The Profiles in Mood States includes scores for anger, anxiety, depression, vigor, confusion and fatigue. Eight patients received methadone treatment and 5 took antidepressants in stable doses throughout the study. Participants did not differ at baseline in body mass index, energy consumption or low intake of n-3 LC-PUFAs.

After 3 months of treatment, participants consuming the n-3 LC-PUFAs had a significant decline in their anger scores, whereas scores in those taking the placebo capsules increased slightly. Scores reached their maximum after 2 months of n-3 LC-PUFA consumption. Anger scores continued to decline throughout the 3-month period for those on the active n-3 LC-PUFA treatment. A similar pattern of significantly improved anxiety scores was observed for substance abusers consuming the n-3 LC-PUFAs, but scores were unchanged in those taking the placebo.

When the investigators examined the relationship between the anger and anxiety scores at the end of the study with the percent change in plasma fatty acids over the 3-month study, they observed significant associations (Figure 1). Lower anger scores were associated with increased DHA, n-3 docosapentaenoic acid and total n-3 LC-PUFAs, but not with increased EPA concentrations. In contrast, reduced anxiety was associated with increased EPA, but not with changes in DHA. Changes in anger and anxiety scores were not associated with changes in any n-6 PUFAs.

It is worth noting that improved scores for anger and anxiety related to increased DHA and EPA, respectively, but neither behavior related to both DHA and EPA when these fatty acids were examined individually. However, both behaviors were significantly associated with increases in the total concentration of n-3 LC-PUFAs measured in µg/ml.

Only a few studies have reported an association between low plasma DHA and hostility in violent men and young urban males. Students under the stress of school exams who consumed 1.5 to 1.8 g/day of DHA exhibited no aggression against others compared with increased aggression observed in those taking a placebo. This study adds another link between DHA and aggressive behavior as reflected in anger scores.

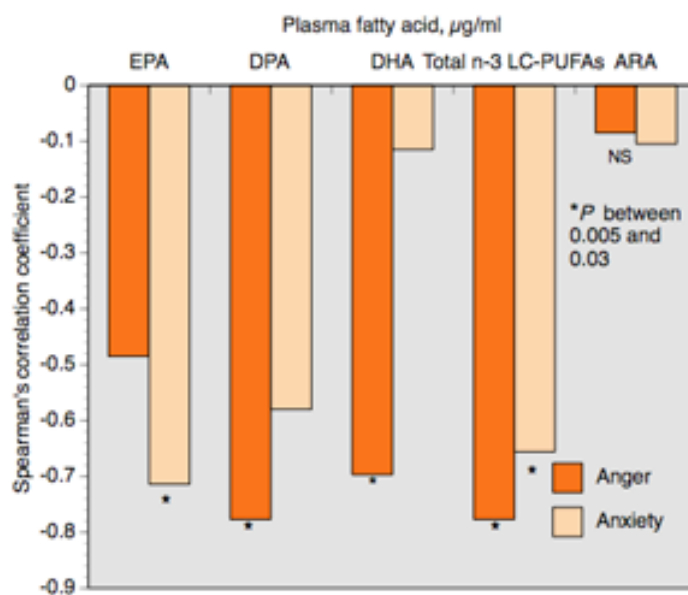


Figure 1. Spearman's rank correlation coefficients for anger and anxiety scores and percent changes in plasma LC-PUFA levels in substance abusers after 3 months of consuming 3 g/day n-3 LC-PUFAs.

As with anger, studies of anxiety and n-3 PUFAs are scarce. A few have suggested a link between increased anxiety and low levels of EPA or n-3 LC-PUFAs. The admin-

istration of a mixture of n-3 and n-6 PUFAs to anxious college students was associated with improved appetite, mental concentration and academic organization. Patients with social anxiety disorder had significantly lower concentrations of n-3 PUFAs compared with those not having this disorder. Moderate fish consumption (83 to 112 g/day or 3 to 4 oz/day) or n-3 LC-PUFA intake was associated with a significant 30% lower risk of incurring depression, anxiety or stress in a 2-yr prospective study of Spanish adults. The present report identified increased EPA specifically with improved anxiety scores and higher DHA with less anger. Although it may be tempting to suggest that EPA and DHA influence anxiety and anger behaviors through different mechanisms, and plausible explanations could be proffered, these specific associations need more robust confirmation before reaching firm conclusions. Investigation of EPA and DHA administered individually without the other would be useful across an array of mental disorders. This study supports others in demonstrating that anxious or angry substance abusers who have low blood levels of

Lower anger scores were associated with increased DHA, while reduced anxiety was associated with increased EPA in this study of substance abusers.

n-3 LC-PUFAs achieve significant improvements in their mental health when treated with a moderate dose of EPA and DHA. This finding could ease the mind and, one hopes, encourage additional research.

Buydens-Branchey L, Branchey M, Hibbeln JR. Associations between increases in plasma n-3 polyunsaturated fatty acids following supplementation and decreased anger and anxiety in substance abusers. Prog Neuropsychopharmacol Biol Psychiatry 2008;32:568-575.

LC-PUFA Imbalance: Low EPA, High ARA Linked to Neuroticism in Healthy Adults

The involvement of long-chain polyunsaturated fatty acids (LC-PUFAs) in psychological and psychiatric conditions has attracted considerable attention in the past several years. This is partly because patients with various personality and mental disorders have been reported to have lower concentrations of LC-PUFAs, especially those of the omega-3 (n-3) family than people without such conditions, and because treatment with n-3 LC-PUFAs has improved symptoms in several studies. Others have reported associations between low concentrations of n-3 LC-PUFAs in red blood cells or plasma lipids and increased anger, anxiety, hostility and risk of suicide.

Associations between n-3 LC-PUFAs and mental disorders have been more widely reported than links with

n-6 PUFAs, mainly linoleic or arachidonic acids. Thus, it is unclear whether dietary intake or tissue concentrations of n-6 PUFAs are associated with the development of mental disorders or whether they simply reflect the effects of low intakes of n-3 LC-PUFAs. Because arachidonic acid (ARA) and docosahexaenoic acid (DHA) sometimes have opposite effects, imbalances between these LC-PUFAs may contribute to risk.

In the cross-sectional study described here, Sarah Conklin and colleagues at the University of Pittsburgh School of Medicine, USA, determined whether a relationship existed between serum fatty acid concentrations and assessments of depression and neuroticism in 116 healthy volunteers recruited from the community. Participants were between the ages of 30 and 55 years (mean age 45 years) and 43% were male. Study participants were free of clinically apparent chronic diseases, psychotropic, cardiovascular or diabetic medications, fish or flax seed oil supplements and diagnosed mental disorders. All completed the neuroticism NEO Personality Inventory and Beck Depression Inventory assessments to provide information about personality characteristics and depressive symptoms. The investigators focused on neuroticism—

Low levels of n-3 LC-PUFAs are observed in various mental and mood disorders. In these healthy adults, those with higher scores for neuroticism, a forerunner of depression, had lower blood levels of EPA and higher ARA.

the enduring tendency to experience negative emotional states—because its presence increases the risk of depression. Facets of neuroticism include anxiety, angry hostility, depression, self-consciousness, impulsivity and vulnerability. Fatty acids were analyzed in serum phospholipids.

Scores for the Beck Depression Inventory were severely skewed, so the investigators grouped the scores into 2 groups, those with no or minimal symptoms (scores ranging from 0 to 9) and those with mild to moderate symptoms (scores 10-17). The n-3 LC-PUFAs were not normally distributed, so values for eicosapentaenoic acid (EPA) and DHA were transformed logarithmically. Data for ARA and the NEO Inventory were normally distributed. The relationships between phospholipid fatty acid concentrations and scores for the components of neuroticism were analyzed by linear regression, controlling for age, race and gender. Depression scores were analyzed by logistic regression.

As shown in Figure 1, the overall neuroticism score was significantly and inversely related to the serum phos-

pholipid EPA concentration ($P=0.004$), but not to DHA ($P=0.23$). Scores for neuroticism increased significantly with higher levels of ARA ($P=0.009$). Four of the 6 individual components of neuroticism—*anxiety, angry hostility, self-consciousness and impulsivity*—were also significantly and inversely related to EPA, but not to DHA. Scores for overall neuroticism, anxiety, depression and vulnerability increased with higher concentrations of ARA. The odds of having mild to moderate depressive symptoms according to the Beck score were significantly increased by nearly 3-fold with higher concentrations of ARA ($P=0.005$). There was also a significant correlation between higher Beck depression scores and overall neuroticism ($P\leq 0.001$) and with each of the 6 facet scores. When the depression scores were reanalyzed taking into account the neuroticism scores, the chance of mild to moderate depressive symptoms was associated with higher levels of ARA, but not with EPA or DHA.

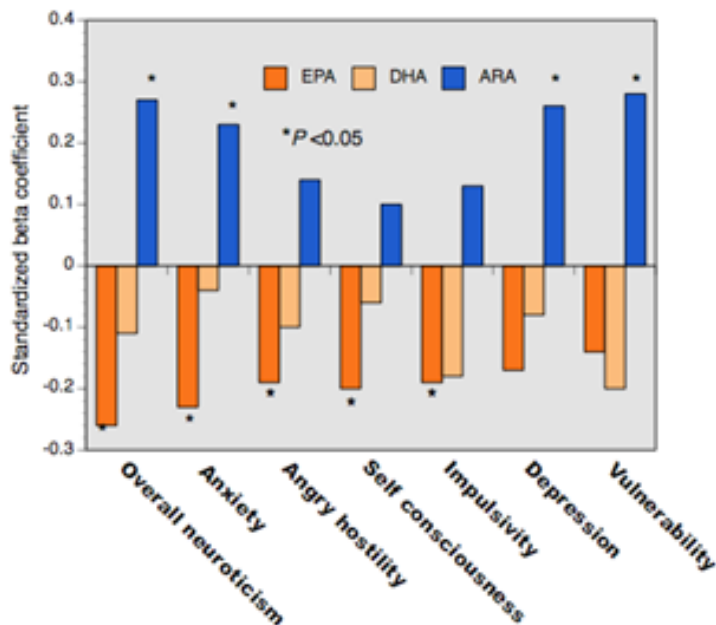


Figure 1. Linear regression coefficients for relationships between serum LC-PUFAs and neuroticism scores.

In this healthy population, higher EPA concentrations in serum phospholipids were linked to lower neuroticism scores, a condition predisposing a person to depression. This finding supports other studies linking EPA status to depression and other mood disturbances, mainly in patient populations. However, few studies have assessed neuroticism itself, but have singled out its components, such as depression, anger and anxiety. It is noteworthy that significant associations between low EPA concentrations and 4 of the 6 facets of neuroticism were observed. The positive association between negative mood traits and mild to moderate depressive symptoms with ARA has been observed previously, but less frequently, and metabolites of the ARA cascade are

In this healthy population higher blood EPA concentrations were linked to lower neuroticism scores. This finding supports other studies linking low EPA status to depression and other mood disturbances. In contrast, higher ARA was associated with mild to moderate neuroticism.

thought to be involved in negative mood disorders.

A higher ratio of ARA to EPA was reported in this study and others, but alterations in both PUFAs could contribute to changes in the ratio, complicating the interpretation of such data. Western diets that provide high intakes of n-6 PUFAs, but relatively small amounts of n-3

PUFAs, especially n-3 LC-PUFAs, would foster higher ratios of ARA to EPA because the denominator is small. For several reasons, ratios of n-6 to n-3 fatty acids are not useful concepts.

Other studies of mood and mental disorders have reported that EPA may be more effective than DHA in treating patients with these conditions. The observation here that serum DHA concentrations were unrelated to neuroticism or depressive symptoms lends indirect credence to these reports. However, without a more detailed understanding of the mechanisms involved in these disorders, it would be premature to conclude that only derangements of EPA metabolism were involved. This point is especially important as DHA and ARA are the primary PUFAs in neuronal membranes and significant DHA deficits in the brains of patients with major depression have been observed.

Conklin SM, Manuck SB, Yao JK, Flory JD, Hibbeln JR, Muldoon MF. High ω -6 and low ω -3 fatty acids are associated with depressive symptoms and neuroticism. Psychosom Med 2007;69:932-934.

■ VISUAL FUNCTION

Alpha-Linolenic Acid Reduces Inflammation and Corneal Damage in Dry Eye Syndrome

Dry eye syndrome, a condition associated with ocular discomfort and insufficient tear flow, affects more than 10 million people in the U.S., mainly women. It occurs more often among people with a history of arthritis, gout, diabetes, thyroid disease and smoking, whereas frequent consumption of tuna is associated with lower risk. Inflammation of the eye's surface and the greater frequency of dry eye among individuals with chronic inflammatory diseases emphasize the importance of inflammation in the condition. Dietary consumption of linoleic acid and gamma-linolenic acid was associated

with significant improvements in symptoms in patients with dry eye living in Genoa, Italy, and in patients with Sjogren's syndrome, an autoimmune disease of the exocrine glands. In the U.S., however, intakes of linoleic acid are high, yet the condition is highly prevalent.

In this study, Saadia Rashid and colleagues at the Schepens Eye Research Institute, Boston, USA, examined the effect of applying alpha-linolenic acid, linoleic acid, or the two fatty acids together in treating mice exposed to low humidity and given scopolamine 3 times every 3 hours to induce dry eye syndrome. Treatment consisted of 0.2% alpha-linolenic acid or linoleic acid or 0.1% of each fatty acid together contained in an emulsified surfactant solution. Treatment began 48 hours after induction of dry eye syndrome and was applied topically once daily for 4 or 9 days. One group received only the vehicle treatment and another no eye drops. Signs of dry eye were measured on days 5 and 10. Corneal fluorescein staining was performed at baseline and on days 5 and 10. Excised corneas were examined for antibody responses to cytokines IL-1 α , TNF- α , IL-2, IFN- γ , IL-6 and IL-10.

After induction of dry eye, corneas exhibited significantly increased fluorescein staining throughout the 10-day study. This finding indicates erosion of or damage to the cornea. At day 5, corneas treated with alpha-linolenic acid had significantly less damage (45%) compared with untreated eyes and those treated with linoleic acid, but were similar to those treated with both linoleic and alpha-linolenic acids. By day 10, only those animals treated with alpha-linolenic acid had significantly reduced fluorescein staining, a 62% reduction compared with vehicle-treated eyes and a 71% reduction compared with untreated eyes. Eyes treated with both linoleic and alpha-linolenic acids showed no improvement compared with controls.

Expression of the pro-inflammatory mediators IL-1 α and TNF- α in the corneas exhibiting dry eye showed slight increases in IL-1 α production from day 2 to day 10, but large and significant increases in TNF- α production by days 5 and 10 compared with baseline and day 2. The cytokines IL-2, IL-6 and IL-10 were not detected in dry eye

corneas. Treatment with alpha-linolenic acid significantly reduced the number of CD11b+ cells in both the center and periphery of the dry eye corneas, on average 40%, compared with controls and other treatment groups. These cells are markers for immune

This study is the first report of significantly reduced inflammation and corneal damage in dry eye syndrome with topical α -linolenic acid treatment.

cells derived from monocytes and macrophages.

In contrast, the conjunctiva—the lining of the eyelid and eyeball excluding the cornea—showed significantly increased cytokine expression in dry eye, with increases varying from 5-fold for IL-2 to 98-fold for IL-10 by 10 days. Interleukin-10 and n-3 PUFAs inhibit innate and T-cell mediated immunity and the production of IL-1 and TNF- α . In this study, treatment with alpha-linolenic acid, but not with any other fatty acid, resulted in a significant decrease in IL-1 α at day 10 and TNF- α at day 5.

This study is the first report of significantly reduced inflammation and corneal damage in dry eye syndrome with topical alpha-linolenic acid treatment. Treatment reduced inflammation in the cornea and conjunctiva and blocked leukocyte infiltration into the cornea. Whether alpha-linolenic acid acts directly on immune responses or is first converted to long-chain polyunsaturated derivatives (n-3 LC-PUFAs) is not known. There are reports that mouse corneas are able to synthesize the n-3 LC-PUFA derivatives lipoxins and neuroprotectin D1, substances with potent anti-inflammatory properties and the ability to increase epithelial wound healing. Although linoleic acid was without effect in these studies, the authors suggest that gamma-linolenic acid, a derivative of linoleic acid previously associated with improved ocular comfort in dry eye, might confer additional benefits. One can easily envision topical treatments for dry eye reformulated to contain n-3 PUFAs that might take the sting out of this condition.

Rashid S, Jin Y, Ecoiffier T, Barabino S, Schaumberg DA, Dana MR. Topical omega-3 and omega-6 fatty acids for treatment of dry eye. Arch Ophthalmol 2008;126:219-225.

■ CLINICAL CONDITIONS

DHA Treatment Immediately After Spinal Cord Injury Rescues Nerve Cells, Limits Damage and Improves Recovery

An intense area of research investigation is how long-chain polyunsaturated fatty acids (LC-PUFAs) in cell membranes affect cell structure and function, particularly in the central nervous system. Two LC-PUFAs comprise most of the polyunsaturates in cell membranes: arachidonic acid, an omega-6 (n-6) LC-PUFA and docosahexaenoic acid (DHA), which belongs to the omega-3 (n-3) family. Both LC-PUFAs affect cell membrane structure, participate in cell signaling, are precursors of highly bioactive substances, such as eicosanoids and neuroprotectins, and are involved in the regulation of cell activation, growth and proliferation, and gene expression. There is evidence, too, that the n-3

PUFAs, alpha-linolenic acid and DHA have neuroprotective properties in some types of brain injury, namely cerebral ischemia, spinal cord ischemia, and induced epilepsy. A derivative of DHA, neuroprotectin D1 has neuroprotective properties in brain under conditions of oxidative stress, ischemia, and inflammation. More recently, n-3 PUFAs were reported to be effective in reducing protein oxidation and restoring normal energy status in the hippocampus of animals that experienced traumatic brain injury.

Treatment of trauma and spinal cord injury is emerging as a potentially important application of n-3 LC-PUFAs, particularly DHA. In this study, DHA enhanced neuronal cell survival and limited some of the damage.

from such injuries results in primary loss of neuronal and glial cells, as well as secondary injury that amplifies the original harm. These secondary injuries include excitotoxicity—the excessive activation of glutamate receptors—oxidative stress and inflammation, and appear to be good targets for intervention. Although there are no restorative treatments for spinal cord injury, combinations of clinical therapies, surgical decompression and novel neuroregenerative approaches have potential benefits. Current evidence suggests considerable potential benefit from n-3 LC-PUFAs, although one report suggested that arachidonic acid and DHA enhanced the injury from experimental cerebral ischemia.

In this report, Wenlong Huang and colleagues at the University of London, U.K., examined the effects of intravenous DHA on experimental spinal cord injury from compression. The middle section of the spinal cord of anesthetized rats was injured at the 12th thoracic vertebra by a standardized compression procedure (Figure 1). Two groups of control animals received either no intervention or laminectomy surgery, a procedure that widens the spinal canal to relieve pressure. Thirty minutes after injury, animals received 1 of 3 treatments: intravenous saline and a control diet, intravenous DHA (250 nmol/kg) and a control diet, or intravenous DHA and a DHA-enriched diet. The DHA-enriched diet provided 400 mg of DHA/kg animal body mass. A second series of experiments examined the effect of delaying the DHA injection until 3 hours after injury. Animals were sacrificed 1 week after injury. In a third set of stud-

Treatment of trauma and spinal cord injury is emerging as a potentially important application of n-3 LC-PUFAs, particularly DHA. In the United States alone, approximately 253,000 people have spinal cord injury resulting from motor vehicle accidents, falls and violence. Damage

ies, animals received either saline or DHA intravenously and were sacrificed 1, 3, 24 or 72 hours later or 1 and 6 weeks following injury. A section of the spinal cord containing the injury or taken from the section at the level of the laminectomy was dissected and preserved for immunocytochemistry. The investigators determined the numbers of surviving spinal cord neurons by counting the neuronal nuclei in the dorsal and ventral horns after photography (Figure 2). Hindlimb motor function was assessed using the Basso, Beattie and Bresnahan scoring system. Lipid peroxidation, protein oxidation and cyclooxygenase were assessed in spinal cord samples.

Intravenous administration of DHA with or without dietary DHA had significant neuronal protective effects. Animals receiving only the DHA infusion sustained 48% neuronal protection and those receiving both intravenous and dietary DHA had 58% of cells protected after 1 week compared with saline controls (Figure 3). After 6 weeks, both DHA-treated groups had about 52% to 70% of neurons protected. In the ventral horn, animals receiving infused and dietary DHA had significantly more neurons than those receiving only infused DHA. A similar protective effect of infused DHA and dietary plus infused DHA was observed in oligodendrocytes, cells that protect the axons of neurons. About 27% to 30% of these cells were protected after 1 week, and after 6 weeks, 26% to 46% were protected. At 6 weeks, there was significantly greater cell protection in the lateral white matter, but not in the ventral white matter, of animals receiving both infused and dietary DHA compared with those receiving only infused DHA.

The investigators also observed significant axonal protection in the lateral white matter 6 weeks post-injury in both groups of DHA-treated animals. In the ventral white matter, only those animals receiving infused plus

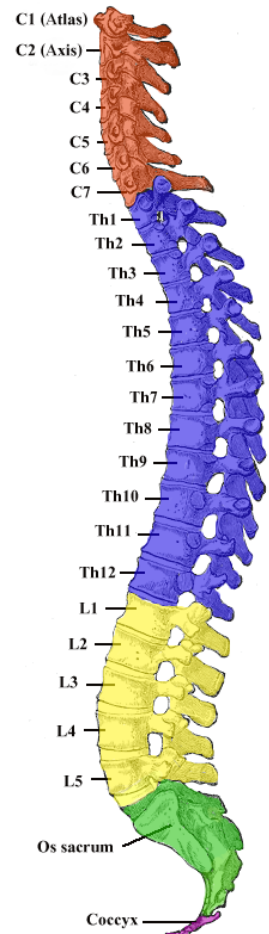


Figure 1. Spinal column showing cervical (C), thoracic (Th) and lumbar (L) vertebrae. Image reproduced from Wikipedia.

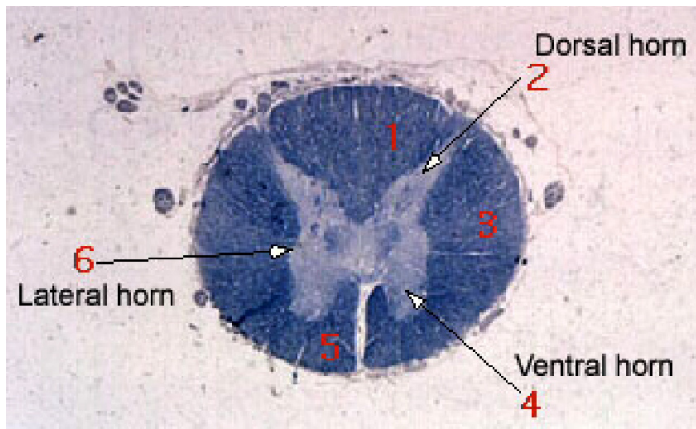


Figure 2. Cross-section of spinal cord at the thoracic level showing the ventral and dorsal horns. Image courtesy of Dr. Robert Jacobs, Colorado College and Dr. Arnold Scheibel, University of California at Los Angeles.

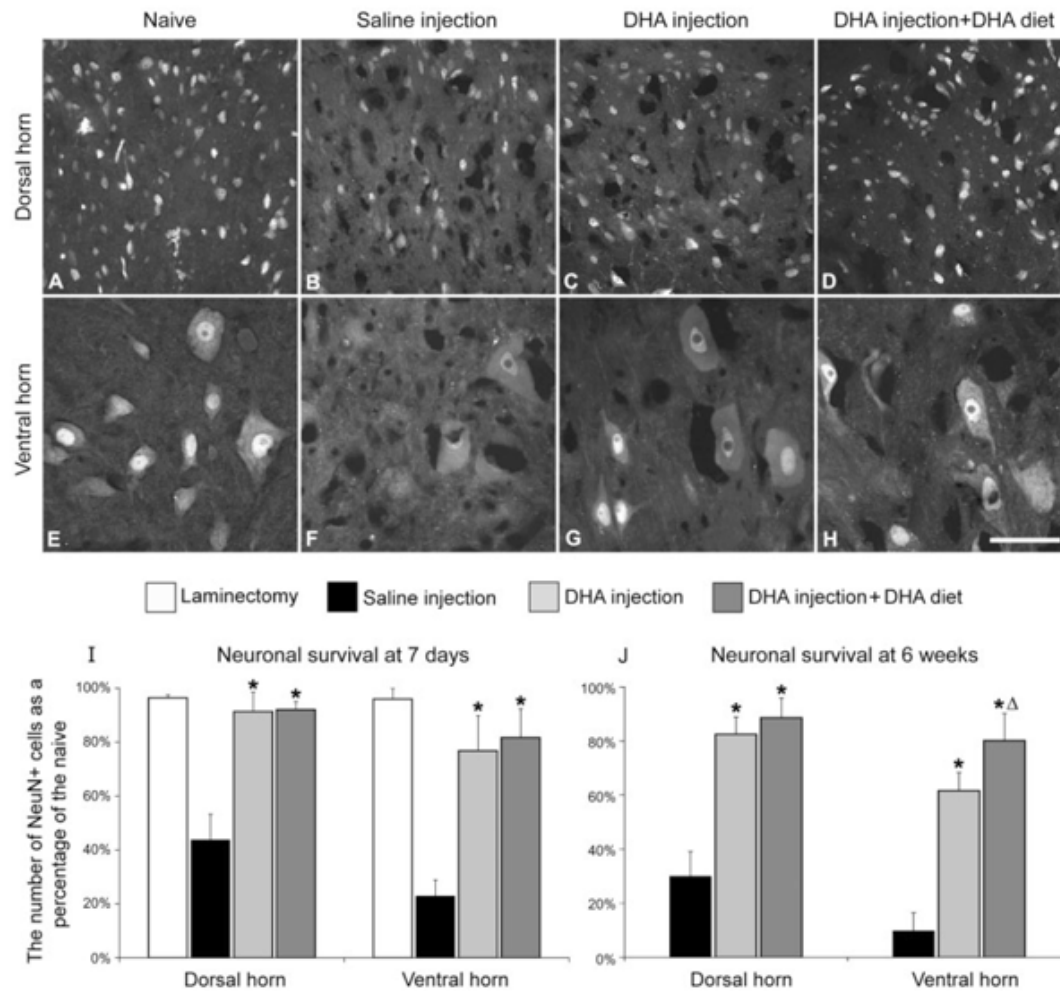


Figure 3. NeuN (cell) labeling of dorsal and ventral horn neurons in animals injected with saline, DHA or DHA plus dietary DHA. At 1 wk, NeuN-labeled cells in saline-treated animals (B, F) was less than controls (A, E). DHA-treated animals (C, D, G, H) had more NeuN-labeled cells compared with saline-treated animals at 1 wk. Quantitatively, cell survival was significantly increased at 1 and 6 wk compared with saline controls, $P < 0.05$ (I, J + SEM). Scale bar=50 μm. Figure from Huang WL et al. *Brain* 2007;130:3004-3009 by permission of Oxford University Press, © 2007 by the Guarantors of Brain.

dietary DHA had significant axonal protection compared with saline controls.

Other protective effects observed in the DHA-treated animals included reduced immunoreactivity in the dorsal and ventral horns, intermediate grey matter and in the corticospinal tract, and lateral and ventral white matter. Provision of DHA in the diet enhanced the protection observed with infused DHA. After 6 weeks, lower immunoreactivity was observed in the lateral and ventral horns, but not in the lateral and ventral white matter. Animals on the DHA-enriched diet had further decreases in immunoreactivity in the grey matter and lateral and ventral white matter compared with saline or DHA-infused animals. Also after 6 weeks, both DHA-treatment groups were free of lesion cavities at the site of injury, whereas saline-treated animals developed multiple cavities.

In terms of motor function, both DHA-treated groups exhibited greater locomotor recovery than the saline controls, an effect that was observed within 48 hours of injury. DHA-treated animals continue to improve their performance from the 2nd to the 6th week, with the injection plus dietary DHA group outperforming the DHA-injection group by the second week post-injury. These observations were confirmed in the higher Basso motor scores. Significant differences between the DHA injection only and injection plus dietary DHA treatment groups were apparent from the 4th to the 6th week post-injury. When DHA infusion after injury was delayed by 3 hours, the beneficial effect of DHA disappeared; infused after 1 hour, DHA exerted a protective effect on locomotion at 7 days. In a pilot trial, higher or lower doses of infused DHA pro-

duced no dose-dependent or toxic effects. Treatment with a DHA-enriched diet alone was without effect on neuronal or oligodendrocyte survival or locomotor activity after 1 week, but did have anti-inflammatory effects in some, but not all, tissues examined.

Acute and chronic administration of DHA is associated with significantly improved neurological outcomes—more neurons and oligodendrocytes survive, more axons are protected and recovery of motor activity is hastened and extended.

DHA infusion moderated other responses to spinal cord injury, reducing the extent of lipid peroxidation and the expression of cyclooxygenase-2 compared with saline-injected animals, although lipid peroxidation and cyclooxygenase-2 expression continued to increase in the 24-hours following

injury. Similarly, protein oxidation—proteins damaged by unstable oxidation products—increased after injury, but with DHA injection, protein oxidation was significantly reduced across a range of protein sizes at 3 and 24 hours post-injury.

These extensive investigations of the effects of DHA on experimental spinal cord injury have shown that acute and chronic administration of DHA is associated with significantly improved neurological outcomes. More neurons and oligodendrocytes survive, more axons are protected and recovery of motor activity is hastened and extended. Treatment with DHA conferred significant protection to axons in the lateral and ventral white matter, which could be especially important in limiting the damage from spinal cord injury. DHA treatment was

also associated with significantly reduced lesions, as observed in the absence of cavities at the site of injury. The authors suggested that the lack of cavities might have resulted from increased survival of neurons and oligodendrocytes.

In addition, DHA treatment led to significantly reduced lipid and protein oxidation and lower inflammatory responses. These processes and the cellular damage that results from them are believed to underlie much of the damage observed in neurodegenerative diseases. Although several immune responses aid in healing and tissue regeneration, excessive inflammatory responses have damaging consequences. DHA is associated with reduced inflammation and the formation of protective substances such as neuroprotectins. In this experimental model, DHA exhibited protective effects when given within 30 minutes or 1 hour after injury, suggesting that immediate treatment of spinal cord injuries with DHA injection could have long-term benefits for improved recovery and damage control. Further, the effects of acute DHA treatment were enhanced when DHA was included in the diet to provide sustained exposure. The authors commented that n-3 LC-PUFAs have been highly effective in other life-threatening conditions and are without adverse effects, but that n-6 LC-PUFAs may worsen outcomes. One wonders if animals having adequate dietary n-3 LC-PUFAs would be better protected from injury?

Huang WL, King VR, Curran OE, Dyllal SC, Ward RE, Lal N, Priestley JV, Michael-Titus AT. A combination of intravenous and dietary docosahexaenoic acid significantly improves outcome after spinal cord injury. Brain 2007;130:3004-3019.

