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Dear reader of Fats of Life,

In case you’ve been having doubts about keeping the faith in science, this issue of Fats of Life will help revive your confidence that science keeps advancing in healthy directions. New findings in biology and medicine keep occurring while we all try to stay oriented in the information jungle. We help you better appreciate a tiny part of the fascinating advances in the world of polyunsaturated fatty acids, a lively growth in the big forest of knowledge. We care for taking a close-up look at new studies, and providing additional background and perspectives, to help you better craft your opinion on polyunsaturated fatty acids – not an easy group of compounds to understand.

This issue of Fats of Life looks at the potential clinical usefulness of a new ratio of two bioactive derivatives from docosahexaenoic acid and arachidonic acid. It may help clinicians better appreciate deranged inflammation pathologies and decide when there is a need to promote the resolution of inflammation. Next, a methodological evaluation of a study carried out with EPA/DHA supplementation shows that careful attention has to be paid to measuring omega-3 tissue status in all study groups of intervention trials.

We evaluate a study that reports on a new immune system-activating mechanism that is mediated by a little-known omega-3 fatty acid, hexadecatetraenoic acid n-3. A possibility for improved chemotherapy in cancer patients could lie ahead when the chemoresistance-promoting activity of this low-abundance, but highly active, fatty acid is fully understood.

We take a close look at a nutritional intervention study that aimed at overcoming recognized limitations in study design. It is an illustrative example of the challenges nutrition researchers face, while we can learn new aspects of how our bodies react to the food we eat. This study looked for the first time at the endogenous PUFA status during weight loss achieved with diets of different macronutrient composition.

If you still need convincing, read on. Whereas in recent years we have seen a thinned appreciation for the cardiovascular benefits of EPA/DHA, a meta-analysis published last week in the Journal of Clinical Lipidology (Maki and colleagues), and supported by the Global Organization for EPA and DHA Omega-3s, has revealed that a well-defined look taken at cardiac mortality as a primary end-point, omega-3 intake, compared to placebo, shows an 8.0% reduction in risk of cardiac death, with larger effects demonstrated in groups with elevated levels of triglycerides and LDL cholesterol, as well as in groups with intakes higher than 1 g EPA/DHA daily. This renewed appreciation for the “cardiovascular” benefit of EPA/DHA is of importance given the large numbers of people that die every year from cardiac deaths.

Science takes time. Constructing the evidence for measurable and reproducible effects in biology is a very lengthy process. Interpreting science also takes time, and the need for improvements in removing subjective interpretation and drawing on the opinions of researchers with experience in their fields is highlighted in our Guest Article. Now that you are curious, move on to explore this issue of Fats of Life. While I wrote this Editorial, the field has already advanced, so keep reading and maintain your confidence.

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CARDIOVASCULAR HEALTH

The RvD1/LTB₄ Ratio in Saliva – Assessing the Usefulness of an Innovative Biomarker for the Resolution of Inflammation in Vascular Disease

THIS ARTICLE AT A GLANCE

• The potential utility of the ratio of resolvin D1 (RvD1), a pro-resolving lipid mediator derived from DHA, to leukotriene B₄ (LTB4), a pro-inflammatory mediator derived from AA, in saliva was determined to predict the level of non-resolving vascular inflammation.

• The study reports that patients with a higher carotid intima-media thickness as a marker of subclinical atherosclerosis had a significantly lower RvD1/LTB₄ ratio.

• The ratio integrates resolution and inflammation processes using lipid mediator levels that can be determined by an ELISA method in readily collected saliva.

• Future studies may validate its use for predicting a range of disorders with unbalanced inflammation, and address the contribution of local oral conditions.

Inflammation is the organ and system response that puts tissues in a state of alert, in order to defend their integrity against infection and physical disruption. However, many non-communicable diseases have a component of unregulated inflammation in their pathology. In a range of inflammatory disorders, the physiological stimuli that trigger the inflammatory response or actively turn inflammation off, i.e. resolution, are not in a proper balance. The inflammatory response may be unrestrained and too intense, with neutrophils damaging the inflamed tissue, or even triggering a systemic overactivation, with multiple organs being compromised in their function. Or the response may be too long-lasting and chronic, causing a slow process of incremental tissue remodeling and gradual loss of tissue function.

The development of atherosclerosis is a good example of a chronically activated inflammatory response that over the course of many years leads to profound remodeling of sub-endothelial vascular tissue, leading to vascular changes and the formation of hardened plaques that can rupture and form dangerous emboli. A thicker vascular intima is a telltale sign of vascular atheroma formation, which is still at a sub-clinical stage of development, and involves the trans-differentiation of endothelial cells, vascular smooth muscle cells, and stem cells and the net uptake of large amounts of cholesterol by smooth muscle-like cells and macrophages. Two broad sets of mediators are maintaining a dynamic balance of vascular tissue homeostasis: These include i) pro-inflammatory mediators, which are signalling substances that are formed by the tissue to activate immune cells that normally remove infectious organisms and recruit or activate cells that help repair damaged tissue; and ii) pro-resolving mediators that limit the extent of the activity of the pro-inflammatory mediators and actively shut down inflammatory responses when metabolic and physical disruption has abated, and there is no more infectious material present anymore, and thus allow and actively promote tissue repair. Atherosclerosis development represents a chronic maladaptive inflammatory state over the course of years, in response to some forms of continuous stress on the vasculature.

In atherosclerosis development, a chronic maladaptive inflammatory response is not being turned off.

One key pro-inflammatory mediator is leukotriene B₄ (LTB₄), which is produced by macrophages from arachidonic acid (AA) through the activity of the 5-lipoxygenase enzyme, very early in the inflammatory process. LTB₄ promotes the recruitment of additional phagocytes and activates local tissues to produce various cytokines that act in concert to optimize microbial clearance and thus initiate the process for tissue healing. Counteracting the activity of LTB₄ and other pro-inflammatory mediators is important, to limit overactive inflammatory responses that would be destructive to the body’s own tissues. Counter-regulation and active termination of inflammatory responses is executed by pro-resolving mediators that are formed locally during self-limiting inflammation. A superfamily of pro-resolving molecules formed during self-limiting inflammation is derived from long-chain polyunsaturated fatty acids (LCPUFA), in particular lipoxin A₄ from AA, and resolvins, protectin, maresins and other specialized pro-resolving lipid mediators (SPMs) from the omega-3 LCPUFA, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA).

Carrying potent inflammation-resolving activity, resolvins
D1 (RvD1) is one of the best-characterized SPMs to date. Exposure of macrophages to RvD1 switches their activity towards a less inflammatory state that is characteristic of a cell type that regulates a variety of processes necessary for orderly resolution. In vascular inflammation, RvD1 reduces the activation of smooth muscle cells by inflammatory stimuli and inhibits the hyperplasia of the intima. In contrast, LTB₄ promotes intimal hyperplasia. A heightened inflammatory response may result from inadequate availability of substrate for SPM formation due to low membrane levels of omega-3 LCPUFA or the reduced expression or activity of the enzymes involved in their biosynthesis. It has been suggested that activating resolution may be a better approach than attempting to only suppress inflammation. Similar indications that the rebalancing of inflammation and its resolution may have significant therapeutic value has recently been drawn for other chronic inflammatory diseases, such as rheumatoid arthritis.

But how do we know when inflammation resolution needs to be promoted? The levels of lipid mediators in the circulation are generally very low, likely due to rapid metabolic inactivation, and they are very challenging to measure reliably in blood. Measuring these substances within inflamed tissues is often not feasible for internal organs. In a recent study, Thul and colleagues from the Department of Medicine, Karolinska Institutet in Stockholm, Sweden, in collaboration with researchers from INSERM/University of Lorraine, Nancy, France, and Le Telomere Cardiology Center in Ghardaïa, Algeria, have reported a study that examined the relationship of the RvD1/LTB₄ ratio in saliva with intima-media thickness (IMT).

The researchers made use of samples and data available from 271 participants in the ERA (Etude de la Rigidité Artérielle) study, a prospective cohort trial carried out in Paris between 1992 and 1999. The ERA study followed one group of normotensive adults and another group of hypertensive adults that received a free health check covered by the French social security system every five years. The data used in the present report are based on the pooled recorded data at a second visit in 1998-1999, when participants had an average age of ~66 years. IMT of the right common carotid artery was determined by ultrasound scanning as an indication of subclinical carotid atherosclerosis. Saliva was collected after an overnight fast and prior to performing any oral hygienic interventions (saliva sample storage conditions were not reported).

The concentrations of LTB₄ and RvD1 in saliva were determined by enzyme-linked immune assay (ELISA), and could be determined for 254 participants. When these participants were divided into two groups according to their RvD1/LTB₄ ratio ≥1 or <1, a significant difference (P=0.006) in IMT was observed: individuals with RvD1/LTB₄ ≥1 had an average IMT of 0.71 mm, whereas in those with RvD1/LTB₄ <1, mean IMT was 0.76 mm. The 32 individuals (13% of the entire group) with RvD1/LTB₄ ≥1 were not significantly different compared to the other group with respect to gender, average age, blood pressure, heart rate, disease prevalence or pharmacotherapy. However, participants with a ratio ≥1 had a lower body mass index (25.1 compared to 26.6 kg/m² (P=0.048; no correction for multiple comparisons was applied)). The median RvD1/LTB₄ ratio in the ratio ≥1 group was 1.37, and 0.28 in the ratio <1 group, showing the separation in ratios (based on median LTB₄ levels of 131 vs 336 pg/ml, and RvD1 levels of 184 vs 97 pg/ml, respectively).

The results of this study suggest that adults with a high RvD1/LTB₄ ratio in saliva have a significantly lower carotid artery intima-media thickness than people with a low RvD1/LTB₄ ratio. This study assessed if the ratio of the pro-resolving lipid mediator RvD1 to that of the pro-inflammatory lipid mediator LTB₄ in saliva was associated with carotid artery intima-media thickness. The results of this study showed that adults with a high RvD1/LTB₄ ratio had a significantly lower carotid artery intima-media thickness than people with a low RvD1/LTB₄ ratio.
tion from an easily accessible fluid such as saliva may be a helpful additional diagnostic aid in identifying the need for restoring a healthy inflammatory response or the need for turning inflammation off, potentially using novel future pro-resolving therapeutic approaches. In peripheral atherosclerosis IMT can be measured non-invasively, but the salivary RvD1/LTB₄ ratio may be particularly helpful for assessing inflammation and inflammation-associated pathologies in organ systems that are more challenging to monitor.

Previous studies by these researchers had indicated that salivary C-reactive protein (CRP) correlates well with systemic circulating CRP levels, a widely used biomarker reflecting inflammation. However, CRP levels are up to 5000-fold more abundant in blood than in saliva. Similar correlations have been obtained for other mediators of inflammation, as well as for metabolic products such as creatinine. Saliva may thus be considered an informative means of monitoring aspects of systemic inflammation and metabolism. For the purpose of using salivary RvD1/LTB₄ ratio as an easily-measurable biomarker of inflammation-resolution balance, it is fortunate, but also surprising, that saliva contains measurable levels of various lipid mediators, as the levels of many lipid mediator autacoids in the circulation are extremely low. Further studies will need to delineate the origin of RvD1 and LTB₄ in saliva. For example, LTB₄ and RvD1 may be formed within the oral cavity by cells that through yet unclear mechanisms mirror systemic inflammatory status, or they may diffuse from the circulation into the oral cavity where they might be protected from metabolic inactivation. An additional important question, pointed out by the authors, is whether other factors determine the levels of target lipid mediators such as LTB₄ and RvD1 in the oral cavity. For example, does the oral cavity microbiome, dental cleaning frequency, smoking and dietary habits have an influence on these lipid mediator levels in saliva?

The development of the RvD1/LTB₄ ratio concept to assess the inflammation-resolution balance for clinical purposes is also an interesting development with respect to currently known read-outs of PUFA status, such as the omega-3 index (which reports the combined abundance of omega-3 LCP-PUFA as a percentage of total fatty acids in red blood cell membranes), or the ratio of omega-3 to omega-6 LCP-PUFA abundance in different tissues and fluids. These provide different aspects on the abundance of LCP-PUFA substrates potentially available for lipid mediator biosynthesis, with higher omega-3 LCP-PUFA levels correlating well with general health and a propensity for lower intensity inflammatory reactions in chronic or acute disease. In contrast, or complementary to this information, the ratio of the levels of two bioactive lipid mediators with opposing activities in the inflammatory response provides an indication of how AA and DHA, respectively, are transformed by the biosynthetic enzymes involved in their formation plus the net metabolic inactivation up to the time of sampling. It will be interesting to see in future how generally applicable the new RvD1/LTB₄ ratio will be to gauge the state of inflammation in the body, and how it may guide future approaches to inflammation control.


**Worth Noting**


Elajami TK, Colas RA, Dalli J, Chiang N, Serhan CN, Welly FK. Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot


FOL
CARDIOVASCULAR HEALTH

Evaluating the Value of Stratification on Omega-3 LCPUFA Levels when the Randomized Controlled Testing Methodology is not Sufficiently Well-Controlled

THIS ARTICLE AT A GLANCE

• This pilot study evaluated the concept that stratification of study participants can be useful for improving interpretations of the relationship between long-chain polyunsaturated fatty acid (LCPUFA) intake and biological effect.

• In patients scheduled for coronary artery bypass graft and/or heart valve replacement surgery, one group of individuals randomly assigned to receive pre-operative fish oil supplementation displayed largely overlapping erythrocyte membrane omega-3 levels with placebo-treated patients.

• After stratifying the pooled patient groups by high and low omega-3 status, or their omega-3 to omega-6 ratio, statistically significant changes in cardiac tissue responses could be measured.

• The measurement of LCPUFA status of people participating in interventional studies, and control over their background ingestion behaviour, is absolutely required to be able to link study outcomes to dietary or supplemental intake. Stratification of participants based on their fatty acid status can become instrumental in assigning clinical effects to dietary and supplemental intake of LCPUFA.

The outcomes of earlier intervention studies (~1980-2000) on the secondary prevention of cardiovascular disease (CVD), as well as the results from epidemiological studies documenting the effects of omega-3 long-chain polyunsaturated fatty acid (omega-3 LCPUFA) intake from dietary sources over long time periods, had largely supported beneficial effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake in reducing coronary events, sudden cardiac death, and mortality from cardiovascular disease-related origins. In contrast, several recent prospective cohort studies and randomized controlled intervention trials that have assessed the associations and effects of omega-3 LCPUFA intake with cardiovascular health, have reported neutral outcomes. The relatively recent appearance of studies that have been unable to support the conclusions of the earlier studies has stimulated specialists to take a closer look at the factors that may lead to the observation of marginal effects of omega-3 LCPUFA intake on cardiovascular health.

Several reasons have been proposed to explain the recent lack of an effect of dietary and supplemental omega-3 LCPUFA. These encompass better overall patient care and improved concurrent pharmacotherapy for cardiovascular disease treatment and prevention, treatment periods that are too short, the use of too low doses, small sample sizes, and a background omega-3 intake that has gradually increased over the years, which would have made it harder to raise omega-3 LCPUFA tissue levels as a result of the intervention. Although the systematic evaluation of these factors will take time, it is expected that lessons learned about how to best test the effects of dietary fatty acids in cardiovascular disease, will also be illustrative for studies addressing the roles of LCPUFA in organ systems and disorders beyond the cardiovascular system.

Another important aspect to take into account when using a randomized controlled intervention to study the clinical effects of omega-3 LCPUFA intake is that investigators should carefully consider the dose at which a specific response can be expected, based on previous data. Otherwise,
they risk using a dose that is unlikely to be sufficient to demonstrate any changes in response. The dose-response relationships for EPA+DHA intake for different aspects of cardiovascular disease have been estimated, and show for example that a linear dose-dependent reduction in arrhythmia development occurs up to a daily dose of 250 mg. Blood pressure-lowering and heart rate-lowering occurs over a much wider dose range, up to several grams a day, in a non-linear fashion, and triglyceride-lowering occurs in a linear fashion up to 2.5 g a day. The anti-thrombotic effect of EPA/DHA does not become apparent up to 2.5 g a day. Additionally, tissue content of DHA, as measured by the content in erythrocyte membranes, relates to the risk of post-operative atrial fibrillation in a U-shaped fashion. Also, the time needed to achieve these effects differs, ranging from daily dosing for several weeks, to sustained intake for years.

Another important factor to consider is the use of composite primary endpoints to test the efficacy of omega-3 LCPUFA. Composite end-points are frequently used, given the practical challenge of enrolling a sufficient number of participants to achieve sufficient power to detect changes in a primary outcome. However, this approach can obscure the true efficacy of a particular daily dose of omega-3 LCPUFA on one of the aspects that form part of the composite, if at that dose the other endpoints fall outside a dose range where a change in response can be observed.

In addition to the above-mentioned challenges that may obscure the detection of omega-3 LCPUFA intake-induced effects, it is important to note that the body and most tissues already contain omega-3 LCPUFA, therefore studies should consider the background diet and tissue levels of LCPUFA. A premise of a randomized controlled clinical trial for a drug is that the background tissue level of the test substance and its metabolites are absent. Furthermore, placebo treatment should not contribute to the introduction of the test substance. However, it has been shown that background omega-3 LCPUFA intake in partic-

ipants in both control and active treatment groups can be of sufficient magnitude to bring tissue omega-3 LCPUFA levels to the same level as achieved in the active treatment group. In addition, the incorporation of EPA and DHA following dietary or supplemental intake demonstrates a marked inter-individual variability. Thus, summarising the challenges to improve experimental study design for evaluating the relevance of omega-3 LCPUFA intake for human health, there exists an absolute need to measure the tissue status of omega-3 LCPUFA in study participants, at study onset, during the study and at study end. Background intake should be carefully controlled, should ideally be nil (no fish intake or supplements during the study), and should be reported.

Atrial fibrillation is a serious and frequently-occurring complication of cardiac surgery. Post-operative atrial fibrillation is usually addressed by rhythm- or rate-management, which aims to restore a normal sinus rhythm. Relatively short-term pre-operative supplementation with omega-3 LCPUFA prior to scheduled cardiac surgery has been shown to improve surgical outcomes, but the results of studies that aim to evaluate the potential use of omega-3 LCPUFA as a preventive approach to lower atrial fibrillation have been highly variable. In one study, patients scheduled for coronary bypass artery grafting or heart valve replacement and receiving three weeks of EPA/DHA prior to surgical intervention had significantly increased mean levels of omega-3 LCPUFA compared to individuals receiving a placebo not containing omega-3 LCPUFA. The mean length of hospital stay decreased, whereas post-operative atrial fibrillation incidence was not significantly changed. When the omega-3 LCPUFA levels in erythrocytes were looked at in detail, it was found that approximately half of the patients receiving placebo for three weeks had omega-3 levels that were overlapping with the levels of half of the participants of the study group that had received omega-3 LCPUFA. The level of EPA and DHA is ideally measured in atrial tissue, or if samples from this tissue are not accessible, erythrocyte EPA and DHA levels can be determined, given the good correlation between levels of LCPUFA in the two compartments.

In effect, the observations suggested that the two groups were not following expectations - a very substantial section of the participants in the treatment and control groups were not different from each other. Furthermore, participants that had been randomly assigned to the control group, but that reported eating fish, were separated from the control group and were allowed to continue eating fish and take omega-3 supplements. This special observation group had,
after three weeks, omega-3 fatty acid levels that were indistinguishable from the treatment group. This means that participants in both control and treatment groups need to be followed closely during any intervention, and instructed not to ingest any fish or take supplements. Inter-individual omega-3 fatty acid blood and tissue levels vary considerably, due to environmental and genetic effects, and their incorporation into tissues is highly variable following dietary or supplemental intake. As another example, in a study with pregnant women, DHA supplementation from week 16 to 36 of gestation led to a mean increase in erythrocyte membrane DHA, but again a significant number of individuals in the placebo group had as good DHA levels as those in the treatment group.

Without testing omega-3 LCPUFA status and rigorously controlling the background omega-3 LCPUFA dietary intake, many studies have been carried out and others are likely in progress, without any knowledge whether the study groups are in fact different. Following the awareness of these general and omega-3 LCPUFA-specific study design limitations, a post-hoc regression analysis that determines the relationships between tissue levels of omega-3 LCPUFA and outcomes was recently suggested to be a potentially useful approach. However, a formal demonstration that such an approach has any value has not been made.

To test the possibility that clinically-relevant effects may be detectable after stratifying patients based on their omega-3 LCPUFA status, Ip and colleagues recently carried out a pilot study with 20 patients that were scheduled to undergo coronary artery bypass graft (CABG) surgery or heart valve repair or replacement. The research was carried out at the Rheumatology Unit at the Royal Adelaide Hospital, and the Disciplines of Medicine and Public Health, at the University of Adelaide, in Australia. EPA/DHA ingestion and tissue incorporation in this study was measured as the percentage of EPA plus DHA of total fatty acids in erythrocyte membranes (i.e. the omega-3 index). Only patients showing a stable sinus rhythm at pre-admission inspection were included in this study. Patients were randomly assigned to one of two study groups of ten people each. The average age of the study participants was 64 years. The treatment group received three capsules of tuna oil per day during the pre-surgical period, providing a daily intake of 1.1 g EPA and DHA combined. Patients in the placebo group received three capsules containing high-oleic sunflower oil daily. The daily supplementation lasted for an average period of 14.5 and 11.5 days prior to surgery, in the treatment and control groups, respectively.

At surgery, venous blood was collected and used for the analysis of erythrocyte membrane lipids. Biopsies were taken from the right atrial appendage, and used for the determination of the gene and protein expression of several proteins responsive to surgery-induced ischaemia and involved in tissue-protective pathways. The incidence of post-operative atrial fibrillation was determined from electrocardiogram records, and scored as present or absent.

The mean age of the patients in the placebo and fish oil groups was similar, 67 ± 3 and 61 ± 4 years, respectively. No significant differences were reported between the groups with respect to gender (80 and 70% male, respectively), the length of the treatment period up to the day of surgery, and the occurrence of post-surgical atrial fibrillation (50 and 30%, respectively). No differences were observed in the gene expression in the myocardium of the apoptosis-related proteins Bax and Bcl2, or the transcription factor PPARα. There were no differences in protein expression in Bax, Bcl2 or phospho-Akt (the activated form of Akt, a kinase involved in cell proliferation and signal transduction) between the two patient groups. No associations between the level of EPA and DHA in erythrocyte membranes and the duration of treatment or patient age were found.

An extensive overlap in the levels of EPA and DHA in erythrocyte membranes between the patients of the two groups was observed. The authors next examined the associations between EPA and DHA levels and clinical markers in the pooled cohort of control and treated patients. Several associations were evident between the level of EPA, DHA or both fatty acids together, and gene and protein expression of the tested atrial myocardial markers. DHA content in erythrocyte membranes was significantly and negatively correlated with the gene expression of PPARα, and with the ratio of Bcl2 to Bax gene expression.

The finding of significant associations in the entire pooled
patient cohort that could not be distinguished as effects between the randomly generated groups pointed to the need to better stratify the participants of this intervention trial. It has been proposed that robust differences in the clinical outcome of chronic cardiovascular disease-associated illness can be appreciated at a threshold level of >8% EPA plus DHA in erythrocyte membranes. Upon stratification, 44% of fish oil-treated patients were reassigned to the below threshold level, whereas 20% of control patients were located above the threshold. After stratification, 12 patients fell below the threshold, with an average omega-3 index of ~7.1%, whereas the remaining patients above the threshold had an average index of ~8.4%. A significantly lower omega-6 to omega-3 ratio was also observed after stratification. Much smaller differences in average omega-3 index or omega-6 to omega-3 ratios were seen in the randomized groups, indicating that stratification can provide enhanced sensitivity to distinguish groups of patients based on fatty acid levels.

The value of stratification by omega-3 LCPUFA membrane incorporation became evident when the expression of the myocardial surrogate markers for atrial tissue activation and risk of cardiac arrest were re-evaluated. In contrast to the randomized groups, the status of patients above the 8% threshold displayed a significantly lower expression of PPARα. Stratification did not modify the Bcl2 to Bax or p-Akt to total Akt protein expression ratios. Atrial fibrillation incidence was marginally lower in the stratified group with an omega-3 index >8% compared to what was observed in the randomized fish oil-treated group, but any changes in atrial fibrillation incidence in both the randomized and stratified groups were not statistically significant.

This study has provided a clear example that in a randomized placebo-controlled and double-blind intervention study, a large proportion of patients who had received fish oil as a source of omega-3 LCPFA did not have erythrocyte omega-3 levels higher than those in the placebo group. Likewise, one out of five placebo-controlled patients had such a good omega-3 status that they exceeded a >8% EPA plus DHA threshold associated with a low risk cardiovascular disease profile. This study, although small, has shown the effectiveness of randomizing patients to produce clearly distinguishable treatment groups, for which a treatment effect could be tested by measurement of a marker of omega-3 LCPUFA ingestion. The authors indicate that this pilot study did not have enough power to demonstrate that a reduction in post-operative atrial fibrillation may have occurred. Stratification when applied to a larger study population promises to provide a definitive answer to the long-standing question whether increased pre-operative dietary or supplemental intake has an impact on post-operative atrial fibrillation.

The authors also provide suggestions for future studies, notably that regular fish consumption and study entry erythrocyte lipid composition should be documented, in order to make decisions on the design of treatment groups that, for example, have a clearly defined or contrasted LCPUFA background. In addition, participants with a low omega-3 index, or low omega-3 to omega-6 ratio, should ideally be recruited when participants are treated towards a pre-specified target omega-3 index range, for example 8-11%, or they should be treated with multiple doses to gain information on non-linear responses. This study was not carried out on patients that had a very poor omega-3 profile. The average omega-3 index in the placebo group, which would be reflective of background intake (on the premise that patients did not markedly adjust their dietary behavior prior to cardiac surgery), was about 7.3%.

Now that the power of post-hoc stratification on omega-3 index, or on the tissue omega-3 to omega-6 LCPUFA relationship has been demonstrated, future studies with more participants will be interesting to carry out. Such intervention studies are likely to provide clearer answers whether dietary intake or supplementation with omega-3 LCPUFA influence clinical outcomes, for which study approaches involving randomization alone, and without knowledge of LCPUFA status, are clearly limiting and obscuring individual clinical responses.

Worth Noting


A large portion of patients with cancer treated with chemotherapy agents develop resistance to the used drugs, significantly lowering their efficacy over time and allowing the recurrence of tumor growth. Understanding the molecular mechanisms of chemoresistance is of importance as it may allow the development of improved antitumoral interventions that overcome tumor chemoresistance development, increasing the efficacy of cancer treatment. Resistance to chemotherapy can involve different mechanisms that are intrinsic to tumor cells, such as increased repair of drug-induced tumor DNA alterations, and diminished drug uptake and augmented efflux that reduce the level of a chemotherapeutic drug in tumor cells. Extrinsic mechanisms of resistance are those that occur outside malignant cells, such as immune system-mediated survival signals that permit neoplastic growth in the face of chemotherapy, or increased metabolic inactivation of chemotherapeutic drugs by augmented metabolism and excretion from the body. The signal for extrinsic mechanisms of chemoresistance may still originate from the tumor, but precisely how these are initiated is often obscure.

Cis-platin is a frequently employed chemotherapeutic drug that intercalates into the DNA structure and brings replication of dividing tumor cells to a halt. In the course of addressing the extrinsic mechanisms of chemoresistance to cis-platin, researchers at the Netherlands Cancer Institute in Amsterdam, and the University of Utrecht, The Netherlands, made a particular observation a few years ago. Mesenchymal stem cells (MSCs) were found to release two relatively poorly studied fatty acids in response to platinum-based chemotherapeutic drugs used in cancer chemotherapy.

The two PIFA were identified as 12-oxo-heptadecatrienoic acid, and hexadecatetraenoic acid n-3 (16:4 n-3) (Fig 1). The former is a dehydrogenated derivative of 12-hydroxy-heptadecatrienoic acid (12-HHT), an arachidonic acid derivative.
acid-derived lipid mediator formed during the enzymatic formation of thromboxane A₂, a well-known vasoconstricting agent. 16:4 n-3 is a poorly documented omega-3 polyunsaturated fatty acid that is sometimes detected in animal tissues, but at far lower concentrations than the omega-3 PUFA alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Since the initial report in 2011, the researchers have gradually carried out a characterization of the chemoresistance-inducing activity of PIFAs. PIFA did not affect the levels of cis-platin inside tumor cells, but did reduce the genotoxic (DNA-damaging) effect of cis-platin in tumor cells isolated from the mouse model. 16:4 n-3 acted in very low doses as a chemoresistance-inducing compound in mice with implanted tumor cells, but did not appear to be directly responsible for the abrogation of cis-platin-induced reduction of tumor growth. PIFA were shown to activate a specific macrophage population in the red pulp of the spleen, with high F4/80 and low CD11b surface markers, which was necessary for the chemoresistance effect. This observation suggested that MSCs secreted PIFAs in response to cis-platin, which subsequently acted on splenic macrophages to trigger the release of other mediators that would act systemically to allow tumor cells to grow in the presence of platinum-drugs.

The receptor involved in mediating the chemoresistance effect of 12-HHT was confirmed to be BLT2, the type-2 leukotriene B receptor, which is a known low-affinity receptor for 12-HHT. In contrast, the site of recognition for 16:4 n-3 had remained unclear. Interestingly, splenic macrophage activation by PIFAs led to the formation and secretion of several lysophospholipids, detected in the conditioned medium that could induce chemoresistance in the mouse model. The specific lysophospholipid that mediated chemoresistance had remained unidentified. Notably, non-genotoxic cancer drugs such as paclitaxel or sorafenib did not trigger the chemoresistance response, suggesting that PIFAs might be mediating an extrinsic chemoresistance response that is initiated by drugs that are genotoxic and/or cause cell-cycle arrest.

In another separate line of research that has expanded in recent years, it has been established that the omega-3 LCP-UFA docosahexaenoic acid (DHA) activates a membrane receptor called GPR120. Whereas initially linked to macrophages and the mediation of the anti-inflammatory actions of dietary omega-3 LCP-PUFA in mice on a high-fat diet, the involvement of GPR120 is now known in several processes including the regulation of insulin homeostasis, thermogenesis, satiety and bone growth. GPR120 is found on cells in the gastrointestinal tract, adipose tissue, as well as on different immune cell types. The receptor also mediates the anti-inflammatory effects in adipose tissue in response to dietary eicosapentaenoic acid (EPA) in a model of diet-induced obesity in mice. GPR120 was recently found to function in the hypothalamus as well, the brain’s appetite control center, mediating energy homeostasis and reducing hypothalamic inflammation following the intake of a high caloric diet. While GPR120 is being evaluated as a promising drug target, for example in type 2 diabetes, the original notion that GPR120 is a specific receptor for DHA appeared increasingly less tenable, since a variety of unsaturated fatty acids, including alpha-linolenic acid (ALA), have also been found to activate GPR120. Interestingly, all act as agonists in their free fatty acid form, hence GPR120 is also called free fatty acid receptor 4, a member of a family of receptor proteins that sense the levels of various types of free fatty acids in tissues.

In the present study, the same group of researchers that had uncovered the PIFAs tested the possibility that 16:4 n-3 might activate splenic macrophages by binding toWhereas GPR120 was originally identified as a receptor for DHA, several fatty acids are now known to activate this receptor protein. This study confirmed 16:4 n-3 as a new agonist of GPR120. GR120 was found to be expressed on one type of macrophage in the spleen of mice and humans.
GPR120, or to another receptor for free fatty acids, GPR40 (free fatty acid receptor 1). Houthuijzen and colleagues first determined whether 16:4 n-3 could activate these receptors by employing a macrophage cell line in which the expression of these receptors could be induced. 16:4 n-3 activated both receptors, and did so more potently than ALA. Both GPR120 and GPR40 were confirmed to be expressed nearly exclusively by the F4/80 high/CD11b low macrophage cell type in the spleen.

In a functional assay, the intravenous administration of the cell culture medium of mouse spleen cells stimulated with 16:4 n-3 produced chemoresistance to cis-platin in the murine tumor model. Furthermore, synthetic agonists of GPR120 and GPR40 were able to do this, indicating that both GPR120 and GPR40 could be involved in tumor cell growth and the overcoming of the inhibition of tumor growth in mice induced by cis-platin. The chemoresistance effect initiated by exposure of macrophages to 16:4 n-3 could, however, only be inhibited by an antagonist of GPR120, but not by blocking GPR40. Additionally, the chemoresistance imparted by 16:4 n-3 was confirmed using mice that had the GPR120 receptor genetically “knocked-out.” The results strongly suggest that some factor that renders tumor cells resistant to the antineoplastic activity of cis-platin can be secreted by spleen cells upon stimulation by 16:4 n-3 through the activation of GPR120.

The researchers had already determined that 16:4 n-3 was able to trigger the formation of several lysophospholipid species by spleen macrophages. The production of lysophospholipids requires the involvement of a phospholipase, an enzyme that cleaves one of the two fatty acid moieties from a phospholipid. In the present study, the involvement of a cytosolic phospholipase A₂ (cPLA₂) in the chemoresistance response to 16:4 n-3 was confirmed. Furthermore, among three lysophospholipids found in the conditioned medium of spleen cells exposed to 16:4 n-3, only one was reduced by an inhibitor of this cPLA₂. This lysophospholipid, nervonoyl-lysophosphatidylcholine (LPC(24:1)) is rather unusual because it contains nervonic acid (C24:1), a long-chain monounsaturated fatty acid that is present in tissues at relatively low levels, with very little known about its physiological function. The treatment of tumor-bearing mice that had their spleens removed with LPC(24:1) did induce chemoresistance to cis-platin. A structurally similar lysophospholipid, LPC(24:0) did not have this effect, suggesting a tight structural requirement to induce resistance of tumors to platinum-based chemotherapy.

The study next made an effort to translate the fundamental insight obtained in mice to the human situation, by confirming that human spleens contain a very similar immunologically-defined macrophage population that also expresses GPR120. Initiating the treatment of cancer patients with platinum-containing chemotherapeutics increased the levels of LPC(24:1) by about 25% in plasma four hours after treatment onset. This was not observed in patients placed on treatment with non-platinum-based chemotherapeutics. Although the increase was not statistically significant, the preliminary results are concordant with the possibility that a chemoresistance response to cis-platin in humans may be mediated by the formation of LPC(24:1) in the spleen.

This study provides remarkable new insight in the mechanism of extrinsic tumor chemoresistance to the widely used chemotherapeutic substance cis-platin. The loss of sensitivity of tumors to cis-platin appears complex, involving several cell types and organs mediating a cascade of events at a systemic level, and involves “new” lipids that had not been implicated previously in other biological functions. Future research will need to confirm these findings, and further es-
The relative impact of dietary 16:4 n-3 as a minor fat remains to be studied. A physiological response to genotoxic substance exposure, stem cells in other organs also employ PIFA production as pal sites of 16:4 n-3 production, or normal mesenchymal enchymal cell-like properties within tumors are the princi- 
resistant cancer cells. Whether cancer stem cells with mes-
plasticity, allowing dedifferentiation into stem cells with invasive and self-renewing properties, and redifferen-
tiation into more differentiated cells. This has also offered a new interpretation of cancer development, incorporating the concept of clonal expansion of mutant stem cells. The dedifferentiation into cancer stem cells, also called the epithelial-mesenchymal transition, has recently been doc-
tumented to have particular importance in the metastasis (dissemination of tumor cells to other organs) of chemotherapy-resistant cancer cells. Whether cancer stem cells with mes-
mesenchymal cell-like properties within tumors are the principal sites of 16:4 n-3 production, or normal mesenchymal stem cells in other organs also employ PIFA production as a physiological response to genotoxic substance exposure, remains to be studied.

The relative impact of dietary 16:4 n-3 as a minor fatty acid affecting susceptibility to chemotherapy has not been systematically addressed. This fatty acid is biosynthesized by green microalgae for algal galactolipid formation, and all animals in the food chain that rely on preformed marine algae-derived PUFA in their diets are expected to contain low levels of 16:4 n-3 in their tissues. The description of 16:4 n-3 as a mediator that can act at a distinct site of action in its free fatty acid form to activate splenic macrophages further contributes to the idea that PUFA have signalling functions as free fatty acids, as was initially suggested for DHA acting on GPR120 to regulate macrophage inflammatory phenotype. The relative contribution of 16:4 n-3 to endogenous GPR120 activation compared with other much more abundant omega-3 LCP-PUFA, such as EPA and DHA, has however not been estab-
ished. The pharmacology of Free Fatty Acid receptors is complex: not all ligands act as full active-site agonists, and the agonist selectivity of GPR120 is permissive with several PUFA of both the omega-3 and the omega-6 fam-
iliaries being described to function as agonists at the receptor, including the relatively abundant fatty acid linoleic acid. A recent study has also cast doubt on GPR120 being uniquely involved in mediating the anti-inflammatory ef-
effect of dietary DHA, in the context of a high fat intake. Furthermore, dietary DHA has recently been found to increase the expression of GPR120 in mice. With the discovery of 16:4 n-3 as a ligand for GPR120, new research on the importance of this hitherto overlooked omega-3 PUFA of low abundance will gather interest. The selective involvement of 16:4 n-3 as a mediator of a potentially protective response of stem cells to genotoxic agents will be interesting to explore further, and this may lead to improvements in cancer treatment.

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Worth Noting

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Galluzzi L, Vitale I, Michels J, Brenner C, Szabadkai G, 


The global obesity epidemic is still getting worse in most parts of the world, as highlighted in recent reports. Addressing the obesity problem is important because being overweight is associated with increased mortality from a range of metabolic disorders, but on the other hand it potentially can be addressed. Obesity-associated disease is not uniform across populations, stimulating interest in understanding how weight gain prevention and promotion of weight loss can be best addressed. An unresolved issue is whether chronic excessive intake of easily available carbohydrates promotes obesity prevalence. Similarly, it is important to know if lowering the intake of carbohydrates in overweight individuals can contribute to weight loss. Further, with carbohydrates being important macronutrients, would lowering their intake in overweight people have any unintended effects?

Hyperglycemia and hyperinsulinemia following chronically-elevated dietary intake of carbohydrates are responsible for increased fat storage and insulin resistance due to accelerated oxidation of circulating glucose to reactive advanced glycation end-products in vascular tissues. The inflammation of metabolically-active tissues has also been implicated in dysregulated metabolic responses to elevated carbohydrate intake. In contrast with these hypotheses, however, are views that indicate that these mechanisms are not of sufficient magnitude or duration to modify the processes the body uses to handle excess energy intake or sugars specifically. Furthermore, much of the evidence linking carbohydrate intake and weight gain is based on observational and cross-sectional studies, whereas intervention studies that could provide a more definite answer have been small in sample size, of too short duration, or did not control for potential confounders. Although intuitively a simple relationship, at present it is still a leap to conclude that a diet with a low glycemic load can prevent weight gain or significantly contribute to weight loss. Several methodological limitations need to be overcome to allow drawing solid conclusions on the relationship between carbohydrate intake, glycemic index and load, and weight gain or loss.

For a start, prospective studies need to adequately monitor food intake. Food records are preferred over the use of food-frequency questionnaires that rely on people’s (generally poor) memories of past dietary habits, and cognitive ability. Food records also allow a better recording of the
specific details on how food was prepared, which affects the glycemic index and load of food items rich in carbohydrates. However, maintaining a food record by study participants carries a risk that dietary habits may change, generally towards currently fashionable dietary recommendations. Furthermore, measurement error should be eliminated through professional supervision guiding participants on how to fill out their records. Ideally, multiple dietary records should be taken that span a sufficiently long period and cover the different seasons in studies lasting more than a year.

A well-recognized limitation of observational studies in evaluating the association of individual food nutrients with outcomes is the need to control for many other variables that co-vary with the nutrient of interest. However, randomized controlled studies may also suffer from this limitation if the assigned diets vary in dietary components that are not being controlled. For example, a diet aiming to evaluate low-glycemic index carbohydrates may have exchanged one food component with another containing less fiber or with a different amount of protein. All macronutrient classes, as well as micronutrients, should ideally be controlled in order to draw meaningful conclusions.

Observational studies are generally limited to establishing associations. These are not causal to the endpoint, and sometimes reversed causation is at play where the outcome affects the intake of the nutrient being investigated. For example, in an observational trial that lasted 6.5 years, an increase in pancreatic cancer risk associated with high available carbohydrate and low fat intake was thought to reflect the dietary changes that are associated with subclinical disease.

Controlled intervention studies are preferred when intending to dissect the relationship between carbohydrate intake and body weight changes, but these need to be of sufficient duration. Carrying out a study with sufficient study duration, incorporating the necessary measures to reduce confounding factors while maintaining participant compliance, however, constitutes a significantly greater logistical endeavour than the mostly prospective observational trials and cross-sectional studies that have been employed to date.

In 2014, the results of the GLYNDIET trial were published. This randomized controlled dietary intervention trial lasted six months and had aimed to systematically address these major limitations. The study was carried out by researchers at the Human Nutrition Unit, Faculty of Medicine and Health Sciences, Institute of Health Pere Virgili at the University of Rovira i Virgili, and the University Hospital Sant Joan, in Reus, Spain, in collaboration with the Instituto de Salud Carlos III, Madrid, Spain. Participants were assigned to one of three groups, all receiving an isocaloric energy-restricted diet with the same content of fiber, but distinguished by the diets either containing carbohydrates with a low-glycemic index (n=41 participants), carbohydrates with a high-glycemic index (n=41), or a reduced fat content (n=40), respectively (Table 1). The study participants were non-diabetic overweight-obese adults (body mass index (BMI) of 27 to 35 kg/m2) between 30 and 60 years old. The applied energy restriction was 500 kcal of total dietary energy intake for all participants, with the total daily energy intake adjusted for body weight according to four groups of body weight ranges.

The groups were balanced for gender (~24% women) and age (mean age of ~45 yrs). No differences in mean values of baseline clinical characteristics were apparent between groups, including average fasting-plasma glucose levels of ~100 mg/dl, indicating that participants were normoglycemic. There was a small difference in energy from protein intake (18.8%, 18.4% and 17.0%, respectively).
Adherence to the diets was executed by registered dietitians providing specific recommendations, in particular on the type of carbohydrates and cooking methods used. Biweekly menus and recipes were provided to promote adherence. Energy and nutrient intake was calculated using a food composition table for Spain. Several participants did not complete the study, with 36, 37, and 31 persons in the above-mentioned study groups finishing the study, respectively.

The results of the GLYNDIET study showed that the decrease in BMI in participants following the low-glycemic index diet was greater than with the low-fat diet (2.5 vs 1.5 kg/m$^2$ decrease in BMI, respectively). The decrease in BMI in people receiving a high-glycemic index diet was borderline significant (P=0.061) compared to the low-fat diet, and comparable to that achieved with the low-glycemic index diet. Decreases in insulin resistance and improvements in beta-cell function were significantly greater in the low-glycemic index diet than the other two diets. Participants on the low-glycemic index diet also reported feeling less hungry when challenged to a breakfast test compared to those on the high-glycemic index diet.

With the three diets achieving weight loss, and the low-glycemic index diet having a more favorable effect on glucose regulation, one aspect that had not been addressed was if the distinct differences in macronutrients would affect other metabolic aspects of weight loss. While the effect of dietary fat quality on fatty acid composition of tissue membranes has been studied extensively, the effect of foods with different carbohydrate qualities is virtually undocumented.

In the present study, Giardina and colleagues report on the effects of the three energy-restricted diets with different macronutrient compositions studied in the GLYNDIET trial on fatty acid composition. The analysis was carried out by the original GLYNDIET researchers together with colleagues from the Lipid Clinic at the Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer, in Barcelona. Blood samples had been taken after an overnight fast at the start of the study and at the end of the 6-month intervention, and washed red blood cells (RBCs) prepared from EDTA plasma had been collected. Fatty acid profiles were determined by gas chromatography-flame ionization detection of fatty acid-methyl esters, following trans-methylation of membranes prepared from lysed RBCs. No information was provided on how long after completion of the GLYNDIET trial the RBC fatty acid profiles were determined, or on how samples were stored.

Results on fatty acid levels in RBCs were available from 30, 31 and 26 participants of the high-glycemic index, low-glycemic index, and low-fat diet groups, respectively. These study participants showed similar characteristics to those of the overall study cohort. In the participants for which fatty acid results were available, the low-glycemic and high-glycemic index diets promoted greater weight loss than the low-fat diet (7.4, 7.2 and 4.4 kg, respectively). Participants in the low-glycemic index group displayed a significantly higher dietary intake of polyunsaturated fatty acids (~30% increase) over the study period. The assessment of the dietary intakes of individual PUFA species revealed no significant changes in intakes of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha-linolenic acid (ALA), total omega-3 PUFA or total omega-6 PUFA between groups. Higher baseline intake of EPA and DHA in the low-fat diet group suggested that groups were not

<table>
<thead>
<tr>
<th>Table 1. Macronutrient composition of the tested diets</th>
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<tr>
<td><strong>Low Glycemic Index diet</strong></td>
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<tr>
<td>Energy from protein (as % of kcal)</td>
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<td>Energy from carbohydrates (as % of kcal)</td>
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<td>Energy from total fat (% of kcal)</td>
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<td>Glycemic index</td>
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(* may also be called “medium”-fat diet, as other studies consider 20% total fat a low-fat diet)

This is the first randomized controlled intervention study to explore the effect of dietary carbohydrate types on fatty acid composition, of interest to explore if different energy-restricted diets may have undesirable effects on the endogenous levels of polyunsaturated fatty acid.
completely matched at baseline, or that the precise dietary intake of individual fatty acids was challenging to estimate from food records. Fiber intake was significantly decreased in the low-glycemic index group.

Objective values of LCPUFA resulting from longer-term dietary intake can be obtained from measuring their levels in RBC membranes. After six months, significant decreases in EPA, DHA and total omega-3 LCPUFA level in RBC membranes (20%, 8.2% and 8.0%, respectively), as well as in the omega-3 index (0.5% point decrease from a baseline omega-3 index of 6.9%), were observed in the participants of the low-fat diet group. These changes were not observed in the high- and low-glycemic index groups, except a 13% decrease in EPA level in the high-glycemic index group. In the low-fat diet group, the levels of the omega-6 LCPUFA adrenic acid (C22:4 n-6) and docosapentaenoic acid (C22:5 n-6) increased over the study period (6.7% and 20%, respectively), a change not seen in the other study groups. Reductions in ALA levels were observed in all groups, although the baseline values were low.

The present study is among the first to indicate that energy restriction on a low-glycemic index diet does not affect LCPUFA status (at the achieved level of weight loss in the study). Although weight-loss can be achieved employing diverse diets with different macronutrient composition, a reduction in total dietary fat content had a significantly more marked effect on endogenous LCPUFA status than modifying the carbohydrate quality of the diet. The results of this study may contribute to a optimization of the weight-loss approach which is to be used for an overweight person. Recent studies have provided marked new insight that no single weight loss approach fits every individual, with fasting glycaemia and insulin sensitivity predicting an individual’s potential for weight loss or gain for different types of diets. Normoglycemic individuals appear to benefit more from an energy-restricted low-fat high-carbohydrate diet for losing weight, whereas pre-diabetic and diabetic individuals will lose weight on diets characterized by a high-fat low-carbohydrate diet or focusing on carbohydrate intakes with a low glycemic index, high fiber content and whole grain consumption. Maintaining a reduced bodyweight, once achieved, also depends on glycemic load, with even further weight loss possible in prediabetic people with a low glycemic load maintenance diet. Maintenance of the achieved lower body weight is sustainable in normoglycemic persons using this approach. The results of the present study also suggest that overweight-obese individuals who do not yet show signs of diabetes and who aim to lose weight through a reduced fat intake, should consider additional intake of omega-3 LCPUFA in order to maintain sufficient dietary levels of these fatty acids. On the other hand, the study indicates that fiber intake may also need to be corrected in individuals losing weight on a low-glycemic index diet.

A limitation of the current study is that the results are restricted to the population in which these were obtained, namely in Spain. The average omega-3 index is high, with one study reporting a value of 9.1% in the Spanish population. EPA+DHA intake clearly contributes to high RBC levels of EPA and DHA in this population, but only partly explains why the levels are relatively high, and other (unknown) environmental and dietary factors are likely contributing as well. The omega-3 index of the GLYNDIET participants was lower (~6.8%) and did not change during the 6-month weight loss intervention, except on the background of a low-fat diet, when the omega-3 LCPUFA status decreased. Further attention needs to be paid to the observation that ALA levels decreased in all tested weight loss protocols. The latter may relate to the fact that the Spanish population has a relatively high vegetable intake, and a lower energy intake overall may lead to reduced ALA-intake.
A further limitation of this study relates to the estimation of fatty acid intake based on 3-day food records, which may significantly underestimate or skew the estimated intake of specific fatty acids that are infrequently consumed. Future studies that employ longer food records may possibly provide more reliable dietary intake estimates for specific fatty acids. It needs to be acknowledged that it is essentially impossible to alter any aspect of the diet without affecting something else, including the composition of fatty acids in the diet. Whereas olive oil was the main reported source of dietary fat in the low- and high-glycemic index diets, dairy was a main source of fat in the low fat diet group. Therefore, it remains a possibility that the changes in endogenous FA profile associated with the low fat diet could have been due to changes in the composition of ingested fatty acids, and which were not detected by diet records due to their lack of sensitivity.

Weight loss itself has been reported to be associated with changes in RBC fatty acid composition. In a small study, weight loss following subtotal gastrectomy markedly improved DHA tissue levels and omega-3 LCPUFA biosynthesis capacity of obese patients. A strong influence of body weight on omega-3 LCPUFA status following supplementation has also been recently uncovered in young people. The current results indicate that body weight reduction by itself was, however, not the main driver of changes in fatty acid composition, but rather that weight loss achieved by a low-fat diet may be associated with reductions in omega-3 LCPUFA, whereas a change in the type of carbohydrates during weight loss did not have such marked effects on LCPUFA status in non-diabetic overweight-obese adults.

This study has provided a useful insight into the effect of different macronutrient compositions that can be provided during a weight loss schedule. Further studies that reduce confounding may improve the already available approaches for achieving weight loss in individuals and different medical conditions, by minimizing the potential adverse effects on essential nutrient intake and their endogenous levels.


**Worth Noting**

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Juanola-Falgarona M, Ibarrola-Jurado N, Salas-Salvadó


Cardiac societies, like the American Heart Association (AHA) or the European Society of Cardiology (ESC), periodically publish guidelines, among them on primary or secondary prevention of cardiovascular disease (AHA) or cardiovascular prevention (ESC) (1,2). In addition, AHA also sometimes publishes “scientific statements” or “science advisories” on certain topics, of which a recent one dealt with supplements containing the two omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (3). In all these documents, the same grading systems are used: one for the level of evidence by which the recommendations are supported, and another for the classes of recommendation (Table 1). While the grading of the level of evidence is straightforward, transparent, and puts opinion last, it is surprising how important opinions are in the classes of recommendation: as important as evidence. The opinions of guideline authors are formed by a multitude of factors, most of them intransparent and uncontrollable. As illustrated in Table 1, another important parameter for the class of recommendation is the balance of risk vs. benefit.

In the recent science advisory of the AHA on omega-3 supplements, EPA+DHA are recommended for “Secondary Prevention of Outcomes in Patients with Heart Failure” with a Class IIa recommendation, which is in keeping with current evidence: one large trial with clinical endpoints had a positive result, fitting the positive evidence from other pertinent studies on EPA+DHA in congestive heart failure (3,4,5). In contrast, and incomprehensibly, the current AHA guidelines on congestive heart failure do not mention EPA+DHA, while the current pertinent ESC guidelines recommended EPA+DHA with a level of evidence B, and a Class IIb recommendation (6,7). In the AHA science advisory, “the majority of co-authors concluded that treatment with EPA and DHA supplements is reasonable for the secondary prevention of CHD death (Class IIa Recommendation); a minority of coauthors preferred a slightly lower strength of recommendation for treatment of patients with this indication (Class IIb Recommendation)”(3). For patients at high risk for cardiovascular disease, a minority of co-authors suggested a Class IIb Recommendation, while the majority concluded that EPA+DHA are not indicated (Class III Recommendation). Of note, some of the authors of the AHA science advisory have a track record in the field of omega-3 fatty acids, while this was not true for any of the authors of the guidelines mentioned. In the current AHA guidelines on secondary prevention of cardiovascular disease, omega-3s are not thought to be effective (1). The triglyceride-lowering effect of EPA+DHA is mentioned in passing in the science advisory, and not in the secondary prevention guidelines, while the ESC prevention guidelines think that EPA+DHA are effective (1,2,3).

In the recent cardiovascular prevention guidelines of the ESC, it is recommended to eat “Fish 1–2 times a week, one of which to be oily fish,” and “The protective effect of fish on CVD is attributed to the n-3 fatty acid content.” It is also noted that “A recent meta-analysis of 20 trials, mostly prevention of recurrent CV events and mostly using fish oil supplements, showed no benefit of fish oil supplementation on CV outcomes.” (2). Twice, the recommendation contradicts itself: if the protective effect of fish is the omega-3 fatty acids, why recommend non-oily fish? If there is no effect of omega-3 supplements, why recommend oily fish? The recommendation is not qualified according to the system in Table 1, and incomprehensible (2).

How can it be that guidelines are not unanimous, given that they are written based on the same evidence?

As illustrated by Table 1, authors of guidelines weigh risk against benefit. What is the risk of EPA+DHA? In the large cardiovascular trials, with >70,000 patients participating for 2–7 years, adverse effects and tolerability of EPA+DHA were almost identical to placebo (8,9). Aside from mild gastro-intestinal disturbances and very rare cases of allergies
against capsules or their content, the main risk perceived for EPA+DHA is the risk of bleeding. However, in the large cardiovascular trials, with almost all participants on platelet inhibitors, some even on dual platelet inhibition, the rate of bleeding in the verum groups was identical to the rates of bleeding in the placebo groups (8,9). We recently published three year data from a cohort of 826 patients, all anticoagulated with phenprocoumon, and reported that higher levels of EPA+DHA in whole blood were associated with a lower mortality, but not with a higher risk for bleeding than lower levels (10). Earlier, it had been demonstrated that the risk of bleeding during a myocardial infarction, a situation treated with a combination of bleeding-inducing drugs, did not depend on the levels of EPA+DHA in erythrocytes, measured as the HS-Omega-3 Index (11). Moreover, the European Food Safety Authority (EFSA) considers a daily dose of up to 5 g EPA+DHA to be safe, while its US-American counterpart Food and Drug Administration considers up to 3 g EPA+DHA/day to be safe (12). All this puts case reports of bleeding associated with EPA+DHA into perspective (e.g. 13). In other words: according to the regulatory authorities, and based on current systematic clinical evidence, at up to 3-5 g EPA+DHA daily, there is no risk to weigh the benefit against, questioning the process of weighing risk against benefit in the case of EPA+DHA, and questioning the classification system in Table 1 for EPA+DHA.

In contrast to most nutritional approaches to treatment or prevention of cardiac disease, large trials with omega-3 fatty acids provide evidence on clinical endpoints, like death, myocardial infarction or rehospitalizations (8). As discussed in more detail elsewhere, in cardiovascular disease, neutral results of many large trials with EPA+DHA on clinical endpoints were mostly due to issues in trial methodology and bioavailability that became apparent by measuring levels of EPA+DHA (12,14). A meta-analysis including only trials not affected by these issues, either by using EPA+DHA in a bioavailable manner (e.g. fish in DART, ref. 15), or using a high dose of EPA+DHA (16), or by recruiting study participants with low baseline values for EPA+DHA (e.g. congestive heart failure, 17,18, own unpublished data), demonstrates the effectiveness of EPA+DHA in reducing total mortality and non-fatal cardiovascular events. In contrast, evidence for other nutritional approaches in cardiovascular prevention, e.g. salt restriction for high blood pressure, is often not based on clinical endpoints from pertinent trials, but rather on surrogate endpoints, like blood pressure (19). Evidence provided for fruit and vegetables is largely observational, and not from intervention trials (20). Taken together, for EPA+DHA, evidence is provided in a form that cardiologists consider the hardest: intervention trials with clinical endpoints, and not only from trials with surrogate endpoints or observational data unable to establish causality.
At face value, however, the results of large trials with EPA+DHA on clinical endpoints are inconsistent or neutral, except in congestive heart failure (e.g. 8). In contrast, almost all trials on surrogate endpoints, like triglycerides, heart rate, heart rate variability or blood pressure, or intermediate endpoints, like progression of coronary lesions, have had consistently positive results (12). Especially in cardiology, guideline authors tend to disregard results from epidemiologic and mechanistic studies, or trials on surrogate and intermediate endpoints, as soon as results from trials with clinical endpoints are available. This is at odds with the true scientific approach of weighing all evidence before reaching a conclusion.

Admittedly, matters are complicated for guideline authors. There is a large inter-individual variability in uptake of EPA+DHA, and there are large differences in bioavailability between various preparations of EPA+DHA: e.g. bioavailability of EPA+DHA emulsified vs. non-emulsified can differ by a factor of 20 (21-24). These differences in bioavailability are not explained by differences in the chemical form of EPA+DHA (except for the free fatty acid form) (23,24). Moreover, intake of EPA+DHA with or without a fatty meal has a huge impact on bioavailability (25). These issues have only recently become apparent by measuring levels of EPA+DHA, specifically in erythrocytes by use of the standardized method “HS-Omega-3 Index® (12,21-24).

Using a standardized method makes comparisons across all sorts of study designs possible, and therefore advances the field substantially. More importantly, this approach makes the clinical use of EPA+DHA much more targeted and thus more promising. Taken together, these developments are likely to change the field of EPA+DHA from a dose-driven field to a level-driven field. Although mentioned in passing in the AHA science advisory, it will take some time, however, before these developments are reflected in guidelines (3).

The guidelines of the cardiac societies mentioned completely and systematically ignore any extracardiac benefits of EPA+DHA, e.g. on vascular function and blood pressure, on prevention and treatment of psychiatric disorders like major depression, or on arthritic pain, to name a few (26 - 29). This is in striking contrast and to the detriment of cardiac patients that consist clearly not only of a heart, but also of other organs like brain and joints. A typical example is congestive heart failure, not only characterized by low levels of EPA+DHA, but also characterized by co-morbidities like major depression or impaired cognition (7). Patients with the latter conditions have been demonstrated to benefit from EPA+DHA in pertinent meta-analyses (26-28). Moreover, EPA+DHA improve quality of life in patients with congestive heart failure (30). Since mean levels of EPA+DHA are higher in cardiovascular disease than in congestive heart failure, things are similar, but in a less pronounced manner (12,18,31, own unpublished observations).

As discussed in more detail elsewhere in relation to trial design (12,14), but in a similar logic: guideline committees, and, by extension, patients with cardiac disease, would benefit from guideline committee members with expertise in the field of omega-3 fatty acids. Moreover, limitations of guidelines, like the ones discussed here, need to be weighed critically, disclosed, and discussed openly for the benefit of cardiac patients. The AHA scientific advisory on omega-3 supplements is an example for a step in this direction (3).

Taken together, current guidelines or scientific advisories from cardiac societies systematically underestimate the value of EPA+DHA:

- the system for classes of recommendations is inadequate for EPA+DHA,
- the safety of EPA+DHA is ignored
- neutral results of many large trials are incorporated into meta-analyses, usually an important basis of guidelines, although issues in trial methodology invalidate but a few of these trials
- known positive effects of EPA+DHA on known comorbidities of patients with cardiac conditions are ignored.

In future guidelines, chapters on safe interventions are needed. Topics to be discussed in such a chapter would be the benefits and relevance of EPA+DHA, vitamin D, smoking cessation, and other safe approaches towards reducing mortality and morbidity. Clearly, the classes of recommendation listed in Table 1 do not apply. Experts from the respective fields are needed to contribute their expertise.

References


15. Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC, Deadman NM. Effects of changes in fat, fish, and fibre intakes on death and myocard-


