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Welcome to 2017 and a new issue of Fats of Life, which brings exciting changes in our design. First of all, we have renamed the PUFA Newsletter to Fats of Life Newsletter, a change that had been pending for some time. We wanted to continue with the name “Fats of Life,” a memorable name and one that is better aligned with the title of this platform for information on lipids that are important for health. The focus will in any case remain on highlighting scientific advancements in understanding the role of polyunsaturated fatty acids to health.

Secondly, our website has a new look, now providing direct access from the homepage to the latest summaries of recent research on polyunsaturated fatty acids. While the newsletter archives will still offer you all the material written over the past 12+ years, we wanted to make it simpler for you to access the most recent research quickly and easily. Research will now be posted more frequently on the site, so plan to check back often. We’ll also continue to compile a group of summaries into pdf form that will be send to subscribers on a regular basis.

All in all, Fats of Life continues to provide an interesting resource for information on PUFA. The main areas that we will continue to review are cardiovascular health, maternal and infant health, cognitive health, the brain and central nervous system, immune function and visual function. Under the topic “Clinical Conditions” we highlight research that does not fall within these topics, and within the category “New Developments” we highlight research articles that address novel applications and shifts in understanding of how PUFA act in our bodies.

In this Fats of Life newsletter issue, we feature two guest articles. The review by Brun and Field highlights the current understanding of how omega-3 fatty acids can support breast cancer treatment, an area of growing preclinical and clinical research activity. The second guest article by Durand and colleagues is an educational entry into the intricate world of non-enzymatic cyclic oxidation products of EPA and DHA, a research area dominated by seemingly hard-hitting chemistry but with interesting learnings for understanding the biological activity of EPA and DHA.

The first summary also looks at non-enzymatic oxidation products, in the context of markers for omega-3 LCPUFA intake in a population at risk for insufficient EPA/DHA intake. A study is highlighted that took a detailed look at the temporal changes in PUFA levels during gestation, providing new insight into optimal ways to supporting lung development in preterm infants. A third summary describes a multicenter clinical study addressing the preventive role of EPA/DHA intake on transition to psychosis in young adults at high-risk. In this case, the researchers met with a failure in replicating earlier results – providing a stimulus to gain even further insight.

Also in this issue, two recent studies made detailed metabolomic analyses of the molecular changes occurring in type 2 diabetics following increased omega-3 LCPUFA intake, aiming to learn more about the effects on glucose homeostasis in this metabolic disorder. And finally, we take a look at a new bioreversible biomaterial that is capable of sustainably delivering inflammation-resolving substances, permitting the potential development of implantable devices with a high device patency.

Gerard Bannenberg
Editor, Fats of Life Newsletter
gerard@goedomega3.com
This study addressed the relationship between omega-3 status in the Inuit and the plasma levels of omega-3 LCPUFA-derived isoprostanes.

- EPA-derived isoprostane levels were positively related to omega-3 fatty acid status, and unrelated to cardiometabolic risk factors, unlike isoprostanes derived from arachidonic acid.
- Older Inuit that adhere more strongly to traditional marine food sources had higher levels of EPA-derived isoprostanes, possibly reflecting a contribution of higher omega-3 intake to reducing cardiometabolic disease risk.

A diet rich in omega-3 long-chain polyunsaturated fatty acids (LCPUFA), such as EPA and DHA, can reduce the risk for development of cardiometabolic disease, and attenuate the intensity of a range of chronic inflammatory conditions. The angio-protective, anti-inflammatory and anti-thrombotic properties of PUFA that contribute to risk reduction are mediated in part through their enzymatic conversion into an array of oxygenated lipid mediators that tone down inflammatory responses and help regulate several fundamental physiological functions such as blood pressure, lipid homeostasis, and hemo-stasis. Interestingly, some oxidation products of PUFA that are formed without the involvement of oxygenating enzymes have also been recognized to have biological activity, and can be detected in vivo. The phytoprostanes, isoprostanes (IsoPs) and neuroprostanes belong to such non-enzymatic oxidation products of alpha-linolenic acid, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These substances can be used to monitor the non-specific chemical oxidation of PUFA contained in membranes, and AA-derived isoprostanes (so-called F2-isoPs) are used as biomarkers of oxidative tissue injury. Augmented F2-isoP levels have also been correlated with risk factors for obesity and cardiometabolic disease, such as blood pressure and C-reactive protein (CRP) levels. Interestingly, isoprostanes derived from EPA (F3-IsoPs) and neuroprostanes derived from DHA do not seem to share the adverse effects of arachidonic acid-derived F2-isoPs, and may mediate protective effects in cells and tissues.

In the Inuit, a population of the Arctic, the absolute requirements for dietary EPA and DHA are higher than in most other human populations, since over the course of their evolutionary history their dietary pattern has been dominated by a high proportion of marine foods containing preformed EPA and DHA. The absence of a need to convert plant-derived α-linolenic acid to longer chain PUFA has rendered Inuit people highly dependent on preformed EPA and DHA in their diet for maintaining high tissue levels of omega-3 LCPUFA. From a perspective of understanding the effects of dietary insufficiency of EPA/DHA on health, the Inuit constitute an interesting human population to study the relationships between diet type, changes in fatty acid status, and the development of risk factors for disease in humans. In addition, a relatively recent shift away from traditional food and lifestyle over the last 50-100 years has led to an increased incidence of cardiometabolic disease in Canadian Inuit from a historical baseline level that was typically much lower than in most Western populations. The relationship and effect of diet and lifestyle, and the role of PUFA intake in particular, may be studied with
higher clarity in the Inuit than in other populations.

A cross-sectional study was recently carried out in 36 Canadian Arctic Inuit communities to gain insight into the relation between omega-3 status, EPA-derived F3-isoprostane formation and cardiometabolic risk factors, including F2-IsoPs. The study was performed by Alkazemi and colleagues from the School of Dietetics and Human Nutrition and Centre for Indigenous Peoples’ Nutrition and Environment at McGill University, Québec, Canada, together with colleagues at Kuwait University, Kuwait, Vanderbilt University in Nashville, TN, and the University of Ottawa, Canada. A total of 233 Inuit adults (56% women) were studied with respect to dietary habits, physical activity, psychosocial factors, medical history, blood pressure, anthropometric indices, fasting blood lipids and various clinical indices. People with high CRP values (≥10mg/l), constituting 21% of the randomly selected individuals, had been excluded. The mean body mass index was 27.8 kg/m2, 34% were overweight, 30% were obese, and 29% had hypertension. Taken together, the study population can be considered at significant risk for developing, or is already displaying, cardiometabolic disease. Plasma F2-IsoP levels were on average at the high end of the concentration range considered normal, and 28% of participants had elevated levels of F2-IsoPs.

The researchers first assessed how various measured variables correlated to each other. F3-IsoPs were positively related to F2-IsoPs, suggesting that as the chemical oxidation of AA increases, so does that of EPA. However, the relation between isoprostane levels and selenium levels was completely different: at higher Se concentration, lower levels of F2-IsoPs were found, but F3-IsoPs levels were higher. The results suggest that the function of selenium, typically thought to support an antioxidant role through Se-dependent glutathione peroxidase, is differentially related to different isoprostanes. Older individuals were found to have higher F3-IsoP levels, but this did not correlate with any metabolic measures (blood lipids, CRP, blood pressure or fasting glucose). The ratio of F3-IsoPs to F2-IsoPs was strongly negatively related to F3-IsoP levels, which the authors suggest could mean that F3-IsoPs may affect F2-IsoPs formation. Although correlations do not signify any causal mechanisms, these results suggest that F3-IsoP formation shows some fundamental differences with F2-IsoP formation, and that a mere passive chemical oxidation of AA and EPA to the respective isoprostanes may be a too simplistic view. However, as expected, F3-IsoP levels were positively associated with total omega-3 LCPUFA levels, and negatively associated with the ratio of AA to EPA levels in red cell membranes.

To better quantify the association of omega-3 LCPUFA status with various other variables, such as isoprostanes, different fatty acids, Se, and the environmental contaminants Hg (total blood) and PCBs, study participants were stratified by low (<4.36%), medium (4.36-6.89%) and high (≥6.9%) levels of omega-3 LCPUFA in red blood cell membranes. Although no significant differences between F2-IsoP levels were found between tertiles, all other measured variables showed significant differences between at least one and the other two tertiles. Hg and PCB levels increased with increasing omega-3 LCPUFA levels. Se was higher in the highest tertile, total omega-6 LCPUFA level was lower in the lowest tertile, and the AA to EPA ratio significantly decreased from the lowest to the highest tertile. The highest tertile of omega-3 LCPUFA had the lowest F2-IsoP to F3-IsoPs ratio, indicating that formation of F3-isoprostanes becomes increasingly dominant over F2-isoprostane formation at higher omega-3 LCPUFA tissue levels. PCBs and total Hg levels, lipophilic environmental contaminants likely originating from the same marine sources, increased as omega-3 intake augmented.

Omega-3 LCPUFA levels were much higher in older Inuit (age ≥40 yrs; 7.37 ± 3.55%) than in the younger (age <40 yrs; 4.49 ± 2.31%), likely reflecting the higher ingestion of traditional (marine) food items. The plasma F2-IsoP to F3-IsoP...
Using multiple regression analysis, the researchers found that the best predictors of F3-isoP levels were Hg, omega-3 LCPUFA and EPA intake, as well as smoking and the ratio of AA to EPA levels, the latter two contributing negatively to the variance in F3-IsoP concentrations. The results suggest that several variables, with mutually enforcing and opposing effects, significantly affect F3-isoP levels. Future research taking a mechanistic approach is needed to determine if indeed Hg exposure stimulates F3-isoP formation, whereas smoking depresses this process. In other words, despite a worse cardiometabolic profile in older Inuit, higher Se and F2-IsoP levels associated with higher intake of traditional omega-3 LCPUFA-rich foods may be related to some protection from chronic cardiometabolic disease.

This was an observational study and no causal links between isoprostane levels or activity with changes in risk factors for cardiometabolic disease can be made. The study, however, provides insight into the qualitative changes in EPA-derived non-enzymatic oxidation products compared to those formed from AA, and suggests that there are fundamental differences between their active formation and their participation in different adaptive tissue reactions. It is currently not clearly understood if and how the body purposefully controls the formation of non-enzymatic oxidation products to regulate different cellular and physiological functions. It is plausible that the body monitors chemical oxidation via the formation of non-enzymatic oxidized fatty acids as an indication of a lack of sufficiency in antioxidants (i.e. vulnerability to oxidation), sensing the need for adaptive corrections in physiological function when oxidative damage to cellular structures becomes appreciable. The results are also compatible with formation of F3-IsopPs being stimulated by mercury, possibly as a protective mechanism. Distinct PUFA-derived isoprostanes and neuroprostanes mediating different horneric functions in the body cannot be excluded.

Future studies are needed to substantiate some of the underlying assumptions made in this investigation. The evidence that F3-isoprostanes relate to hard cardiovascular disease endpoints, or that the notional potent proinflammatory effects of F2-IsopPs are implicated in cardiovascular pathophysiology, is very limited to date. No evidence for an association between increased F2-IsopP levels and increased fatal or non-fatal coronary heart disease incidence was identified in a prospective cohort study that specifically addressed the relationship between markers of oxidation and cardiovascular disease incidence. Rather, changes in F2-isoprostane levels in tissue fluids may relate better to the risk factors of cardiovascular disease, such as diabetes and smoking. The premise that F3-isoprostanes may have anti-inflammatory and protective effects in human tissues also needs to be evaluated, considering the yet poorly documented causal contributions of F2-isoprostanes to cardiovascular disease.

This study also revealed that older Inuit, which more strongly adhere to traditional food sources, display a different pattern of isoprostanes compared to younger generations that have adopted a more Western-type diet, with a much lower omega-3 LCPUFA content. The various parameters that have been identified in this study may help future studies to evaluate the effects of changes in dietary intake of PUFA, particularly in populations that are especially vulnerable to low intake of preformed EPA/DHA and are in the process of abandoning traditional food patterns.


Worth Noting


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Bronchopulmonary dysplasia is a form of lung injury caused by assisted ventilation and oxygen support compounding an incomplete development of lung tissue observed in very early and low birth weight preterm infants.

In a study of fetal fatty acid (LCPUFA) accretion during the third trimester of pregnancy, the level of arachidonic acid contained in phosphatidylcholine (AA-PC) was found to be more abundant in cord blood than in the maternal circulation, and fetal docosahexaenoyl-PC (DHA-PC) level started exceeding maternal levels after week 33.

Bronchopulmonary dysplasia severity was associated with a low AA-PC to DHA-PC ratio in cord blood of very immature infants born before week 28. In particular, higher cord blood levels of DHA-PC were associated with more severe bronchopulmonary dysplasia in these neonates.

This study points at specific requirements for individual long-chain polyunsaturated fatty acids (LCPUFA) for optimal lung development during a specific time period, of relevance to dietary habits during pregnancy and to preterm infant nutrition.

Bronchopulmonary dysplasia can also occur in neonates with respiratory distress syndrome, or develop over the course of the first weeks after birth for unclear reasons, but possibly related to infections in utero. Histologically, it is characterized by unusual abnormalities of the bronchioles, such as metaplasia, a decrease in alveolar number, and formation of cysts, and an incomplete vascularization of lung tissue. A marked inflammatory response with granulocyte sequestration in the lung and formation of cytokines and transforming growth factor-β also typifies bronchopulmonary dysplasia. The disorder can lead to substantial structural remodeling of lung and airway tissue, such as scarring and replacement of normal branched alveolar respiratory surface with consequences for impaired lung function into adulthood. A significant proportion of infants with bronchopulmonary dysplasia recover nearly fully with only small losses in expiratory flow capacity.

There are no effective treatments available and emphasis has been much on prevention, promoting optimal lung growth and keeping neonates safe from infections. Effective treatments for bronchopulmonary dysplasia are furthermore dif-
The development of bronchopulmonary dysplasia has a relatively high incidence in very early and low birth weight preterm infants. Dysplasia has allowed increased attention to approaches that augment the resistance of the immature preterm lung tissue towards mechanical and hyperoxic lung injury, for example through the use of nutritional support that boosts antioxidant enzyme systems and anti-inflammatory responses. In addition, knowledge about the progressive increase in lipid accumulation occurring alongside a relative decrease in protein accrretion during the third trimester, and the detrimental effects of the interruption of placental LCPUFA accretion following preterm birth, has generated interest in evaluating the importance of fatty acid and lipid requirements in fetal development during the third trimester.

A relatively well-studied example for the requirement of a specific LCPUFA in fetal and infant development is that of DHA in the central nervous system. Significant research is being carried out to understand the contribution of the fetal and early-life adequacy of DHA to the proper development of neural tissue and the neurocognitive faculties in childhood. How distinct LCPUFA species serve the fetal development of the peripheral organs is far less studied. Interestingly, in the cerebral cortex of the developing fetus in the third trimester and in term neonates the abundance of another LCPUFA, arachidonic acid (AA), is comparable to that of DHA in total lipids and in specific phospholipids. Together with its chain-lengthened product eicosatetraenoic acid (also known as arenic acid), omega-6 LCPUFAs are nearly twice as abundant in total lipids of the cortex of neonates as DHA. Brain phosphatidyl-inositol, although quantitatively a minor phospholipid species, is far more enriched in AA compared to DHA. Only later, during early childhood, does the concentration of DHA in cerebral cortex overtake that of AA, to remain higher well into late adulthood. AA is obtained more easily from typical foods ingested by pregnant and lactating mothers than DHA, and is produced from dietary linoleic acid (LA) more readily than DHA is from its essential fatty acid precursor alpha-linolenic acid (ALA). The absolute intake of AA is important, however, for the composition of infant formula given to infants that are not breast-fed. The importance of AA, in addition and in relation to DHA, to infant development has been discussed in recent Guest Articles in Fats of Life.

A recent study has investigated the temporal changes in the levels of some phospholipids carrying LCPUFA in the maternal circulation and in the fetal circulation during the third trimester of pregnancy. The study was performed by Bernhard and colleagues from the Departments of Neonatology and Gynecology, and the Center for Pediatric Clinical Studies, Faculty of Medicine, Eberhard-Karls-University in Tübingen, Germany. The researchers obtained serum samples from 108 mothers soon after delivery, of which 94 were from singleton pregnancies. Umbilical cord plasma samples from 121 newborns were collected at birth. The various lipids measured at birth were considered to reflect the spectrum and levels of phospholipid-bound fatty acids of fetuses of equivalent ages. A temporal portrait of lipid dynamics during the last trimester of gestation was thus obtained. The fatty acid composition of two phospholipid species, phosphatidyl-choline (PC) and phosphatidyl-ethanolamine (PE), was determined by liquid chromatography-tandem mass spectrometry. Little information on the analytical methodologies was provided. Bronchopulmonary dysplasia severity was quantified by a scoring assessment according to the categories absent, mild, moderate or severe.

In this study group, 47% of the birth cohort of very immature neonates, born in week 24-28 post-menstrual age, developed bronchopulmonary dysplasia of mostly moderate severity. Beyond week 28 post-menstrual age no cases of bronchopulmonary dysplasia were observed. The overall levels of phosphatidyl-choline (PC), but not those of phosphatidyl-ethanolamine (PE), were correlated between mother and newborn. PC is an important carrier of PUFA to the pla- centa, from where PUFA species are transported to the fetus, whereas PE is believed to be less important in this respect. In mothers, PC levels decreased until week 33 post-menstrual age, after which levels started to rise again. Maternal PE levels did not show this pattern and levels kept gradually increasing throughout pregnancy. In neonates at birth, PE levels in cord plasma remained approximately the same from week 24 until term at week 42, at a low concentration (approximately 9 times lower than maternal values). Total PC levels in newborn cord blood were much higher than PE levels, and were ap-
proximately 3- to 4-fold lower than in the maternal circulation, and showed a slow decrease during the third trimester.

The levels of several PC species containing specific fatty acids displayed positive correlations between maternal and fetal blood. This was the case for PC species containing oleic acid, AA, eicosapentaenoic acid (EPA) and DHA. When levels were expressed as the molar ratio of PC lipid species containing a particular fatty acid, a break-point in the pattern of maternal PC concentration was seen for PC-DHA; a constant ratio of fetal to maternal PC-DHA levels was observed until week 33, followed by a relative increase in fetal PC-DHA levels. This may point at an even more active placental accretion and fetal absorption (bio-magnification) of DHA from week 33 until term. The increased uptake of DHA from PC-DHA was at the expense of other PC species; PC containing saturated fatty acids and LA also showed a break-point at week 33, but from this moment accretion (as measured from the fetus-mother molar fractional ratio) gradually decreased until week 44. A constant ratio was observed for oleic acid, AA and EPA throughout the last trimester. Interestingly, the molar ratio of AA-PC in the fetus was constantly higher than in mothers, suggesting active AA accretion throughout the last trimester. In contrast, the molar ratio of LA-PC was half that seen in mothers, suggesting a restricted placental transport of LA into the fetal circulation.

The researchers next focused on any specific changes in the levels of AA and DHA at the moment of birth of very early preterms (born between week 24 and 27 post-menstrual age), at highest risk for developing bronchopulmonary dysplasia. The ratio of AA-PC to DHA-PC in cord blood was approximately 5 in this group of preterms, whereas it was around 2 in their mothers. This ratio remained constant in mothers that delivered at any time during the third trimester, but the high cord blood ratio seen in very early preterms also gradually decreased up to week 37-41 towards the same ratio of 2. The severity of bronchopulmonary dysplasia in preterms born before week 28 was found to be inversely related to the ratio of AA-PC to DHA-PC in cord blood (and was unrelated to the ratio in maternal blood). Additional analyses indicated that the levels of AA-PC were not related to the severity of BPD, but BPD grade was higher in early preterms with higher levels of DHA-PC levels. The results suggest that, on a background of sufficient maternal-fetal AA delivery, a higher fetal ratio of AA to DHA in PC is associated with a reduced risk of developing BPD.

Taken together, this study has provided new insight into the dynamic changes in LCPUFA during the last trimester of pregnancy. DHA may be even more actively delivered to the fetus via PC from week 33 onwards. AA accretion seems to remain constant throughout the third trimester, as far as its presence in PC is reflecting AA levels in the fetal circulation. The study supports the possibility that specific peripheral organs have specific temporal requirements for sufficiency in specific polyunsaturated fatty acids. Lung and airway development in humans occurs from about 22 weeks of gestation onwards and pulmonary growth remains active even after birth with alveolarization following vascular development. This study suggests that both AA and DHA already need to be available in sufficient amounts from week 24 onwards, with an optimal ratio of AA to DHA within PC that is dominated by AA, to allow healthy development of the lung and small airways. This requirement precedes the ramping up of maternal-fetal delivery of DHA at around week 32, likely serving higher demands for this PUFA in supporting the growth of tissues during late gestation.

This study provides the first indications that AA may be of critical importance to the proper development of the lung during a specific period of gestation. These analyses were based on a limited group of only 19 very early preterm infants and their mothers, and future studies are needed to confirm and understand its role in the developmental biology of the lower airways and lungs. As lung tissue continues its full development after birth, the optimal provision of spe-
specific nutrients that may become limiting to fetal growth, such as choline, AA and DHA, may be very important. A new randomized controlled intervention trial will address the usefulness of supplemental DHA added to a soy oil-based emulsion (devoid of AA) in the enteral feeding given to preterms born before week 29 with bronchopulmonary dysplasia. Given that it is still unknown how relatively higher levels of fetal DHA-PC during the critical period of week 24-27 postmenstrual age may predispose to bronchopulmonary dysplasia, new intervention trials that address the effect of supplemental DHA may also want to consider the insight gained in this new study by Bernhard and colleagues. This may involve supplementation with more AA than DHA in very early preterm infants, to reduce a potential inhibitory effect of DHA on the incorporation of AA into membrane phospholipids. Guaranteeing adequate dietary intakes of both AA and DHA by pregnant women itself already in the beginning of the trimester to enable fetal lung development may further lower the risk for bronchopulmonary dysplasia incidence and severity in neonates.


**Worth Noting**


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COGNITIVE HEALTH

Failure to Replicate Findings on Omega-3 LCPUFA Supplementation on the Transition to Psychotic Disorder in Individuals at Ultra-High Risk

THIS ARTICLE AT A GLANCE

• Psychotic disorders encompass serious psychiatric disorders, manifesting themselves in the altered perception of reality, such as delusions or hallucinations, disorganized or bizarre behaviors and “negative” symptoms, which include flatness of emotion and interpersonal relations and amotivation. Psychotic disorders, when chronic, are characterized by low functioning and a poor prognosis.

• A previous study demonstrated a lowered risk of progression of illness for first episode onset of psychosis in young adults.

• This study aimed to build upon these findings in a multicentre study of individuals at ultra-high risk (UHR) for progression to psychotic disorders. Participants were randomized to receive omega-3 LCPUFA supplementation or placebo. All participants received cognitive behavioral therapy, and antidepressants and benzodiazepines were allowed for the treatment of concurrent depression and anxiety respectively.

• No effect of six months supplementation in young adults also receiving psychosocial treatment was found over placebo. It is unclear if better patient characterization and additional stratification may be necessary to discern potentially beneficial effects.

Psychotic disorders include serious chronic psychiatric disorders, such as schizophrenia and schizoaffective disorder, making progression to a chronic illness in those at high risk of great importance. Common identifying features of psychotic disorders are bizarre subjective mental experiences (delusions and hallucinations), impairments in cognition, failure to correctly interpret perceptions (vision, auditory), and the inappropriate expression of emotion and mood. Some common psychotic disorders are schizophrenia, schizoaffective disorder, bipolar disorder, delirium, and psychosis associated with dementia. Psychosis can also be provoked by psychoactive drugs and medications. Lifetime risk for psychosis incidence is about 8 out of 1000 persons.

Chronic serious psychotic disorders like schizophrenia typically have a relatively early onset, often during late teens and young adulthood. A critical moment in psychotic disorder development is the so-called “transition” of individuals towards experiencing their first psychotic period. The precise moment of onset of the first clear episode of psychosis has long been viewed as somewhat arbitrary since it is preceded by small positive and negative symptoms that are below a clearly measurable threshold occurring during a prodromal (prior to illness) period. Since the introduction of criteria that allowed for the measurement of prodromal symptoms in quantitative terms, an increasing number of studies have been carried out to identify behavioral, pharmacological and nutritional treatment approaches to reduce transition rates. The criteria are useful to identify individuals in the prodromal phase of first-episode psychosis, especially of those that are considered to be at “ultra-high risk” (UHR), i.e. who have an imminent risk for conversion within one year. Controlled intervention studies offer the opportunity to identify approaches that convert psychotic disorders into a group of hopefully preventable, or at least more manageable, illnesses.

In recent studies that have addressed the possibility to modulate the prodromal phase with interventions that may be anticipated to be relatively safe with respect to mental health, the potential efficacy of dietary supplementation with omega-3 LCPUFA has been noted. Supplementation of UHR individuals for six months with omega-3 LCPUFA markedly lowered the risk of transition to psychosis, reduced symptomatology, and improved functioning. These findings are in line with growing recognition that major recurrent psychiatric disorders may be
Chronic serious psychotic disorders like schizophrenia typically have a relatively early onset, often during late teens and young adulthood. A critical moment in psychotic disorder development is the transition of individuals towards experiencing their first psychotic period.

Characterized by omega-3 LCPUFA tissue deficiencies, suggesting that omega-3 supplementation could have beneficial effects in a range of mood and affective disorders, including major depression, schizophrenia, and bipolar disorder. The preliminary evidence that suggested omega-3 LCPUFA supplementation is beneficial in lowering the risk for transition to psychosis was obtained in a randomized double-blind placebo-controlled trial that had been carried out in a single public hospital. The next step in demonstrating efficacy is replication across different populations in multiple clinical centers.

The results of the multicenter study were recently published by McGorry and colleagues, from the National Centre of Excellence in Youth Mental Health, Melbourne, Australia, in collaboration with some 14 other clinical centers in Australia, Asia, and Europe. Study participants at the various study sites were selected based on the following criteria: they were seeking help and met the UHR criteria. Potential participants who had experienced a prior psychotic episode, were taking omega-3 supplements, were being treated with mood stabilizers, or had severe illness or developmental disorders, among other criteria, were excluded from the study. In total, 304 participants (aged 13-40 years), were allocated in a randomized fashion to either of two groups; a group receiving 1.4 g omega-3 LCPUFA per day (n=153), or to the control group that received a similar volume of paraffin oil (n=151). All participants received cognitive behavioral case management, a high-quality psychosocial intervention that can improve day-to-day community functioning.

The measured rates of transition to psychosis in the test group and the placebo group were not different: transition rates were 11.5% and 11.2%, respectively, at the 12-month time point, using the intention-to-treat approach (i.e. extrapolating the effects as if all participants had completed the full study protocol). The transition to psychosis at the six-month time point, the primary outcome, was not significantly different either. A significant improvement in favor of the control group was seen at six months on the Global Functioning: Social and Role scale (P=0.02). At this time point, or at 12 months, none of the primary outcomes evaluated social functioning and psychopathology of study participants. Thereto a range of tests of mental health and social functioning was employed, including the Brief Psychiatric Rating Scale, the Scale or the Assessment of Negative Symptoms, the Young Mania Ratio Scale, The Montgomery-Åsberg Depression Rating Scale (MADRS), the Social and Occupational Functioning Assessment Scale, and the Global Functioning test. MADRS scores at baseline were used for randomization, in order to achieve an equal distribution of participants with respect to depressive symptoms. The results on psychopathology assessed at baseline, at six months and at 12 months were reported.

Nearly half of the participants (45.7%) were male, and the average age of participants was 19.1 years old, with no significant difference between groups. All participants had a low level of functioning, with a score <50 on the Social and Occupational Functioning Assessment Scale for at least one year, or had experienced a more than 30% reduction in this score over the year prior to enrollment. Participants were allowed to continue taking antidepressants (serotonin reuptake inhibitors), and benzodiazepines to reduce anxiety. No difference between the two groups was found with respect to the use of these drugs, with >60% taking anti-depressants. Individuals at risk for psychosis who participate in intervention studies tend to display a relatively high rate of study noncompliance. A relatively high but similar drop-out rate of approximately one quarter of the participants was observed in both study groups. Adherence rates, defined as people returning less than 25% of capsules during monthly study visits, were also low, 43% and 41% in omega-3 LCPUFA and control respectively.
of the other secondary outcomes showed any difference between groups. No adjustment for multiple testing was employed in the comparison of the effect of treatment on the various symptom and functioning measures. When restricting the comparison only to participants that had correctly adhered to daily capsule intake, no difference in transition rates between people receiving omega-3 LCPUFA supplementation or placebo was found either.

Next, the researchers focused on those participants considered to have the highest risk of transitioning to psychosis. These individuals were those with a MADRS score of 14 or higher. The transition rates of this sub-group of participants were again not found to be different among those receiving placebo or omega-3 LCPUFA.

Taken together, this study has provided evidence that in young adults at high risk for transitioning to psychosis and receiving cognitive behavioral case management, the supplementation with 1.4 g omega-3 LCPUFA daily over a six-month period has no added effect on the risk of transitioning to psychosis. The study assessed a relatively large group of individuals at UHR for psychosis onset in distinct geographical locations in Australia, Asia and Europe. The results did not confirm what had been observed previously in a study with a similar intervention approach but that had been carried out in a single Australian public hospital.

Reasons for the failure to replicate the original promising study in this larger study have been considered by the study authors. First, the overall rate of transition (10.5%) was lower than in the previous positive study (16.1%). This may have reduced the power to detect a significant effect. However, the obtained results were not suggestive of this possibility and the authors doubt that having included more participants would have given a different result. It is also possible that with a majority of participants taking anti-depressants and all individuals receiving a high quality psychosocial intervention, it could have been impossible to identify an effect of omega-3 LCPUFA supplementation if a maximum benefit was already achieved under these conditions.

Finally, it is possible that young adults classified at high risk for psychosis still form a heterogeneous group of patients with regards to the type and grades of pathology. Indeed, psychotic disorders are a group of illnesses that each display differences in average age of onset, dependence on external precipitating factors, chronicity and need for maintenance treatment. In the present study, participants consisted of help-seeking individuals that were included if they met criteria for the definition of the UHR mental state, but may nevertheless still compose a group with variations in underlying neural pathologies. Further stratification is also needed with regard to which individuals may be susceptible to supplementation, most importantly their baseline levels of LCPUFA, information on which was not recorded in this study. When the conclusions of this study are reported strictly, omega-3 LCPUFA supplementation does not change the risk for transition to psychosis in high risk individuals in general. The replication study does generate interest in carrying out more detailed studies.

Notably, psychotic disorders such as schizophrenia are highly heterogeneous, and it is possible that a subset of patients may be especially responsive to treatments such as omega-3 fatty acids. Studies that aim at better defining the background and biomarker characteristics of the individuals making up study groups entering this type of studies are already being undertaken. Although the etiology of psychosis remains largely undefined, a link to immune system activation has been made and changes in membrane fatty acid...
composition have been reported in individuals at risk for psychosis. The recently attained insight in the possibility of addressing depressive symptoms with EPA supplementation has suggested that efficacy can be predicted if an individual’s degree of oxidative stress is taken into account. This is of relevance since randomization in the present study was based on achieving uniformity in the allocation of subjects by depression level in both intervention groups, founded on the recognition that depression and antidepressant use has a known effect on illness progression in UHR individuals. New and more refined research will likely tell us more about the extent to which psychosis development is modifiable.


**Worth Noting**


Comprehensive Assessment of At Risk Mental States, The PACE Clinic, University of Melbourne, Australia. http://www.repsych.ac.uk/pdf/Brief%20CAARMS%20with%20SOFAS%202016.pdf


Handbook of Psychiatric Measures: http://www.r2library.com/Resource/Title/1585622184


For a full list of reference links, visit FatsofLife.com
Type 2 diabetes is a metabolic disease in which people cannot adequately control blood glucose levels. Blood glucose values > 6.9 mM (fasting) and > 11 mM (post-prandial) are typical for the diabetic state. The disorder is largely preventable by a balanced diet and a healthy and active lifestyle. Dietary and lifestyle habits for a large proportion of citizens today are unhealthy, and the prevalence of type 2 diabetes has increased steadily in the past few decades, affecting hundreds of millions of people worldwide. Type 2 diabetes is characterized primarily by a deranged glucose homeostasis that stems from a loss in the ability of insulin-dependent tissues to adequately respond to insulin (insulin resistance). Alterations in insulin signaling involved in facilitated glucose transport across membranes, and a reduced vasodilatory and increased vasoconstrictor response to insulin in type 2 diabetics, lead to a reduced ability of metabolically active tissues to extract glucose from blood. In addition, a reduced thermogenic response to insulin promotes weight gain and the ectopic deposition of fat in pancreas, liver and muscle, further deteriorating insulin sensitivity and supporting the development of a reduced ability to secrete insulin. Chronic resting hyperglycemia and increased post-prandial hyperglycemic responses that ensue from inadequate blood sugar control lead to vascular damage over time. Retinopathy, kidney failure, neuropathy, and poor wound healing responses are characteristic sequelae of the microvascular complications in type 2 diabetics. Macrovascular complications include coronary artery disease, cerebrovascular disease and peripheral artery disease. A majority of type 2 diabetics is obese, but the disorder can also appear during normal aging.

In addition to improved focus on prevention as a key factor limiting the increase in type 2 diabetes prevalence, the identification of affordable approaches to reduce the increased risk of microvascular complications in current diabetics is also important. Lifestyle and nutritional changes, including increased intermittent physical activity and intake of foods with a lower glycemic index and load, are beneficial in order to recover normal levels of insulin sensitivity, blood glucose, and body weight. It is less clear though if the vascular injury and increased mortality from cardiovascular causes in type 2 diabetics are modifiable by achieving normoglycemia and a more active lifestyle alone. Aggressive control of glycaemia, blood pressure and LDL-cholesterol that achieve a very marked (>10%) decrease in body weight and a near-normal glycated hemoglobin level is associated with a reduced risk of death from cardiovascular causes and infarction. Hyperglycemia in diabetes imparts a long-term increased risk of microvascular complications and cardiovascular disease, a process termed “glycemic memory,” which is likely mediated by epigenetic changes. Demonstrated proof of efficacy of all the modifiable factors that may improve the short and long-term health of the large global population of type 2 diabetics is therefore of interest.

Adequate omega-3 long-chain polyunsaturated fatty acids (omega-3 LCPUFA) intake and tissue levels can reduce some of the risk factors associated with cardiometabolic disease, including vascular patency and blood pressure, immune cell activation in vascular inflammation, and hypertriglyceridemia. Of particular interest to type 2 diabetes is the ability of omega-3 LCPUFA intake to reduce VLDL-associated hypertriglyceridemia, which may help reduce ectopic fat accumulation in the pancreas and liver, and may contribute to diabetes reversal in patients submitted to a low calorie intake regime. Although the modulatory effects of omega-3 LCPUFA intake on lipid homeostasis and inflammation are relatively well recognized, their effects on glucose homeostasis are considered to be more complex and are still poorly understood. Adequate tissue levels of omega-3 LCPUFA may contribute to normal insulin re-
sistance, as suggested by preclinical research and observa-
tional studies. However, intervention studies in humans that
have addressed the effect of increased omega-3 LCPUFA in-
take on insulin resistance have provided mixed results. Large
systematic reviews have not found convincing evidence for
an effect of omega-3 LCPUFA intake on glycemic control in
type 2 diabetics.

To better understand the reasons for the varied effects of
omega-3 LCPUFA intake and tissue status on glucose me-
tabolism in type 2 diabetics, more detailed studies on the
dysfunctional molecular responses in this disease and their
potential modulation by omega-3 LCPUFA may provide
new insight. Two recent studies have made such an assess-
ment, and used samples from two double-blind randomized
controlled trials. Both studies compared the effects of dietary
supplementation with EPA/DHA-rich fish oil, flaxseed oil
(rich in alpha-linolenic acid) and a vegetable oil that was low
in omega-3 PUFA content (soybean oil and corn oil).

The first study was carried out by Karakas and colleagues
from the Department of Internal Medicine, and the Genome
Center at the University of California at Davis, CA, and the
Department of Veteran Affairs Northern California Health
Care System, Mather, CA, USA. The study focused on US
American women (20-45 years old) with polycystic ovary
syndrome, a disorder characterized by insulin resistance.
Fifty-four women, divided into three intervention groups, re-
ceived 3.5 g a day of EPA/DHA from fish oil, alpha-linolenic
acid from flaxseed oil, or an equivalent volume of encapsu-
lated soybean oil, for six weeks. Metabolites in plasma were
measured by gas chromatography-mass spectrometric (GC-
MS) analysis at the beginning and the end of the intervention
period. An extraction and derivatization step was employed,
which allowed relative quantification (so-called enrichment
analysis) of mostly polar metabolites, which were identified
by comparison of the retention index and mass spectral simi-
arity with a database of >1000 metabolites. In addition, var-
ious parameters of glucose homeostasis and cardiovascular
disease risk were measured.

After six weeks, women that had received fish oil had an im-
proved overall insulin secretion (insulin response) than the
other groups, which showed no change compared to baseline.
Women receiving fish oil had a small but significant decrease
in hemoglobin A1c (HbA1c), a glycated form of hemoglobin,
and a marker of long-term plasma glucose levels. HbA1c
refers to the stable ketoamine formed after intermolecular re-
arrangement (an Amadori rearrangement) of the reaction
product formed between glucose and the amino-terminal va-
line group of hemoglobin (HbA.). Such ketoamines formed
from the reaction of glucose with reactive amine groups pres-
ent in a range of cellular proteins, including hemoglobin and
also specific lipids, can, under conditions of oxidative stress,
further react to form so-called “advanced glycation end-prod-
ucts” (AGE’s). These substances signal tissue damage and
potently activate inflammatory responses. A chronically ele-
vated rate of AGE formation, such as that occurring in dia-
abetes, is believed to be causally involved in vascular tissue
damage. Baseline levels of HbA1c were at the high end of
the normal range in this study.

The intake of fish oil worsened the Matsuda insulin sensi-
tivity index, a measure of the efficacy of glucose removal
from the circulation after a glucose challenge. Fish oil sup-
plementation decreased early insulin release (upon an intra-
venous glucose challenge) as did soybean oil intake, but
increased overall insulin levels in blood upon an oral glucose
challenge compared to soybean oil intake. Flaxseed oil in-
take did not affect insulin release. As expected, fish oil sup-
plementation lowered triglyceride levels, an effect shared
with flaxseed oil intake. Fish oil and flaxseed oil intake had
no significant effect on the Disposition Index, a measure of
β-cell function and compensatory insulin release, whereas
soybean oil intake reduced this parameter. There was no ef-
fect on glucose levels for any of the interventions tested.

Taken together, EPA/DHA intake during six weeks slightly
reduced hemoglobin glycation and further ameliorated in-
sulin sensitivity, but increased insulin blood levels, perhaps
as a result of a slower breakdown of insulin, since pancreatic
function was unaffected.

These intricate modulatory effects of fish oil intake on glu-
cose homeostasis were accompanied by several significant
changes in metabolite abundance, particularly amino acids.
The major changes observed were: increased serum levels of branched chain amino acids (BCAAs) (isoleucine, leucine and valine), decreased levels of cysteine and its oxidized disulfide form cystine, and significant increases in several other amino acids (glycine, serine, glutamic acid, glutamine and proline). These changes were not seen after flaxseed oil intake, except for similar but less pronounced changes in cysteine and cystine levels. Soybean oil supplementation increased the levels of isoleucine, valine, methionine, glycine and proline, and had an effect on cysteine and cystine similar to that of flaxseed oil. Using pathway enrichment analysis, a technique that allows the mapping of metabolite changes onto known metabolic pathways, the researchers concluded that fish oil supplementation affected amino acid synthesis and degradation pathways, pathways involved in the metabolism of small organic substances like formaldehyde, the degradation of amines and polyamines, the degradation of purine nucleotides, and the biosynthesis of alanine, glycine and L-carnitine. The impact of soybean oil intake was limited to amino acid biosynthesis and degradation, and flaxseed oil mainly affected alanine biosynthesis and cysteine degradation.

Other studies have documented the effect of omega-3 LCP-UFA on essential amino acid metabolism. This involves effects on the gene transcription of enzymes regulating BCAA breakdown, forming a link between increased BCAA levels and their known insulin resistance-promoting effect. A biosignature of BCAAs (leucine, isoleucine, and valine) and related amino acids (phenylalanine, tyrosine, methionine, alanine and histidine) consequent to a change in their catabolism, has been proposed as a means to distinguish individuals who are cardiometabolically “unwell” (including displaying insulin resistance) from those who are more metabolically healthy. Insulin resistance may lead to a compensatory release of insulin, a process also known to be promoted by BCAAs and in line with the observations made in this study. In addition to recognizing the impact of fish oil intake on amino acid metabolism, the intake of soybean oil produced a metabolic response that resembled fish oil intake more closely than the intake of ALA-rich flaxseed oil. This observation suggests that the triglyceride-lowering effect of EPA/DHA and plant-derived ALA can be dissociated from the effects of omega-3 LCPUFA (or strictly fish oil as studied here) on amino acid metabolism.

The second intervention trial assessed changes in the metabolomic profile of a group of Chinese type 2 diabetic adults after supplemental fish oil intake. The study was performed by Zheng and colleagues from the Department of Food Science and Nutrition, at Zhejiang University, Hangzhou, China, in collaboration with the Huazhong University of Science and Technology, and the Agricultural University, both in Wuhan, China, and the University of Cambridge, UK. The intervention study was carried out in three geographically different clinical centers in China. Metabolomic analysis was performed on serum samples from 53 participants from one of the participating centers. Participants (age 35-80 years, average age ~70 years) were assigned to one of three study groups that during a period of six months received fish oil (2 gram EPA+DHA in 4 capsules/day; n=20), flaxseed oil (2.5 gram ALA in 4 capsules/day; n=20), or corn oil (4 capsules/day; n=19). A group of healthy controls (n=17) without type 2 diabetes was recruited in parallel.

Untargeted metabolic profiling was carried out by ultra-performance liquid chromatography interfaced with tandem mass spectrometric analysis, as well as by GC-MS analysis of derivatized samples. In total 407 distinct signals were detected, which were matched against a library of >3300 compounds. Biochemical parameters (lipoprotein-associated cholesterol, triglycerides, glucose levels in serum, and fatty acids in erythrocyte membrane phospholipids) as well as heart rate, blood pressure and body mass index, were measured at the beginning and end of the intervention period. The multisite intervention trial showed that marine omega-3 LCPUFA supplementation did not improve insulin resistance or fasting glucose and insulin levels. However, HbA1c levels decreased significantly over time when compared to the corn oil group, as well as triglycerides and LDL-cholesterol.

In the EPA/DHA-supplemented diabetic individuals, significant changes were measured in the levels of omega-3 PUFA: increased serum levels of the free fatty acid form of EPA, DHA and docosapentaenoic acid (ω-3), as well as EPA esterified to phosphatidylcholine (the most abundant phospholipid type). Much less expected, the only other metabolite with a significantly changed level (a four-fold increase) was 3-carboxy-4-methyl-5-propyl-2-furopropanoate (abbreviated CMPF; Figure 1). This substance is known as a metabolite of furan fatty acids (or F-acids), a specific class of fatty acids that contain a methylated furan moi-
Furan fatty acids are formed by microscopic organisms (microalgae and bacteria) and bio-accumulate in marine organisms such as fish, possibly in a similar fashion to the trophic transport observed with omega-3 LCPUFA. Levels of F-acids in food vary, but can be significant in fish tissues (~0.1-4 g/100 g), and levels of 0.1 g/100g have been reported in fish oil. The furan moiety confers free radical scavenging activity to this type of fatty acids, but any specific biological roles remain largely unknown. Upon fish consumption, furan fatty acids are absorbed into the circulation, then metabolized to CMPF and excreted via urine. Bacteria in the gastrointestinal tract of fish may generate furan fatty acids directly.

Increased CMPF levels (from a measurable background level) suggest that this reflects an increased intake of furan fatty acids from the fish oil supplement that was given, in line with a previous study that documented the absorption of this poorly documented group of dietary antioxidants. Linear correlations were found between the change in serum EPA or DHA and CMPF, and increased serum CMPF was associated with lower triglyceride levels. Further studies need to be carried out to elucidate the origin of CMPF in omega-3 LCPUFA-supplemented diabetic patients, to understand whether dietary furan fatty acids may induce any metabolic changes, and to contrast their effects with those ascribed to straight-chain fatty acids. This is of interest as the average CMPF level was furthermore reported to be higher in the healthy controls than in the diabetic subjects, suggesting that some level of furan fatty acids may be of benefit to metabolic health and/or that a decrease in furan fatty acid degradation to CMPF occurs in diabetic individuals.

Interestingly, in both studies insulin resistance was not improved, and was even worsened, by omega-3 LCPUFA intake. It has been suggested that improvements in insulin sensitivity can be observed only at a high omega-3 index, and that the effect is easily missed in intervention studies if omega-3 levels are still inferior to those required to reach a sufficiently elevated omega-3 index. Glycated hemoglobin levels did decrease in both studies, suggesting that on a background of unchanged glucose levels, the glycation of hemoglobin may be diminished, reflecting a lower propensity of glycation reactions of glucose with proteins, a first step in the formation of AGEs that are involved in vascular damage in diabetes. The results point at the possibility that omega-3 LCPUFA intake may reduce oxidative events involved in vascular tissue damage in the diabetic state. Hyperglycemia-mediated cellular injury involves mitochondrial superoxide anion production, and the transformation of Amadori products into reactive AGEs involves an oxidation step. The results of the American intervention study also suggested that changes in amino acid metabolism do not mediate the triglyceride-lowering effects of fish oil intake, but rather may be associated with a decreased glycation.

The limitations of the studies were the relatively small groups for randomized controlled intervention studies. Nevertheless, for the purpose of discovery, these studies have provided new insights that provide indications for renewed focus on the relevant changes that accompany omega-3 LCPUFA intake in type 2 diabetics. These two studies also indicate that amino acid metabolism may play an important role in carbohydrate metabolism and could be a relevant point of modulation of the insulin resistant state of diabetics. Future intervention studies with higher omega-3 LCPUFA intake levels may provide a clearer picture as to whether insulin resistance is also sensitive to improvement. In addition, furan fatty acids present as minor components in specific foods may have been overlooked to date, and may have a potentially interesting activity in metabolic disorders, therefore are worth exploring in more detail.

during omega-3 polyunsaturated fatty acid supplementation in women with polycystic ovary syndrome. *BBA Clin.* 2016;5:179-185.


**Worth Noting**


Stefano GB, Challenger S, Kream RM. Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic


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A Multilayered Biomaterial for Vectorial Release of DHA-Derived RvD1 to Restrain Local Tissue Inflammatory Responses and Disorders

THIS ARTICLE AT A GLANCE

• A thin, pliable, biodegradable multi-layered material incorporating the DHA-derived lipid mediator resolvin D1 (RvD1) was developed.

• The thin-film device allowed sustained diffusion of RvD1 from the surface with the lowest copolymer density, permitting one-sided release.

• RvD1 could be released from the thin-film device into arterial tissue, and activated anti-inflammatory, anti-proliferative and anti-migratory activity in smooth muscle cells.

• Thin-film devices eluting specific pro-resolving lipid mediators (SPMs), such as RvD1, may be further developed for applications in surgical and endovascular interventions to lower or resolve local inflammatory reactions and surgical complications.

A variety of implantable devices are currently being employed in interventional cardiology and other surgical fields, to repair and treat different health-threatening tissue lesions. For example, the placement of stents and bioresorbable scaffolds is a relatively common procedure in myocardial revascularization to reestablish blood flow delivery to a poorly perfused part of the myocardium. Other relatively frequent revascularization interventions, such as in the lower extremities in patients with varying types and degrees of peripheral artery disease, employ different stents and prosthetic vascular grafts. Implants intended to have long residency times in the body, such as bone replacements, artificial mitral and tricuspid valves, and vascular stents, need to have a high degree of patency. They should adequately adhere to surrounding tissue, be compatible with the physical demands of the tissue in which they are implanted, and should not cause chemical and physical irritation that would trigger thrombosis (in intra-vascular implants) or inflammatory responses that would ultimately lead to failure of the device, or stimulate tissue remodeling. Restenosis (narrowing of the lumen of a blood vessel previously opened by some surgical intervention) and neo-atherosclerosis are frequently observed following the placement of an endovascular device or venous or prosthetic graft, and comprise a chronic inflammatory reaction with neo-intimal hyperplasia and fibrosis.

In contrast to implants that should last, an implanted material may need to be fully resorbed, e.g. bioresorbable stents, sutures and some prosthetic materials. Additionally, the implant can be a drug-eluting device with the eluting drug contributing to device patency. For example, the implementation of vascular stents that release immunomodulating drugs has measurably lowered the incidence of restenosis. The sustained perivascular release of such drugs from an implanted biomaterial has also been shown to exert anti-restenotic activity in animal models. Given the variety of different tissues that are potentially subject to repair or treatment using biomaterials, and the distinct demands placed on device patency, interest and space for innovations abound for the delivery of substances that can improve the outcome of surgical interventions or as locally-delivered therapeutic agents.

A particularly attractive prospect is learning how to harness the body’s own mechanisms to control local tissue inflammatory responses and stress reactions to achieve a high degree of device patency. In this regard, the omega-3 LCPUFA eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and DHA, generate a family of bioactive compounds known as the specialized pro-resolving mediators (SPMs), which have been shown to have important roles in modulating inflammatory responses. SPMs, such as DHA-derived D-series resolvins, potently activate the termination of inflammatory responses. Resolution of inflammation involves the modulation of a range of cellular activities of the innate immune system, such as neutrophil and macrophage migration, cytokine release, phagocytosis and apoptosis, as well as the activity of resident tissue cells (such as migration and proliferation). Given the involvement of SPMs in the self-limiting nature of inflammation, a growing body of research has addressed the
ability of SPMs to modify the outcome of a range of acute and chronic inflammatory disorders. Employing SPMs in biodegradable scaffolds may offer new prospects for device patency.

One such SPM, resolvin D1 (RvD1; 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15Z,19Z-docosahexaenoic acid), promotes tissue protection from inflammatory injury in various settings of inflammation, including vascular injury, ischemia-reperfusion injury, acute kidney injury and inflammatory bowel disease. Studies addressing the in vivo activity of SPMs in animal models generally involve the administration of the test substance directly into the circulation, by oral administration (through the drinking water, or by gavage), or topically (onto the skin surface). SPMs are considered to generally function as autacoids, i.e. they exert local hormone-like activity on neighboring cells to activate a range of inflammation-resolving activities. Specific enzymes rapidly degrade SPMs into biologically inactive metabolites that presumably preclude spill-over beyond the cells and tissues where these mediators are formed. Although SPMs are capable of exerting systemic effects upon oral and systemic administration, a way to locally deliver SPMs specifically to tissues where resolution activation is desired in a controlled fashion has not been addressed.

Previous reports have shown that local activity of SPMs in the context of biomaterials may be useful. For example, in experiments with a porous chitosan-based acetylated biomaterial in a murine inflammation model, RvD1 was able to modulate the functional phenotype of macrophages towards one involved in inflammation resolution. Nanoparticles prepared from human neutrophil microparticles enriched with SPMs have also been shown to have anti-inflammatory and proresolving properties. However, a well-defined biomaterial that allows the local release of SPMs in a sustained manner while maintaining biological activity over a long time remains to be achieved. A recent study has addressed this possibility.

The development of such a new biomaterial was carried out by Lance and colleagues from the UCSF Graduate Group in Bioengineering at University California Berkeley, San Francisco, CA, in collaboration with colleagues at the Department of Bioengineering and Therapeutic Sciences, and the Cardiovascular Research Institute and Department of Surgery, at the University of California, San Francisco, and the Department of Anesthesiology, Perioperative, and Pain Medicine at Brigham and Women’s Hospital at Harvard Institutes of Medicine, Boston, MA, USA. The study reports on the development of a thin-film device composed of two or three layers of poly(lactic-co-glycolic acid) (PLGA) each with a different density. Bilayered and trilayered composite films were created by sequential layering of thin films of co-polymer solution using the spin coating technique. Film layers with distinct densities were achieved by using solutions of PLGA in which the ratio of lactic acid to glycolic acid used for the formation of the co-polymer was different. PLGA layers with ratios of lactic to glycolic acid co-polymers of 50:50, 72:25 and 85:15, had average layer thicknesses of 12, 9.9 and 26 µm, respectively. After assembly into a trilayer, an average film thickness of 50.9 µm was obtained. Such devices were transparent and pliant. RvD1 was placed in-between layers and patches of multilayered materials were obtained that would theoretically allow vectorial diffusion of the lipid mediator (100 ng and 200 ng for bilayered and trilayered devices, respectively) towards and out of the side of lowest density. PLGA is biodegradable since it slowly hydrolyzes in the body, and is bio compatible as its aqueous hydrolysis products lactic acid and glycolic acid are endogenous metabolites.

The expected mass transfer behavior was evaluated in practice by several approaches. First, it was confirmed that RvD1 could diffuse from the composite materials, by measuring release over time into buffer or serum-free medium when placed in test tubes at 37°C. Maximum recovery of entrapped RvD1 was determined by homogenization in ethanol. The directional elution of RvD1 was studied by placement of the device in a two-sided diffusion chamber that allowed sampling of medium in contact with either side of the trilayered film.

Release of biologically-active RvD1 from the thin-film device was determined in various ways. The effect of RvD1 on primary human vascular smooth muscle cells isolated from saphenous vein explants was determined by placement of the thin-film device in a permeable transwell insert placed over the cells grown in culture. Modulation of NF-κB activity (as a read out of inflammatory activity) following the addition of tumor necrosis factor-α (TNF-α), smooth muscle cell proliferation, cell viability, and cell migration into an experimental injury site (modelled by “scratching” a thick line of cells growing on the culture dish), were measured to assess the release of biologically active RvD1. An ex vivo flow chamber was de-

This study evaluated the performance of a new biodegradable thin-film device that can deliver the DHA-derived lipid mediator RvD1 in a local environment in a sustained and directional fashion.
signed to test RvD1 release from the lower-density side facing an inner piece of rabbit aorta, which was in turn enclosed by a larger diameter section of aorta. The inner vessel was then perfused in a closed circuit with pulsed flow of serum-free media, in which the accumulation of RvD1 could be measured. Finally, in vivo device functionality was determined in a rat model in which the trilayered material was wrapped around the left carotid artery with the lowest density side facing the artery. After 1 hour, RvD1 levels were measured by enzyme-linked immunoassay in arterial tissue of the left and right carotid artery.

Release of RvD1 that had been enclosed between the layers reached a maximum rate after three weeks when tested in vitro, with rates staying constant up to seven weeks. Release rates were approximately twice as high into a serum-free medium than in a phosphate-buffered salt solution (PBS), indicating that the environment has a marked effect on RvD1 diffusion. The physical disintegration of the thin-film device observed at 10 weeks was visibly more marked in serum-free medium than in PBS, and may also contribute to the higher RvD1 release. The amount of RvD1 that could maximally be released was determined after homogenization of the device into ethanol and constituted 84% of the total dose loaded within the film structure. In non-homogenized devices, release into ethanol did not surpass 35% of total dose, suggesting that total surface area plays an important role in releasing the bulk of the incorporated RvD1. In PBS, the amount eluted did not surpass 14% of total RvD1 load.

Employing the two-sided diffusion chamber, directional RvD1 release occurred after one day, with significantly higher release of RvD1 from the low-density side. After two weeks, 97.9% of the total released RvD1 had been released on the low-density side, demonstrating that the high-density PLGA layer effectively blocked diffusion. RvD1 that diffused from the device was shown to be chemically stable when PBS was used, whereas in serum-free medium at least two other structurally-related substances of unreported identity were observed in addition to RvD1 itself.

Assays of vascular smooth muscle cell responses to trilayered thin-film devices showed that RvD1-loaded devices markedly inhibited a cellular inflammatory response (determined after 20 hours) initiated by TNF-α exposure, nearly abolished serum-induced cell proliferation (determined after 10 days), and significantly reduced cell migration (determined after 24 hours). These effects were observed at RvD1 concentrations of 5-50 nanomolar in the medium. No effect was observed on endothelial cell migration in a similar scratch injury model.

In the ex vivo aorta perfusion model, RvD1 release from the low-density side towards the inner vessel was 0.41 picogram per mg artery in 24 hours. In contrast, release towards the outer vessel was 4.4-fold lower (0.094 picogram per mg tissue), showing that vectorial release could be achieved within a biological tissue. Continuous removal of RvD1 from the inner aortal tissue into the perfusion medium may likely have occurred, and the absolute dose of RvD1 released from the low-density side is likely higher than calculated from tissue content alone. Finally, employing the thin-film device as a perivascular wrap showed that release of RvD1 into the left carotid arterial tissue (0.63 picogram per mg tissue) was significantly higher than the levels measured in the right carotid artery that was used as control tissue (with basal tissue levels of 0.12 picogram RvD1 per mg tissue).

This study has reported on the development of a new multi-layered thin-film drug delivery device made from bioresorbable material that allows vectorial diffusion of an omega-3 LCP-UFA-derived lipid mediator with anti-restenotic and inflammation-resolving activity. Release from the thin-film biodegradable device was sustained over several weeks and could be directed towards one side of the device by using graded layer densities. Biological activity was confirmed and occurred at medium concentrations in the range where RvD1 is expected to act as an agonist (high picomolar to low nanomolar).
Restenosis and thrombosis constitute significant sources of risk to endovascular interventions. Continued efforts are being made in material development to reduce and eliminate these risks. The use of RvD1 in a bioresorbable device that permits sustained localized release may have specific advantages in vascular lesions involving neointimal hyperplasia, such as in restenosis after venous grafting and in-stent restenosis. RvD1 triggers the active down-regulation of vascular smooth muscle migration and proliferation, the activation of local tissue inflammatory response to injury or cytokine stimulation, as well as oxidative stress. The advantage of this new thin-film device, in particular its ability of directional SPM release, for specific applications remains to be demonstrated. The authors indicate that many applications that are surgically-adaptable may be evaluated, for example in bypass-grafting, and reducing neointimal hyperplasia of carotid angioplasty.

The observation that the type of matrix around the thin-film device affected the rates of release, the stability of the device itself, and the formation of structurally-related analogues of RvD1, indicate that the precise kinetics of the release of the enclosed RvD1, or other SPMs, will be challenging to predict in different in vivo applications. Depending on the purpose, sustained release over several weeks may be sufficient to address local therapeutic delivery of an SPM, after which the device is gradually degraded and absorbed. The vectorial diffusion that initially directs RvD1 release towards the intima and vascular lumen when placed around a vessel, may be lost over time as the device progressively disintegrates. How resolvins affect the resorption of the otherwise biodegradable PLGA thin-film device will need to be tested. Combinations of this material to improve the grafting of stable implants may also be envisaged. The capacity of SPMs to promote microbial clearance, and improve device patency by lowering risk of infections associated with biomaterial implantation, may also constitute an additional advantage. The road of medical device development is very long, but it will be very interesting to watch how this new functional biomaterial may find its way into clinical practice.


Worth Noting


Thin film spin coating:


F3-Isoprostanes and F4-Neuroprostanes: Non-enzymatic Cyclic Oxygenated Metabolites of Omega-3 Polyunsaturated Fatty Acids: Biomarkers and Bioactive Lipids

Jean-Marie Galano, Camille Oger, Valérie Bultel-Poncé, Guillaume Reversat, Alexandre Guy, Joseph Vercauteren, Claire Vigor, Thierry Durand
Institut des Biomolécules Max Mousseron (IBMM), UMR 5247, CNRS, Université de Montpellier, ENSCM, Montpellier, France

Abstract:
The isoprostanes are non-enzymatic oxygenated metabolites derived from polyunsaturated fatty acids (PUFA) formed in vivo by free radical mechanism. Those cyclic oxygenated metabolites named isoprostanes (IsoPs) were originally discovered from arachidonic acid (AA, C20:4 n-6) in 1990 and since then best known as biomarkers for assessing endogenous in vivo oxidative stress (OS) in humans and animals. During the last twenty-five years, a few chemist groups have successfully synthesized these cyclic oxygenated metabolites derived from omega-3 (n-3) PUFA such as F3-IsoPs from eicosapentaenoic acid (EPA, 20:5 n-3), and F4-neuroprostanes (F4-NeuroPs) from docosahexaenoic acid (DHA, 22:6 n-3), and their availability allowed a better understanding of their potential roles as bioactive compounds but also extended their use as more specific biomarkers of OS. Accordingly, we will discuss the impact of F3-IsoPs and F4-NeuroPs generated from EPA and DHA in this review.

1. Introduction
The understanding of the role of n-3-PUFA peroxidation in the pathogenesis of various diseases is continuously increasing but the biological activity and the biochemical role of the myriad of metabolites generated have been largely undetermined by investigators and remain unexplored for most of them. The reasons for the small number of investigations could be due to the false idea that the rate of non-enzymatic PUFA oxidation in vivo is negligible, and/or to the previously held idea that any oxygenated metabolites derived from lipid peroxidation are undesirable and toxic. Moreover, not all of these metabolites are commercially available and need to be custom synthesized.

Previously, quantification of lipid metabolites 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) was the main assessment for oxidative stress measurement (OS) in biological systems. However, they appear to be not as robust biomarkers when compared to F3-IsoPs measurement and correlated to OS. Since the discovery of Morrow and Roberts, F3-IsoPs have become a “gold standard” for assessing endogenous OS in humans, animal models and in biological fluids [13]. These lipids are oxidized in situ on the phospholipid membranes and hydrolyzed via phospholipase A2 (PLA2) and platelet activating factor acetylhydrolase into the free form, and finally released in tissues and systemic circulation. Among these metabolites, some have been commonly, and in some cases routinely, measured as OS biomarkers related to vascular systems and neurodegeneration [8, 9, 11]. The discovery and study of isoprostanes have provided a major step forward in the field of free radical research. The quantification of these oxygenated lipids has opened up new areas of investigation regarding the role of free radicals in human physiology and pathology, and appears to be the most useful tool currently available to explore the role of endogenous lipid peroxidation in human diseases. However, as explained below such lipids are not only reliable biomarkers but also exert bioactive properties.

So far, evidence in favor of the bioactive role of isoprostanes from n-6 PUFA were shown in various biological systems [6, 7, 11]. 15-F3-IsoP will be not developed in this review (for references, see recent reviews) [6, 7, 11, 14, 15].

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the major n-3 polyunsaturated fatty acids (PUFA) of marine fish oil. Evidence from epidemiological studies, clinical trials, animal and cellular experiments showed fish oil, and specifically n-3 PUFA, having beneficial effects in numerous diseases [1]. Due to the number of double bonds in the structure of EPA and DHA, they are prone to free radical attack and can undergo non-enzymatic peroxidation to generate cyclic oxygenated metabolites, termed isoprostanes (IsoPs) and neuroprostanes (NeuroPs) [2-5]. The comprehension of the effect of PUFA and their non-enzymatic metabolites has been reported in a number of recent prominent reviews [6-12].
2. Formation, nomenclature and quantitation of \( F_3 \)-IsoPs, \( F_4 \)-NeuroPs derived from EPA and DHA, and isofurans. Morrow et al. discovered in 1990 novel prostaglandin (PG)-like isomers, which were termed isoprostanes (IsoPs) [3]. In contrary to PG initiated by cyclooxygenases, their mechanism of formation proceeds via a non-enzymatic free radical peroxidation of AA bound to phospholipids and not from free AA [3]. The main structural characteristics compared to PGs are the \textit{cis}-relationship of the side chains, the absolute number of potential isomers and the racemic generation of the metabolites. Once formed in the membranes, the IsoPs can then be released by phospholipases in the circulating fluids [16]. Later, it was discovered that other PUFAs such as EPA and DHA can undergo a similar oxidation process leading to \( F_3 \)-IsoPs and \( F_4 \)-NeuroPs respectively [4] [5] (Figures 1 and 2).

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**Figure 1: Biosynthesis of \( F_3 \)-IsoP derived from EPA**

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**Figure 2: Biosynthesis of \( F_4 \)-NeuroP derived from DHA**
In 2002, new oxygenated metabolites were discovered with a furan core as the main feature. Their formation follows the initial same free radical cascade pathway of IsoPs, but a competition between the five-membered ring formation (IsoPs) and attack of further diradical oxygen lead to the competitive generation of isofurans (IsoFs) from AA [17]. It is now well described that both types of metabolites are present in most lipid matrices. Similarly, other PUFAs can also generate isofuranoid derivatives, [6] including neurofurans (NeuroFs) (Figure 3) [17, 18] and dihomo-isofurans [19] (dihomo-IsoFs) from DHA and adrenic acid (AdA, 22:4 n-6) respectively.

There are two nomenclatures proposed by Taber [20] and Rokach [21] to name such metabolites. Taber nomenclature was approved by IUPAC and will be used throughout this review. To avoid confusion, the structure of 4-F4t-NeuroP is presented in Figure 4 and will be described briefly.

3. Chemical syntheses.
A number of talented chemists have developed, all around the world, different chemical strategies to reach these IsoPs and NeuroPs (see few reviews [6, 7, 11], for the general understanding of their in vivo formation and biological functions, but also for diagnostic applications. We will mention briefly in this review only the four groups which performed the total syntheses of F3-, A3-IsoPs derived from EPA and F4-, A4-NeuroPs derived from DHA.

Our group has developed the first synthesis of F4-, NeuroP, 4(RS)-F4t-NeuroP, in 2000, using a radical carbocyclization strategy [24]. Later, in 2010, by using a more flexible strategy using our bicyclo[3.3.0]octene key intermediate [25], we have reached other series of F3- and F4-NeuroPs and deuterated analogues [26]. Rokach and co-workers reported the synthesis of the major metabolites of EPA, 5-F3c-IsoPs and 5-F3t-IsoPs [27]. Cha and co-workers in 2002 reported a total synthesis of 17-F4c-NeuroP using a double cyclization step, with Pd(OAc)2 [28]. Taber and co-workers described in 2008 an interesting approach towards the synthesis of the four diastereomers of 13-F4t-NeuroP, using a thermal diastereoselective en cyclization of 1,6-dienes to the 1,2-cis-cyclopentane skeleton [29]. It is worthy to mention that Zanoni, Vidari and co-workers are the only chemists who have developed a strategy to reach A3-IsoPs and A4-NeuroPs [30].

4. Biomarkers
4.1. Neuroprostane and Isofuran
OS may contribute to the pathogenesis of pre-eclampsia, a life-threatening disorder of pregnancy that adversely affects the mother and the baby [31]. In a recent study, Bardeen et al. quantified F3-IsoP, IsoF and F4-NeuroP in maternal
plasma and cord blood of women with pre-eclampsia and normal pregnancies [32]. Women with pre-eclampsia had significantly elevated maternal IsoF and F4-NeuroP, but no F2-IsoP. Interestingly, cord blood IsoF were approximately 5-fold higher than those found in maternal plasma. This could reflect the oxidative challenge presented at birth, when there is transition from a relatively low intra-uterine oxygen environment to a significantly higher extra uterine oxygen environment.

The brain is vulnerable to oxidative insult because of high oxygen requirements for its metabolism and high PUFA composition, in particular DHA, hence F4-NeuroP was considered to be a specific marker of brain OS. Aneurysmal subarachnoid hemorrhage (aSAH) and traumatic brain injury (TBI) are associated with devastating central nervous system (CNS) injury. We and others have shown a significant increase in cerebrospinal fluid (CSF) IsoF in aSAH and TBI patients compared with their respective age- and gender-matched controls. aSAH patients also had significantly increased levels of CSF F4-NeuroP and F2-IsoP. Patients with TBI had significantly increased CSF F4-NeuroP, but F2-IsoP levels were similar to control [33]. These data confirm that CNS injury, in case of aSAH or TBI, results in increased OS and as DHA is the brain major PUFA, F4-NeuroP levels in CSF could be a much more specific indicator of neurological dysfunction than F2-IsoP. Hsieh et al. have shown that increased F4-NeuroP in CSF of patients with aSAH correlated with poor neurological outcome [34]. They suggested that F4-NeuroP might be more useful than F2-IsoP in CSF to predict outcome and interpret the role of hemorrhage in aSAH.

The anti-atherogenic effects of omega 3 fatty acids EPA and DHA are well recognized but the impact of dietary intake on bioactive lipid mediator profiles remains unclear. Gladine et al. studied the impact of DHA supplementation on the profiles of PUFA oxygenated metabolites and their contribution to atherosclerosis prevention [35]. A special emphasis was given to the non-enzymatic metabolites knowing the high susceptibility of DHA to free radical-mediated peroxidation and the increased OS associated with plaque formation. Targeted lipidomic analyses revealed that both the profiles of EPA and DHA and their corresponding oxygenated metabolites were substantially modulated in plasma and liver. Notably, the hepatic level of F4-NeuroP was strongly correlated with the hepatic DHA level. Moreover, unbiased statistical analysis revealed that the hepatic level of F4-NeuroP was the variable most negatively correlated with the plaque extent (p<0.001) and an important mathematical positive predictor of atherosclerosis prevention.

4.2. Dihomo-Isoprostane
F2-Dihomo-Isoprostanes belong to the family of Isoprostanes deriving from the non-enzymatic oxidation of adrenic acid (C22:4 n-6, AdA), a polyunsaturated fatty acid distributed in the body, but also a specific component of myelin in the brain of primates [36, 37]. Rett syndrome (RTT) is a pervasive abnormality of development affecting almost exclusively females, which is included among the autism spectrum disorders. RTT is caused, in up to 95% of cases, by mutations in the X-linked methyl-CpG binding protein 2 (MeCP2) genes [38]. The disease shows a wide phenotypical heterogeneity, with at least four distinct major clinical presentations, i.e., typical, preserved speech, early seizure variant, and congenital variant. Clinical evidence indicates that F2-Isoprostanes and F4-NeuroP are involved in the intimate pathogenetic mechanisms of RTT. Plasma levels of free F2-IsoP are significantly higher in the early stages of RTT, as compared with the late natural progression of typical RTT. Until recently it was thought that the predominant central nervous system damage in RTT occurred in gray matter. However, the relative abundance in myelin of the precursor AdA and the increased level of F2-dihomo-Isoprostanes, strongly confirm an early and severe damage to the brain white matter as suggested by previous brain MRI evidence. Thus F2-dihomo-Isoprostanes can be considered early markers of lipid peroxidation in RTT [39]. F4-NeuroP also appear to be important biomarkers in RTT. Plasma F4-NeuroP levels correlate with disease severity in RTT and are significantly related to neurological symptoms severity, mutation type and clinical presentation. Therefore, F4-NeuroP may play a major role along the biochemical pathway from MeCP2 gene mutation to clinical evidence, proving that a DHA oxidation process occurs.

4.3. Dihomo-Isofuran / Neurofuran
Neurofurans (NeuroF) and dihomo-isofurans (dihomo-Isoprostanes) are produced in vivo by non-enzymatic free radical pathways from DHA and AdA, respectively. As these metabolites are produced in minute amounts, their analyses in biological samples remain challenging. We performed syntheses of NeuroF and dihomo-Isoprostanes thanks to an enantiomerically enriched intermediate, which allowed, for the first time, access to both families: the alkenyl (4(RS)-ST-Α5-8-NeuroF) (Figure 3) and enediol (7(RS)-ST-Δ5-11-dihomo-Isoprostanes) [19, 40] and their quantitation in rat brain and heart tissues. It is also the first report to show concentration of known NeuroF and dihomo-Isoprostanes in the heart tissue. These DHA and AdA metabolites are presently in testing for various pathological models as OS biomarkers and bioactive compounds.

5. Biological activities
The biological roles of oxygenated metabolites from the per-
oxidation of n-3-PUFA mainly emphasize their formation by enzymatic pathways, especially with respect to anti-inflammatory activities and the reduction of pro-inflammatory eicosanoids stemming from AA [7, 41]. For example, lipoxigenases mediate formation of metabolites such as resolvins, protectins and maresins [42, 43] that have shown a large range of potent anti-inflammatory activities in diseases. Nevertheless, recent studies showed that isoprostanes per se derived from n-3-PUFA (mainly from EPA, DHA and α-linolenic acid) are new actors to be considered [5, 15, 41], suggesting that bioactive roles of oxygenated n-3-PUFA are not limited to those released through enzymatic pathways.

Metabolites of EPA

Over a decade ago, one study highlighted that unlike the 15-F3t-IsoP derived from AA, 15-F3t-IsoP from EPA does not activate platelet aggregation [44]. This notable difference of activity between cyclic oxygenated products derived from n-6-PUFA and n-3-PUFA suggests a very subtle structure-activity relationship [7]. More recently, Jamil et al. [45] investigated the ability of 5-F3t-IsoPs to regulate glutamatergic neurotransmission. Hence, 5-F3t-IsoPs could have important pharmacological implications in neurology since EPA, its precursor, is rich in the brain and retina. Glutamate serves as the primary excitatory neurotransmitter in several vertebrate retinal cells, including ganglion cells. The group also investigated the modulatory role of 5-epi-5-F3t-IsoP on K+-induced glutamate release in isolated bovine retina. They found that 5-epi-5-F3t-IsoP attenuates K+-induced [3H] D-aspartate release in a concentration-dependent manner and indicated that the mechanism involved is due to, in part, pre-junctional prostanoid EP1-receptors activation. This result displays the beneficial role of 5-epi-5-F3t-IsoP by reducing excitatory neurotransmitter release, thereby retarding the progression of ocular neuropathic disease.

A4/J3-IsoPs, the EPA-derived cyclopentenone isoprostanes, were also identified for their biological qualities in vivo under an OS environment. Two studies observed that these EPA-cyclopentenone derivatives possess anti-inflammatory activities and have antioxidant properties. Indeed, the 15-A3t-IsoP inhibited in a concentration-dependent manner the expression and the activity of iNOS and COX-2 in mouse macrophages when pre-treated for 30 minutes with 15-A3t-IsoP. It is postulated that 15-A3t-IsoP exerts an anti-inflammatory activity via the inhibition of the nuclear factor kappa B (NFkB) by blocking the degradation of inhibitory subunit IκBα [46]. The second study was performed in hepatocarcinoma cells and found that the compound J3-IsoP, isomers of A3t-IsoP from EPA, induced the nuclear related factor 2 (Nrf2)-based antioxidant response through the inhibition of Keap-1, a negative regulator of Nrf2 [47].

Metabolites of DHA

The group of Morrow and Roberts, which pioneered the in vivo identification of NeuroPs, also demonstrated the biological effects of A4/J4-NeuroPs, cyclopentenones derived from DHA, to be mainly anti-inflammatory mediators in murine macrophage cell line [48]. Notably, they reported that in particular 14-A4t-NeuroP suppressed the effect of pro-inflammatory mediators such as lipopolysaccharide in macrophages, and confirmed the inhibition of the NFκB pathway as the major mechanism of action of DHA as well as EPA peroxidized metabolites.

The most recent study on the biological effects of a DHA metabolite, 4(RS)-4-F4t-NeuroP (Figure 3) specifically was performed in the cardiovascular system [49]. It is proposed that the oxidation of DHA and generation of 4(RS)-4-F4t-NeuroP is necessary to prevent ischemia-induced arrhythmias in mice with myocardial infarction [50]. As previously observed in different oxidative conditions [51], we proposed that in oxidative stress conditions such as ischemic diseases, non-enzymatic cyclic oxygenated metabolites of DHA formed by peroxidation in cardiac membrane lipids, namely 4(RS)-4-F4t-NeuroP, are responsible for the anti-arrhythmic properties of DHA by counteracting the cellular stress by ROS. Importantly, it appears that non-enzymatically released cyclic oxygenated metabolites of n-3 PUFA regulate cell communication, exert a physiological role and potentially act as a therapeutic agent [51]. Besides 4(RS)-4-F4t-NeuroP, Le Guennec’s group associated with our group evaluated the in vitro anti-arrhythmic properties of other oxygenated metabolites of EPA and DHA on single cardiac cells isolated from mice hearts. They observed that several cyclic oxygenated metabolites showed anti-arrhythmic (4(RS)-4-F4t-NeuroP, and 15-F3t-IsoP from EPA) or pro-arrhythmic (5(RS)-5-F3t-IsoP and 8-F3t-IsoP from EPA) properties, opening the way for likely biological roles; in depth validation is required to further substantiate the bioactive roles. Finally, Le Guennec et al reported in a recent patent that 4(RS)-4-F4t-NeuroP is suitable for the treatment of acute myocardial infarction [52, 53]. Finally, a recent study by Gladine and co-workers reported that A4t- and F4t-neuroprostanes possess anti-inflammatory activities similar or even more pronounced than neuroprotectins (PD1, PDX), supporting that neuroprostanes should be considered as important contributors to the anti-inflammatory effects of DHA [54].

6. Conclusion

The experimental evidence outlined here supports the notion
that several of the biological activities of n-3 PUFAs in OS conditions could be explained by the action of non-enzymatic peroxidation products. In general, it appears that non-enzymatically cyclic oxygenated metabolites of n-3 PUFA could exert a physiological role. It highlights that in diseases which involve ROS production, some non-enzymatic oxygenated metabolites of n-3 PUFAs could be produced and prevent deleterious consequences of diseases, such as arrhythmias.

Care should be taken by scientists not to overlook the production of non-enzymatic metabolites of n-3 PUFAs, which could have traits equally or more active than the well-known enzymatic metabolites. Their production is very sensitive to the environment (diet and oxidative status) and may play advantageous or disadvantageous roles in many diseases, where oxidative status is highly related to the severity of the diseases such as cardiovascular, neurodegenerative, pulmonary, developmental, and metabolic disease and cancer.

References


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In North America and Europe, breast cancer accounts for over 25% of all new cancer diagnoses in women, and approximately 1 in 8 women will be diagnosed with breast cancer in their lifetime (1-3). Despite ongoing advances in screening, prevention, diagnosis, and treatment, breast cancer remains the second leading cause of cancer-related death in women, and one of the most expensive to treat (4). Relapse and metastasis remain high despite treatment with surgery and chemotherapy (5). Treatment itself carries risks. The side effects of chemotherapy include dose-limiting toxicity to the cardiac, immune, and nervous systems (6). Improving treatment without increasing toxic side effects is crucial to successful outcomes and long-term health.

Omega-3 Fatty Acids and Breast Cancer

Fish oil and high dietary consumption of fatty fish has consistently been associated with reduced incidence of breast cancer (7, 8). Fish oil is a rich source of omega-3 long chain polyunsaturated fatty acids (LCPUFA), predominantly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The majority of omega-3 LCPUFA in the diet is alpha-linolenic acid (ALA). EPA and DHA can be synthesized in the body from ALA, and EPA itself is a precursor of DHA. However, endogenous synthesis is very low and the only way to significantly increase these LCPUFA is to directly consume them. Consequently, the primary dietary sources of EPA and DHA are fatty cold water fish, supplements, or foods fortified with DHA/EPA. There is a growing evidence that EPA and DHA have distinct but overlapping physiological effects (9) and this might also apply to anti-cancer functions.

Breast cancer is divided into subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), and the human epidermal group factor receptor 2 (HER2). The three main types of breast cancer are i) ER+/PR+ (also referred to as hormone sensitive), ii) HER2+, and iii) triple negative breast cancer, which does not express ER, PR, or HER2 (10). Treatment depends on several factors including subtype and extent of disease, and can include a combination of surgery, radiation, and systemic therapy (11). Systemic therapy includes hormone therapy, targeted therapies, and chemotherapy. Hormone therapy, including treatment with the anti-estrogen drug tamoxifen, is indicated for patients with ER+/PR+ tumors. HER2+ tumors are treated with Herceptin (trastuzumab), a targeted therapy that binds and inhibits the HER2 receptor. Chemotherapy with cytotoxic agents is indicated for triple-negative breast cancer and advanced disease. There is convincing experimental evidence that incubating the different subtypes of human breast cancer cells with DHA alone or in combination with EPA decreases the survival of tumor cells (12). Similarly, when rodents with mammary tumors are fed a combination of EPA and DHA or DHA alone, tumor growth is decreased (13). This anti-cancer effect is specific to cancer cells, as the growth of normal mammary cells is not negatively altered by DHA and EPA (14).

DHA and Breast Cancer Treatment

Reducing side effects of treatment

Cytotoxic agents are toxic to cells, inhibiting growth. Unfortunately, cytotoxic agents are rarely specific to cancer cells. Consequently, chemotherapy in cancer treatment is often limited by dose-limiting toxicity or side effects. Side effects of chemotherapy include short-term side effects like fatigue, hair loss, and pain. Other side effects including cardiotoxicity and nerve damage can result in long-term health problems. DHA may ameliorate the side effects of some chemotherapeutic agents. Taxane drugs are commonly used to treat breast cancer, as well as ovarian, lung, and other solid tumors. These drugs inhibit cell proliferation. Treatment with some taxane drugs are limited by neurotoxicity, termed peripheral neuropathy, characterized by numbness, tingling, and pain in the hands and feet (15). In a small randomized control trial, omega-3 LCPUFA supplementation (640 mg/day gelatin capsule, 54% DHA, 10% EPA) decreased the incidence of pe-
Peripheral neuropathy in breast cancer patients being treated with the taxane drug paclitaxel (16). Other clinical trials report that supplementation with omega-3 LCPUFA (0.6g-8.6 g/day) increased the ability of patients to tolerate a variety of different chemotherapeutic drugs in a range of cancers, including lung cancer, pancreatic cancer, and colorectal cancer (17). In these patients, supplementation with DHA was associated with decreased weight loss, improved quality of life, and reduced anemia (17).

**Increasing sensitization to cytotoxic drugs**

There is increasing evidence that pre-treatment with DHA can increase the sensitivity of breast cancer cells to chemotherapy drugs. Doxorubicin, a drug that interferes with DNA replication, is commonly used in breast cancer treatment. In breast cancer cell lines, pre-incubation of cells with DHA followed by treatment with doxorubicin increases the effectiveness of this drug (14). A similar effect is seen in vivo in mice implanted with human breast tumors. Dietary supplementation with fish oil increased the effectiveness of doxorubicin, resulting in a greater reduction in tumor size compared to mice fed the control diet (18).

Similar results were seen when human breast cancer cells were treated with DHA and taxane drugs, commonly used as first line therapy in breast cancer (19). This increased sensitivity to taxanes has been confirmed in a rat model of breast cancer. Rats treated with a carcinogen to induce mammary tumors were fed either a control diet, or a diet rich in omega-3 LCPUFA (1% EPA, 2.5% DHA), and treated with docetaxel, a widely used taxane drug. Tumors in rats fed a DHA rich diet had a significantly greater reduction in size compared to rats fed control diet (20). Thus, not only was DHA able to decrease side effects of taxanes, it may also improve the effectiveness of these drugs.

**Increasing sensitization to hormonal and targeted therapy**

In addition to sensitization to cytotoxic drugs, omega-3 LCPUFA may also be beneficial for hormone and targeted therapy in breast cancer. Tamoxifen is a hormone treatment used in ER+/PR+ breast cancer. It binds to ERα, preventing activation of this receptor by estrogen. This in turn prevents the activation of estrogen responsive genes including genes involved in proliferation, and other genes that can enhance tumor growth (21). In rats treated with a carcinogen to induce mammary tumors, a diet high in n-3 LCPUFA in combination with tamoxifen increased the regression of pre-cancerous growths compared to tamoxifen alone (22).

Herceptin is a monoclonal antibody that specifically recognizes and binds to the HER2 receptor, which is imbedded in the cell membrane. When HER2 is overexpressed or mutated, as it is in some breast cancers, it can signal to cells to proliferate (23). While HER2 is expressed by many cells in the body, a subset of breast cancer patients have tumors that overexpress this protein. When these patients are treated with Herceptin, the breast cancer cells that specifically overexpress this protein are targeted. Binding of Herceptin to HER2 on breast cancer cells inhibits proliferation (24). Recent studies have observed that when breast cancer cells expressing HER2 were treated with Herceptin, incubation with DHA increased cell death, and reduced growth compared to cells treated with Herceptin alone (25, 26).

**Possible explanations as to how DHA and EPA can improve breast cancer treatment**

The exact way that omega-3 LCPUFA inhibit breast cancer growth is not completely clear but there is evidence for several different possible paths. Incubating breast cancer cells with DHA and EPA reduces cell proliferation, and induces cell death (12). This anti-cancer effect is specific to malignant cells, as normal breast cells are not sensitive to similar concentrations of EPA or DHA (14). EPA and DHA are fatty acids that incorporate into the membrane. This results in a number of changes. Firstly, the membrane is more susceptible to oxidative damage.

Tumors are not well equipped to repair this damage and it leads to a suicide-like death called apoptosis. Secondly, changes in the amount of EPA and DHA in the membrane can alter permeability to other molecules, perhaps even chemotherapy drugs. It also changes the amount, location and function of receptors and signals in the membrane that regulate growth and death of the tumor cells (27, 28). DHA has also been demonstrated to decrease the migration of breast cancer cells, and metastasis in mouse models of breast cancer (29-31). This could be due to changes in the cell membrane due to incorporation of DHA and EPA and/or changes in expression of proteins that promote metastasis. There is also evidence that DHA may eliminate breast cancer ‘stem’ or initiating cells. These are the cells within a tumor that are widely believed to be respon-
sible for treatment resistance, cancer relapse and metastasis. Treatment of breast cancer cells with DHA can decrease the number of these cancer stem cells (32, 33).

DHA and other omega-3 LCPUFA have well established roles in the immune system (34). During development, these LCP-UFA play essential roles in the proper establishment of the immune system. Omega-3 LCPUFA, specifically DHA and EPA can be metabolized to give rise to anti-inflammatory signaling molecules that mediate and reduce the inflammatory response (34). Omega-3 LCPUFA have been demonstrated to reduce inflammation and improve the ability of the immune system to recognize and target cancer cells in a variety of cancer models (35). In lung, colorectal, and pancreatic cancer, dietary DHA has been demonstrated to downregulate inflammation, and may improve anti-cancer immune targeting (36). This has not been well studied in breast cancer, but preliminary evidence suggests that DHA can alter the inflammatory response within mammary tumors (13), resulting in improved response to, and ability to tolerate, current treatment.

Clinical evidence to support omega-3 LCPUFA supplementation in breast cancer patients
A small phase II clinical trial was published in 2009 that examined the potential beneficial effect of DHA in combination with chemotherapy. In this trial, 25 patients with advanced, metastatic breast cancer were given DHA supplements in the form of capsules containing DHA-enriched triglyceride oil (1.8g/day total DHA), taken three times daily with meals. DHA supplementation started 7-10 days before beginning chemotherapy with a combination of cytotoxic drugs, and then continued with DHA supplementation for five months of chemotherapy (37). While all patients were given the same amount of DHA, incorporation of DHA after a one week loading period was highly variable between patients, as measured by the amount of DHA incorporated into plasma phospholipids. Importantly, patients with high incorporation of DHA had significantly better response to chemotherapy than patients who had lower levels of DHA incorporation. In patients with high DHA incorporation, overall survival was 34 months, compared to 18 months in patients with low DHA incorporation. A similar study examining the potential benefit of DHA in combination with chemotherapy in advanced non-small cell lung cancer observed an increased response rate in patients receiving fish oil supplements containing DHA (38).

These results are supported by findings from a small prospective study of patients with non-metastatic, invasive breast cancer. In this study, the fatty acid content of breast adipose tissue obtained during biopsies was analyzed for fatty acid content. Increased DHA correlated with increased response to chemotherapy and the study determined that DHA was an independent predictive factor of sensitivity of the tumor to treatment (39).

While these results are promising, additional work is necessary for inclusion of supplementation with DHA into the current treatment for breast cancer and other cancers. Of note, while patients who had high incorporation of DHA had improved response to chemotherapy, presently, there is no way to predict which patients will have high DHA incorporation. The phase II clinical trial contained only a small number of patients with advanced breast cancer. DHA supplementation in patients with early breast cancer, and patients undergoing treatment with hormone and targeted therapy remains to be examined. Nonetheless, the pre-clinical evidence suggests that DHA has the potential to help all breast cancer patients who undergo chemotherapy.

Conclusions
There is considerable interest by the public and patients for advice on nutritional interventions to treat cancer. Breast cancer patients have very high rates of reported use of nutritional supplements after diagnosis, with studies reporting 45-85% of breast cancer patients reporting supplement use (40-42). The anti-tumor effects of the nutrient DHA in pre-clinical models is well established and a number of potential ways to explain this effect have been identified. This anti-cancer effect is supported by human studies that observe decreased incidence of breast cancer with increased intake of fish oil containing omega-3 LCPUFA. Omega-3 LCPUFA, and specifically DHA, have been demonstrated to sensitize breast cancer cells to current treatment, without harming normal cells. This is supported by preliminary studies in advanced breast cancer patients undergoing chemotherapy. Current chemotherapy is limited by dose-limiting toxicity. Improving current treatments while sparing non-cancerous cells is essential to improving patient outcome. Using omega-3 LCPUFA to improve current breast cancer treatment provides a promising avenue of investigation and a new market for supplements and functional foods.

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