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Journal of Lipid Research
2016 SEP ; 57(9): 1608-1611
Doi: 10.1194/jlr.C071084

Publisher: American Society for Biochemistry and Molecular Biology

commentary

EPA and/or DHA? A test question on the principles and opportunities in utilizing the therapeutic potential of omega-3 fatty acids[1]

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Decades of research and certainly more than 20,000 papers have been dedicated to the health benefits of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs). We have learned that n-3 LC-PUFAs modulate multiple molecular processes and exert pleiotropic beneficial effects that, depending on the pathophysiological context, may range from anti-inflammation and triglyceride-lowering to cardioprotection and anti-arrhythmia, or even improved cognitive function (1, 2). However, a challenging question remains about how molecular events translate into physiological responses and finally to desired health benefits. Currently, there is also the issue that a series of recent clinical trials showed insufficient evidence for the therapeutic efficacy of n-3 LC-PUFA supplements. Taking the otherwise overwhelming epidemiological, preclinical, and mechanistic evidence for granted, we are forced to critically analyze what we know about the principles, opportunities, and limitations of n-3 LC-PUFA actions. This is an ongoing process that will have consequences for designing appropriate future clinical trials, stimulating research on important open questions, and improving our understanding of the therapeutic potential of n-3 LC-PUFA supplements.

In this commentary, only two general topics will be discussed; namely, the features of n-3 LC-PUFA supplements that differentiate them from "normal drugs" and the role of n-3 LC-PUFAs as precursors of novel lipid mediators with highly potent cardiovascular actions. This is to provide some background for the specific "EPA and/or DHA" question, and how this question has been addressed by the recent study of McManus et al. (3) published in this issue of the *Journal of Lipid Research* that deals with the differential effects of EPA vs. DHA on postprandial vascular function and the plasma oxylipin profile in men.

n-3 LC-PUFA supplements. The n-3 LC-PUFA supplements typically used in clinical trials and already prescribed for triglyceride-lowering or cardiac protection contain EPA (eicosapentaenoic acid; C20:5 n-3) and DHA (docosahexaenoic acid; C22:6 n-3), in a ratio of approximately 3:2. Whereas drugs can be given or not given to a patient, n-3 LC-PUFAs are essential constituents of the body. At least some basic, but frequently suboptimal, supply is secured via the intake of vegetables providing the respective C18 precursor (α-linolenic acid; C18:3 n-3) or directly through EPA- and DHA- containing marine food components. Similar considerations apply to arachidonic acid (AA; C20:4 n-6), the physiologically most important n-6 LC-PUFA. Meat and dairy products deliver AA directly. Moreover, the respective C18 precursor (linoleic acid, LA; C18:2 n-6) is supplied in overly large amounts in the vegetable oils and cereal products. Beyond the nutrition that determines the n-3/n-6 balance, there are gene-diet interactions, hormonal and epigenetic regulation, as well as disease state-related factors that modulate an individual's capacity to synthesize n-3 and n-6 LC-PUFAs from their C18 precursors. Intriguingly, functional genetic polymorphisms in this pathway were probably subject to "positive selection" during human evolution in adaptation to specific nutritional environments (4). The current distribution of the corresponding genetic variants is different in different populations, and excitingly, also when comparing, for example, coronary artery disease patients with healthy controls (5). In consequence, all these factors contribute to the fact that patients enrolled to receive EPA/DHA-supplementation will exhibit individually different baseline levels of n-3 vs. n-6 LC-PUFAs. Similarly important, these factors might also modulate the effect size of EPA/DHA-supplementation with regard to the achieved changes in endogenous fatty acid profiles. Furthermore, the time course of n-3 LC-PUFA induced changes merits indication-specific attention. Unlike the acute postprandial increases of plasma EPA- and DHA-levels to be discussed below, the incorporation and wash-out kinetics of EPA and DHA proceeds on a time scale ranging from days (plasma phospholipids in fasting state) to weeks (red blood cells reflecting also cardiac tissue) to months in adipose tissues (6). Unfortunately, data on intervention-induced changes in fatty

DOI 10.1194/jlr.C071084

[1]See referenced article, *J. Lipid Res.* 2016, 57: 1720–1727.

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acid profiles exist for some but not all clinical trials, including the GISSI-P study (7) that largely established our belief in the benefits of n-3 LC-PUFAs. Without recognizing and overcoming these obvious confounders, it will remain difficult to define the target EPA/DHA-levels and treatment times for desired therapeutic effects and also to understand why there are such large discrepancies in the outcomes of different human studies with n-3 LC-PUFAs. Moreover, we need to learn more about the genetic polymorphisms in PUFA metabolism that potentially predispose to cardiovascular disease in a diet-dependent manner and may also modulate individual responses to n-3LC-PUFA supplementation.

n-3 LC-PUFAs as precursors of novel lipid mediators. The other principle to be considered stems from experimental evidence indicating that many of the beneficial cardiovascular effects attributed to n-3 LC-PUFAs are associated with their precursor roles in lipid mediator formation. This notion is currently facing a revival and is still expanded in ongoing experiments. Classical work suggested that EPA competes with AA for the conversion by cyclooxygenases (COXs) and lipoxygenases (LOXs), resulting in the formation of less potent pro-inflammatory eicosanoids (8, 9). This concept has been further refined for the COX and LOX pathways (10, 11) and extended by identification of EPA- and DHA-derived lipid mediators with powerful biological activities. These most recently recognized lipid mediators include the "specialized pro-resolving metabolites" (SPMs: resolvins, protectins, and maresins) (12) and the "omega-3 epoxyeicosanoids" generated by cytochrome P450 (CYP) enzymes (13). In line with the substrate- and regioselectivity of the CYP enzymes, 17,18-epoxyeicosatetraenoic acid (17,18-EEQ alias 17,18-EpETE) becomes the predominant endogenous epoxyeicosanoid after EPA/DHA supplementation both in rodents and man (14). Less is known about the diet-dependent formation of SPMs because analytical methods have been reported by only a few laboratories (15). 17,18-EEQ appears to function as a highly potent mediator of cardioprotective and antiarrhythmic effects of EPA (13). As demonstrated in in vitro and animal studies, 17,18-EEQ also displays vasodilatory, anti-inflammatory, and anti-allergic properties that contribute to the beneficial effects of n-3 LC-PUFAs in diverse disease states ranging from bronchial disorders (16), fatty liver disease (17), and intraocular neovascularization (18) to allergic intestinal inflammation (19). The DHA-derived 19,20-epoxydocosapentaenoic acid (19,20-EDP alias 19,20-EpDPE) shares several of these properties but has attracted the most attention due to its capacity to inhibit tumor angiogenesis and proliferation (20). The much longer known AA-derived omega-6 epoxyeicosanoids have in part similar or less potent and even opposite effects compared with their omega-3 counterparts (21, 22). A limitation for biological activities of the CYP epoxygenase products is their rapid metabolization to less active diols by soluble epoxide hydrolase (sEH), an enzyme known to be increased in several disease states (23). Based on these preclinical studies, much recent research, including the study presented by McManus et al., is aimed at understanding the relevance of the CYP epoxygenase/sEH-pathway in humans. LC-MS/MS-based profiling of both the classical and novel LC-PUFA-derived lipid mediators has been developed in several laboratories. This lipid mediator profiling may yield biomarkers that are closely linked to the cardiovascular actions of n-3 and n-6 LC-PUFAs. It also provides novel opportunities for recognizing genetic as well as disease state- and comedication-related factors potentially limiting the response to EPA/DHA-supplementation, as these could modulate and reduce the formation of specific EPA/DHA-derived bioactive lipid mediators.

The "EPA and/or DHA" question. One of the apparently simple but truly difficult questions to answer in this research field concerns differences in actions between EPA and DHA. Summarizing all the mechanism-, cell-type-, risk marker-, and indication-specific findings, the best general answer currently possible seems to be the following: there are shared and differential as well as complementary and synergistic effects of EPA and DHA (24, 25). This question is also related to current attempts of tailoring the EPA:DHA-ratio to the treatment needs of specific indications. Hopefully, we will see soon whether or not these attempts will be successful to produce reproducible clinical effects and thus evidence-based recommendations.

In the current issue of the *Journal of Lipid Research*, McManus et al. make a rather good case for addressing the "EPA and/or DHA question" in a specific physiological condition; namely, the postprandial state and its effect on vascular function and arterial stiffness. Arterial stiffness was characterized primarily by measuring the "Pulse Wave Velocity and Augmentation Index" (AIx). The interventions included control as well as EPA- and DHA-rich test meals. The resulting changes in vascular function and plasma fatty acid profiles were analyzed by comparing the corresponding values before and 4 h after giving the test meals. Moreover, the authors quantified several oxylipins, in particular, the omega-3 epoxyeicosanoids discussed above, as well as nitrite and hydrogen sulfide, as mediators and biomarkers of the mechanisms potentially contributing to the EPA/DHA-induced-changes in vascular function.

Briefly summarized, the results show that DHA improved postprandial vascular function (reduced AIx) with an effect size that would translate into a meaningful reduction in cardiovascular disease (CVD) risk. A strong trend was also evident following the EPA intervention. Moreover, oxylipin-profiling indicated that postprandial changes in the formation of omega-3 epoxyeicosanoids may play a mechanistic role in mediating the observed beneficial effect on vascular function.

The authors explain the rationale of selecting the postprandial state by referring to the fact that the postprandial state typically covers up to 18 h per day in adults following Westernized dietary patterns. Accordingly, the postprandial phenotype appears more relevant than the fasting one for evaluating CVD risk markers, such as vascular dysfunction and increased arterial stiffness. Moreover, the

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postprandial state is associated with increases in lipemia and glycemia as well as inflammatory stress, providing a challenge for the maintenance of vascular function. The authors, recruited volunteers possessing a moderately increased CVD risk, based on blood lipids, blood pressure, and waist circumference. Taken together, the selection of the postprandial state as well as of participants at risk can be considered to increase the likelihood of success, since the benefits of EPA/DHA-supplementation are frequently only revealed under challenging conditions. However, and potentially influencing the effect size, baseline fatty acid profiles were not considered when selecting the participants.

The participants received high-fat test meals providing 4.16 g EPA or DHA or control oil. The fatty acid composition of the test meals showed that the control meal, containing a palm oil/soybean mixture, was indeed free of any LC-PUFAs. The EPA:DHA-ratios were about 5:1 in the EPA-rich oil (ERO) and 1:6 in DHA-rich oil (DRO). Whereas the control meal did not change the total plasma concentrations of n-3 LC-PUFAs, the ERO meal specifically increased the EPA levels by about 250% and DHA was increased by almost 200% after the DRO meal. Test meals containing other intentionally adjusted EPA+DHA mixtures were not included. This is a shortcoming of this study when asking for potentially additive or synergistic effects of EPA and DHA.

In line with previous studies (26, 27), but nonetheless still impressive, the period of only 4 h was sufficient to generate significant increases in the circulating levels of EPA- and DHA-derived oxylipins. Although some blood cells, like monocytes/macrophages (28), express CYP-enzymes, major sites of CYP-epoxygenase activities are the endothelium, liver, and other organs. Moreover, CYP enzymes need their substrates in a free form, making the release of EPA and DHA, either from lipoproteins in the circulation or later in the cells and tissues, an essential precondition for the enzymatic production of omega-3 epoxyeicosanoids. Without further data, we can only speculate about the postprandial transport and lipase-dependent processes that make EPA and DHA so quickly accessible to the CYP enzymes in blood cells, the endothelium, or other tissues. Once produced, the CYP-eicosanoids themselves can be esterified into phospholipids. At least in the fasting state, the majority of these metabolites circulate esterified primarily with lipoproteins. From this reservoir, the CYP-eicosanoids can be released by lipoprotein lipase, making direct interactions with the vasculature possible (29). The other and more established pathway of CYP epoxyeicosanoid-mediated vascular actions consists in their production in the endothelium followed by paracrine relaxing effects on vascular smooth muscle cells (30). The present study exclusively analyzed the nonesterified circulating oxylipins, leaving open whether or not similarly rapid postprandial changes also occurred with the esterified metabolites.

Furthermore, the observed changes in oxylipin profiles merit some detailed comments. The EPA-derived 17,18-EEQ and its sEH-generated diol (17,18-DiHETE) became the predominant metabolites, surprisingly, not only after giving the ERO but also the DRO test meal. In contrast, the metabolism of DHA via the same CYP epoxygenase/sEH-pathway was detectable only by an increase of the diol metabolite (19,20-DiHDPA) that occurred specifically in the DRO group. Taking the sum of epoxy and diol metabolites, the EPA-derived metabolites exceeded the ones from DHA by a factor of 15 postprandial to the EPA-rich meal and still 8-fold after the DHA-rich meal. Presumably, DRO intervention provided in addition to DHA also sufficient amounts of EPA (supposedly as contaminant or retroconversion product of DHA) for the CYP epoxygenases that generally prefer EPA over DHA (14).

As discussed by the authors, this state-of-affairs suggests that, following DHA intervention, both DHA and EPA and their CYP-generated oxylipins may act in a complementary and additive fashion to mediate the impact on vascular function. Searching for the underlying mechanisms, it might be worth considering that, beyond exerting direct vascular effects, CYP epoxygenase-generated metabolites also have the capacity to limit monocyte activation (28), a process that may also occur in the postprandial state and in turn contribute to the development of vascular dysfunction.

Finally, it also should be noted that many members of the huge oxylipin family were below the detection limit or not accessible through the LC-MS/MS method used by the authors. Beyond the CYP-derived metabolites discussed in detail above, the oxylipin profile presented is rather incomplete, precluding notions about the potential involvement of SPMs and other lipid mediators. It also would have been interesting to learn to what extent EPA and DHA were able to outcompete the postprandial formation of CYP epoxygenase products derived from n-6 PUFAs such as AA and LA. Considering these open questions and also the promises of CYP eicosanoid and SPM lipid mediator profiling discussed in the background part of this commentary, the whole research field would benefit greatly from the analytical experts in different laboratories initiating inter-laboratory tests and joint efforts to improve and standardize the required analytical methods ranging from sample preparation to LC-MS/MS-based quantification.

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