Omega-3 fatty acids: new studies, new data, new questions …

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Abbreviations: CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DHA docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid receptor; GPR, G protein coupled receptor; HDL, high density lipoprotein; LDL, low density lipoprotein; MI, myocardial infarction; SPM, specialized pro-resolving mediator

Twenty years ago, we predicted that the new millennium would bring "more complexity but a better understanding" around the role of fats and fatty acids in human health and disease [1]. Certainly, the metabolism, mechanisms of action at the molecular and cellular levels, and physiologic actions of many fatty acids are now much better described, with a number of important discoveries being made [2]. In this sense, there is more complexity (e.g. multiple interacting mechanisms) but there is better understanding. Nevertheless, the full translation of this understanding into the public health and clinical arenas has not been achieved, largely because of inconsistencies arising from human trials. The bioactive n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) continue to attract significant attention. There is a long history of research in these fatty acids and their multiple actions [3,4]. There is strong and consistent evidence from large cohort studies that they play a role in reducing risk of incidence of, and mortality from, the main non-communicable diseases [5,6,7]. There was also early evidence of reduced risk of mortality from cardiovascular disease (CVD) in at-risk groups (reviewed in [8]), and evidence that they improve outcomes in hospitalised patients receiving artificial nutrition support [9,10]. Major advances in understanding the actions of EPA and DHA have been made.

A plasma membrane G-protein coupled receptor (GPR) called GPR120 (also called free fatty acid receptor 4 or FFA4), which is able to bind long chain fatty acids, is highly expressed on taste bud cells, enteroendocrine K cells of the upper small intestine, adipocytes and inflammatory macrophages. As such, it plays a role in fat "taste", and in the regulation of fat digestion, intestinal hormone secretion, insulin sensitivity and inflammation. Oh et al. [11] demonstrated that FFA4 is involved in anti-inflammatory signalling. EPA and DHA promoted FFA4-mediated gene activation in cultured macrophages, and the anti-inflammatory effects of DHA did not occur in macrophages not expressing FFA4. Thus, EPA and DHA might act on inflammatory cells via FFA4 to reduce inflammatory responses. Oh et al. [11] also demonstrated that DHA-induced translocation of glucose transporter 4 to the surface of cultured adipocytes was abolished by FFA4 knockout, suggesting that FFA4 mediates some of the favourable metabolic actions of DHA. Recent updates on n-3 fatty acid actions mediated through FFA4 can be found elsewhere [12,13,14]. Although FFA4 has become a focus for effects of n-3 fatty acids, Christensen et al. [15] described that the non-methylene interrupted polyunsaturated fatty acid pinolenic acid was a more potent agonist of FFA4 than EPA and DHA.

Although EPA has been long recognised to be a substrate for synthesis of eicosanoids such as prostaglandins, thromboxanes and leukotrienes [16], novel families of lipid mediators

produced from both EPA and DHA have been described. Like eicosanoids, these are produced by cyclooxygenase and lipoxygenase enzymes often working within the same pathway. Several families of these mediators have been described including the resolvins, synthesized from both EPA (E-series resolvins) and DHA (D-series resolvins), protectins (synthesised from DHA) and maresins (synthesised from DHA). Together, these mediators have been termed specialized pro-resolving mediators or SPMs: they have been demonstrated in cell culture and animal feeding studies to be potently anti-inflammatory, inflammation resolving and immunomodulatory [17,18,19]. SPMs have been described in human blood and other fluids including breast milk and in tissues, as reviewed recently [20]. The production of SPMs is favoured by the higher EPA and DHA status brought about by increased oral intake of these fatty acids [20]. SPMs may be responsible for many of the biological actions ascribed to EPA and DHA. Recently conjugates of SPMs with biological activity, such as glutathione-conjugated maresins, have been described [21].

Despite the advances in mechanistic understanding of the actions of EPA and DHA, the question of the functional differences between EPA and DHA remains [22]. DHA has vital roles in the brain and eye [23], which EPA cannot replicate and therefore it is clear that an adequate DHA supply is required at the earliest stages of the life course. Both EPA and DHA give rise to potent SPMs [17,18,19]. In model cell culture systems, DHA is often more potent than EPA [24] and DHA is a stronger agonist of FFA4 [10,14] and inhibitor of nuclear factor kappa B activation [25,26] than EPA. Human trials have attempted to discriminate the effects of these two fatty acids, often in relation to cardiovascular risk factors; data from such trials were subjected to systematic review [27], which identified that both EPA and DHA lower blood triglycerides, with DHA having a greater effect, while DHA, but not EPA, increases high-density lipoprotein (HDL) cholesterol concentration, particularly HDL_2, and increases low-density lipoprotein (LDL) cholesterol concentration and LDL particle size. Both EPA and DHA inhibit platelet activity, whilst DHA improves vascular function and lowers heart rate and blood pressure to a greater extent than EPA. Given these findings, it might be expected that DHA would have a greater effect on incidence of, and outcome from, CVD. However recent large clinical trials do not support that conclusion. This is discussed further below.

One intermediate in the conversion of EPA to DHA is docosapentaenoic acid (DPA). Fish and standard n-3 supplements contain DPA. However, compared with EPA and DHA, the functional effects and mechanisms of action of DPA are underexplored. DPA appears to share many of the properties of DHA [28] and DPA is now known to be a substrate for

synthesis of highly active SPMs [29]. Recent pre-clinical and human trials of DPA are discussed by Ghasemi Fard et al. in an article in this issue. DPA appears to be a fatty acid with some promise.

Human trials of pure or near-pure EPA report enrichment of EPA and DPA in blood and blood cells, suggesting conversion of EPA to DPA in humans. Furthermore, human trials of pure or near-pure DHA report enrichment of EPA in blood and blood cells and this has been interpreted to indicate so-called retroconversion of DHA to EPA. However, a recent study using stable isotope tracing of the fate of DHA indicates that such retro-conversion is minimal in humans [30], with the suggestion that the enrichment of EPA that occurs with increased DHA intake may be due to reduced EPA metabolism. Of interest, not only does long term intake of DHA lead to enrichment of EPA levels, but acute injection of DHA as a component of triglyceride emulsions leads to rapid increases in blood and tissue levels of EPA (and DHA) and its SPM derivatives within two hours [31]. Comparisons of mechanisms that are shared or are different between acute and chronic administration of n-3 fatty acids have been recently reported [32]. In addition to a changing view of the importance of retroconversion, alternative early steps in the pathway of conversion of α-linolenic acid to EPA have been proposed [33], while there is renewed discussion of the precise pathway by which DPA is converted to DHA [34]. Hence, the pathway of endogenous synthesis of EPA, DPA and DHA is coming under increased scrutiny.

The Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT), published in early 2019, included 8,179 participants (29% in a primary prevention cohort with diabetes plus another cardiovascular risk factor and 71% in a secondary prevention cohort) supplemented daily with 4 g of a formulation rich EPA ethyl ester (providing approx. 3.84 g of EPA daily) or mineral oil placebo and followed up for a median of 4.9 years [35]. All patients were being treated with statins and they had triglyceride concentrations of 135–499 mg/dL. The primary outcome was a composite of cardiovascular death, non-fatal myocardial infarction (MI), non-fatal stroke, coronary revascularisation or unstable angina. The patients who received EPA had a statistically significant reduction in the primary outcome compared to placebo (hazard ratio: 0.75; 95% confidence interval (CI): 0.68–0.83; $p < 0.001$), the pre-specified secondary outcome (composite of cardiovascular death, non-fatal MI or non-fatal stroke) (hazard ratio: 0.80; 95% CI: 0.66–0.98; $p = 0.03$) and a whole range of other pre-specified outcomes. This effect was greater in the secondary prevention cohort than in the primary prevention cohort [35]. Although EPA decreased blood triglyceride concentrations, the improvement in the primary outcome was not dependent on the baseline

triglyceride level or the degree of subsequent triglyceride lowering, suggesting that the reduction in cardiovascular risk in this population may be independent of (or in addition to) triglyceride lowering. REDUCE-IT is important as it demonstrates that even in at-risk populations that are well managed with modern pharmacological treatments, a suitably high dosage of EPA (i.e. ~4 g daily) can provide an additional benefit in reducing cardiovascular-related events and mortality.

A meta-analysis published in 2019, included data from 13 randomized controlled trials, including REDUCE-IT [36]. Trials had to have a sample size of at least 500 patients and a follow-up for at least one year to be included. The total sample size of the aggregated trials was 127,477, while the mean duration of follow-up was 5 years. The outcomes of interest included MI, coronary heart disease (CHD) death, total CHD, total stroke, CVD death, total CVD and major vascular events. In the analysis excluding REDUCE-IT, n-3 fatty acids were associated with a significantly lower risk of MI, CHD death, CVD death and total CVD. Inverse associations for all outcomes were strengthened after including REDUCE-IT. Statistically significant linear dose–response relationships were found for several outcomes: for example, every 1 g/d EPA + DHA corresponded to 9% and 7% lower risk of MI and total CHD, respectively. A new meta-analysis published in 2020 combined data from 40 studies in over 135,000 patients [37]. Marine n-3 fatty acid supplementation was associated with a significantly lower risk of MI (13%), fatal MI (35%), CHD events (10%), CHD death (9%) and CVD events (5%), with dose-dependent effects being seen for MI and CVD events. Every 1 g/day EPA+DHA corresponded to 9% and 5.8% lower risk of MI and CVD events, respectively.

The recently published EVAPORATE trial [38] investigated the effect of the same EPA ethyl ester preparation as used in REDUCE-IT and at the same dose on coronary plaque regression over 18 months in hypertriglyceridemic individuals. Once again, the placebo was mineral oil. EPA ethyl ester reduced low attenuation plaque volume compared with placebo and also reduced plaque volume, non-calcified plaque volume, fibro-fatty plaque volume, fibrous plaque volume and calcified plaque volume compared with placebo. These findings demonstrate that EPA may directly affect atherosclerotic plaques and hark back to earlier work demonstrating increased carotid plaque stability in patients receiving combined EPA plus DHA (total 1.8 g/d) [39], an effect which was later linked to EPA rather than DHA [40].

Very recently the main findings from the Long Term Outcomes Study to Assess Statin Residual Risk with Epanova in High Cardiovascular Risk Patients with Hypertriglyceridemia (STRENGTH) randomized controlled trial were published [41]. STRENGTH included

13,078 at risk participants (> 50% in a secondary prevention cohort) supplemented daily with 4 g of a formulation that includes highly purified EPA and DHA as free fatty acids (providing approx. 2.2 g EPA and 0.8 g DHA daily) or corn oil as placebo. All patients were being treated with statins and had triglyceride concentrations of 180–499 mg/dL. The primary outcome was a composite of cardiovascular death, non-fatal MI, non-fatal stroke, coronary revascularisation or unstable angina requiring hospitalisation. The trial was stopped early for futility (no difference in the primary outcome), after a median follow up of 42 months [41]. There was also no difference between groups in the individual components of the primary outcome or in one of the secondary outcomes (composite of cardiovascular death, non-fatal MI or non-fatal stroke). However there was a trend to a reduction in another secondary outcome (coronary events: cardiac death, MI, coronary revascularisation or hospitalisation for unstable angina) (hazard ratio: 0.91; 95% CI: 0.81–1.02; $p = 0.09$) and a significant reduction in coronary events in patients with established CVD at study entry (hazard ratio: 0.85; 95% CI: 0.75–0.97; $p = 0.02$). Of concern, there was a highly significant increase in atrial fibrillation (hazard ratio: 1.69; 95% CI: 1.29–2.21; $p < 0.001$), a finding that contributed to cessation of the trial.

So, we are left with two recent trials of large size in comparable patient groups with long follow-up and using a similar dose of n-3 fatty acids but reporting divergent findings [35,41]. How can this be? REDUCE-IT used ~3.84 g/day EPA as an ethyl ester. Positive findings in this trial are supported with those from the large JELIS trial [42] which also used EPA ethyl ester although at a dose of 1.6 g/day. STRENGTH used a mix of EPA and DHA as free fatty acids; it is stated that this formulation contains 0.55 g of EPA and 0.2 g of DHA per g [43]. Therefore, STRENGTH provided 3 g of EPA plus DHA per day (2.2 g as EPA). Given the findings of JELIS, the dose of EPA used should be sufficient to have an effect. Thus, differences in findings between REDUCE-IT and STRENGTH are most likely not related to either the dose of n-3 fatty acids or of EPA used. However, the different dose of EPA used in REDUCE-IT and STRENGTH (3.84 vs 2.2 g/d) explains why the elevation in plasma EPA was greater in REDUCE-IT (400% vs 270%). REDUCE-IT did not provide DHA but STRENGTH did. An explanation for the different findings of these studies could be that DHA has adverse effects and these outweigh the benefits of EPA. This seems unlikely since DHA generally has beneficial effects on cardiovascular risk factors [27] and also because the dose of DHA used was modest. Nevertheless, in REDUCE-IT EPA lowered LDL-cholesterol by an average of 6.6% or 0.13 mmol/L relative to placebo at 1 year ($p < 0.001$), while in STRENGTH EPA plus DHA increased LDL-cholesterol by 3% relative to placebo at 12 months ($p < 0.001$). This is in keeping with observations that DHA but not EPA raises LDL-

cholesterol [27]. It is possible that this modest elevation of LDL-cholesterol in STRENGTH offset modest benefits of EPA so that an overall null finding occurred. Both REDUCE-IT and STRENGTH reported lower triglycerides (both by ~18%), higher HDL-cholesterol and lower C-reactive protein with n-3 fatty acids. Overall, it is not likely that DHA had an adverse impact that became clinically meaningful. Another difference between REDUCE-IT and STRENGTH is the comparator, mineral oil in REDUCE-IT and corn oil in STRENGTH. It is possible that mineral oil lowers the absorption of dietary fatty acids that contribute to cholesterol lowering or slowing atherogenesis. It has been argued that mineral oil had adverse impact in REDUCE-IT, for example raising LDL-cholesterol by an average of just over 10%, creating the impression that EPA-ethyl ester was beneficial in comparison [41,44]. This argument has some merit and must be explored further to fully understand the inconsistent findings between these two large trials.

In conclusion, recent research has established multiple interacting mechanism by which n-3 fatty acids regulate molecular and cellular responses and tissue physiology in ways that are consistent with improved heath and a reduced profile of risk for major non-communicable diseases, as supported by large cohort studies. However, two large trials in patients at risk of poor cardiovascular outcomes report different findings [35,41]. Reasons for this are not immediately apparent but clearly require exploration. So where are we now. More complexity? Yes. Better understanding? Not yet…..

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