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EDITORIAL

Weighing Up Fish and Omega-3 PUFA Advice with Accurate, Balanced Scales: Stringent Controls and Measures Required for Clinical Trials

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Your patients seek sage health and therapeutic advice. However, focussing on strong evidence-based advice is challenging while wading through disparate clinical studies *and* dispelling the confusing, sometimes apparently contradictory, out-of-context or incorrect messages trafficking to the public via news and internet media, fuelled by the noisy plethora of commercial 'nutriceutical' and 'food pharmacy' products. The cardiovascular benefits of dietary omega-3 long chain polyunsaturated fatty acids (LC PUFA) have been of increasing public and health professional attention and interest for the past several decades. It is therefore timely and important that such benefits are reviewed by Nestel et al., in this issue of Heart Lung and Circulation [1]. In this update of the 2008 National Heart Foundation of Australia (NHF) recommendations on clinical evidence for omega-3 PUFA in prevention and treatment of cardiovascular disease, Nestel et al. conclude that whilst there are clear benefits of eating fish, there is no additional support to recommend the use of refined fish oil supplements.

This overall conclusion is based upon further studies published since 2008 supporting regular intake of fish in prevention or treatment of cardiovascular disease. However, as neither beneficial nor adverse effects of omega-3 LC PUFA supplementation were found in primary or secondary prevention of coronary heart disease, omega-3 supplements are not supported. On the basis of this report and ahead of its publication, the popular news media in Australia has already run with headlines that fish oil does not protect against heart disease. But can that conclusion be drawn from the evidence reviewed?

The evidence around supplements is derived from randomised controlled trials (RCT) and meta-analysis of RCTs and is, therefore, Level I and II evidence. On the other hand, the evidence in favour of fish consumption is derived from observational studies and meta-analysis of observational studies such as prospective cohort studies and is regarded as high Level III evidence. The simplistic view is that Level I trumps Level III. However, consistent evidence from observational studies now goes back 30 years [2,3] supporting an inverse relationship between omega-3 LC PUFA intake and cardiovascular mortality. Then we must ask, why is there a difference between the observational studies and the clinical trial supplement studies? Is there a true difference? Or is interpretation confounded due to trial design?

A properly designed RCT relies upon the dichotomous separation of the treated group from placebo or control. In a clinical trial of (for example) a new statin or a new indication for an existing beta-blocker, it would be unacceptable for the control group to be exposed to significant circulating or tissue concentrations of the test drug. Yet in RCTs of omega-3 LC PUFA supplements, every control group subject

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will have a baseline whole body omega-3 status that is significantly different to zero.

Observational studies consistently demonstrate the differences between individuals eating fish regularly and those not eating fish. That is, they are reporting the effects of omega-3 LC PUFA consumption and resulting omega-3 status against those who have a low intake or low omega-3 status. Most effects of fish oil appear dependent upon the incorporation of the omega-3 LC PUFAs eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) into tissues [4,5]. None of the post-2008 supplementation studies evaluated by Nestel et al. measure baseline or study-end omega-3 levels reflective of dietary intake and tissue incorporation, and few report exclusion of regular fish eaters. James et al. [6] demonstrated that patients specifically excluded from a clinical trial based on high fish consumption were indistinguishable from the fish oil supplement group with regard to their omega-3 status, and even after the exclusions there remained considerable overlap in omega-3 levels between the supplement and control groups at the end of the study. Their conclusion: *''unless modern n-3 studies with cardiac patients exclude potential subjects on the basis of their background fish or fish oil intake, it is unlikely that any dietary n-3 intervention will create a distinct group with regard to tissue EPA+DHA levels''* [6], is highly pertinent as is their broader discussion.

The recent RCTs since 2008 show no design change that would improve capacity for the detection of treatment effects over previous studies. The ORIGIN study [7] at least estimated EPA+DHA intake in their study population and in describing an interquartile range of 40-568 mg/d, they clearly demonstrated (but then ignored) the potential for overlap in the treatment and placebo groups and an increased risk of missing a real effect (type II error), confirming the premise of James et al. [6]. Mozaffarian and Rimm [8], pooling both RCT and cohort studies, estimated an intake of 250 mg/d EPA+DHA for maximum reduction of risk of cardiovascular death and found negligible further benefit from higher doses. Streppel et al. [9], in the long-running Zutphen cohort study, also found reduced risk of sudden death associated with fish consumption delivering less than 250 mg/d EPA+DHA but more than none, and lack of dose-response beyond 250 mg/d. The omega-3 overlap within the ORIGIN study, which found no significant effect of supplements, is clearly a problem. Without the reporting of omega-3 status the extent of this risk with most RCTs is unclear.

Similarly to beta-blockers, where the extent of heart rate lowering is significantly related to survival in heart failure whereas the dose is not [10], the tissue incorporation of omega-3 LC PUFA (omega-3 status) is related to cardiovascular survival [5]. Until randomised controlled trials focus on verified omega-3 tissue measures, or at least report a surrogate for tissue incorporation, such as erythrocyte membrane or adipose composition and account for background intake, it is unlikely that we will have anything other than confounded trial outcomes.

Another primary difference between epidemiology and clinical trials is the nature of the omega-3 intake. One could easily assume that fish intake would vary more in the provision of omega-3 LC PUFA and it does, from the low total content of South Australian King-George whiting to the high content of fatty fish such as salmon, tuna and sardine. Ironically, even with farmed fatty fish such as salmon, marked fluctuation of omega-3 LC PUFA content has recently alerted the requirement for the stringent control of fish feeding protocols and quality of their dietary source. However, what is most consistently different is that most table fish (irrespective of high or low fat content) contain more DHA than EPA [11]. On the other hand, most fish oil supplements contain more EPA than DHA and the JELIS trial used a pure EPA supplement. Whilst it is still not clear which of EPA and DHA is the critical active component of fish oil, there is no doubt that myocardium incorporates DHA in preference to EPA in humans [12] and animals [13], however they may in fact each actively influence different endpoints [4,5,11].

Understanding the pleiotropic mechanisms of action of omega-3 PUFA is important for establishing biological plausibility and garnering clinical support for following any recommendations [1,4,5,14]. Since the 2008 NHF report, further support has come for actions directly within the myocardium and pacemaker tissue. Omega-3 PUFA lowers resting heart rate [14]. Often assumed to be due to altered vagal activity, it is observed even in transplant patients devoid of vagal input [15] and in healthy fit young adults in the form of lower exercise-related heart rate and more rapid post-exercise heart rate recovery, also without vagal contribution [16]. There is additional support also for omega-3 LC PUFA benefiting patients with heart failure, both through supplementation and dietary fish consumption [1]. Like the heart rate effect, this is most likely through direct cardiac effects such as improved LVEF [17,18] attributable to the incorporation of DHA into myocardium. The effects of supplements on triglyceride lowering (Level I evidence) have also been confirmed by post 2008 evidence [1], however it is important to note that this is very much a therapeutic effect associated with high doses only, where there is little risk of overlap between dietary intake and resultant omega-3 status and effective dose [5]. Cardiac related endpoints such as heart rate lowering and improved heart function associated with low doses can be categorised as separate from the vascular related endpoints, which include triglyceride lowering, vascular function, lipoprotein oxidation and atheromatous plaque, and it is plausible that omega-3 PUFAs may function across quite distinct nutritional (cardiac) and therapeutic (vascular) intake ranges [5].

Of particular concern in RCTs is the use of combined vascular and cardiac endpoints, such as stroke or sudden death, as this assumes a common mechanism of action and the combination of vascular and cardiac patients in secondary prevention assumes a common substrate. For example post-MI patients present as a vulnerable cardiac substrate and are at increased risk of sudden death or heart failure, whereas stroke patients are considered more at risk of further stroke. Blended patient groups with varied disease aetiology, clinical history, pharmacotherapeutic management, and

having endpoints dependent upon different mechanisms of action, can only add to the variability in outcomes and diminish the likelihood of significant effects being observed. A notable example of such confounding is the double blind, RCT by Galan et al.[19], which not only mixed patient aetiology and status (history of myocardial infarction, unstable angina, or ischaemic stroke) with multiple compound primary and secondary endpoints, but also introduced late timing of entry to the RCT, with a mean of 101 days. In contrast, the GISSI-P study subjects were recruited with a post-MI mean of 16 days and the observed effect on fatal arrhythmia prevention was evident by 120 days (early) [20]. Thus, in addition to inclusion of well-defined patient treatments (agent type/dose, timing of therapy) and testing single clinical endpoints (that include mechanistic and molecular evidence of effect), there is no question that the design of prospective RCT requires distinct stratification of patients that are carefully characterised for varied confounders in greater detail and sample size. A challenge even for industry funded medical device or pharmaceutical trials!

Two recent controversies have beset dietary omega-3 PUFA. These relate to a reported association between omega-3 PUFA and prostate cancer and the proposed adverse influence of dietary omega-6 PUFA. Both of these controversies have gained publicity in the popular press. With respect to prostate cancer risk, the lack of association highlighted in the NHF review [1] is further supported by an additional more recent meta-analysis [21]. The omega-6 to omega-3 ratio was not considered in the review but is worthy of discussion in conjunction with the NHF recommendations, since it often attracts media and scientific attention based on theoretical competition for tissue incorporation. The proposed interactions between omega-6 and omega-3 PUFA and their ratio in the diet was directly analysed prospectively within the Cardiovascular Health Study [22]. Not only is there no adverse effect of the omega-6 PUFA on omega-3 LC PUFA action but total mortality and cardiovascular mortality is lowest in conjunction with both high omega-3 and high omega-6 PUFA. This demonstrates the fallacy of attempting to improve omega-3 LC PUFA status by reducing omega-6 PUFA intake and supports continuing the long-standing recommendation to maintain omega-6 PUFA intake for cardiovascular health.

The update of evidence since 2008 supports the continued National Heart Foundation of Australia recommendation to eat fish but does not support the benefit of omega-3 supplements. However considering the lack of defined clarity regarding disease substrate and clinical endpoints, omega-3 LC PUFA source and type, there is also no direct evidence to hastily dismiss omega-3 supplements. Unproven does not equate with non-existent, particularly when definitive, well-designed RCTs are still required. For whilst it is recognised that there is consistent benefit derived from regular fish consumption, and increased fish consumption should be recommended and encouraged for prevention and treatment of cardiovascular disease, it is also the case that many Australians do not eat enough fish to derive this benefit from diet alone. Thus it remains crucial that amply sized, stringently controlled clinical trials are performed based on the status of measured omega-3 levels rather than dose (analogous to warfarin studies based on INR [23]), and grounded in a better understanding of omega-3 LC PUFA's complex pleiotropic mechanisms of action, in addition to the molecular basis of cardiovascular disease. In the interim, adequate omega-3 LC PUFA intake should be recommended, irrespective of its dietary source.

Conflict of Interest

Both authors state that we have no financial or commercial interest in fish oil supplements or fish products though we do consume fish as per National Heart Foundation of Australia recommendations (!).

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