Effect of fish and fish oil-derived omega-3 fatty acids on lipid oxidation

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There is evidence that omega-3 (ω3) fatty acids derived from fish and fish oils reduce the risk of cardiovascular disease via mechanisms underlying atherosclerosis, thrombosis and inflammation. Despite these benefits, there has been concern that these fatty acids may increase lipid peroxidation. However, the in vivo data to date are inconclusive, due in part to limitations in the methodologies. In this regard, our findings using the measurement of F2-isoprostanes, a reliable measure of in vivo lipid peroxidation and oxidant stress, do not support adverse effects of ω3 fatty acids on lipid peroxidation.

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There is now considerable evidence that a diet rich in ω3 fatty acids derived from fish and fish oils, specifically eicosapentaenoic acid (EPA, 20:5 ω3) and docosahexaenoic acid (DHA, 22:6 ω3), protects against atherosclerotic heart disease, myocardial infarction and sudden death.1 ω3-Fatty acids have a wide range of biological effects, including benefits on lipoprotein metabolism, platelet and endothelial function, blood pressure, vascular reactivity, cytokine production, coagulation and fibrinolysis.1–3 Recent evidence also has demonstrated that, in humans, EPA and DHA have differential effects on lipids and lipoproteins,4 blood pressure5 and heart rate,5 and vascular reactivity.6

Despite the benefits associated with increased ω3 fatty acid consumption, there remains a theoretical concern that these fatty acids may increase the unsaturation index, consequent to the incorporation of EPA and DHA into membranes and lipoproteins, leading to increased lipid peroxidation.7 The significance of this relates to the fact that there is much evidence supporting a role for lipid oxidation and oxidative stress in the pathogenesis of cardiovascular disease.8 It is thought that oxidative stress and oxidized lipids play a critical part in the genesis and progression of the atherosclerotic lesion.8 The hypothesis that increased intake of ω3 fatty acids may lead to increased lipid peroxidation is based on the premise that fatty acid oxidizability increases with an increase in the number of double bonds in the fatty acid chain.9 Whilst this may be true of in vitro studies of lipid peroxidation in homogeneous solutions, in vivo systems are more complicated and influenced by additional factors. In support of this, Visioli et al.10 showed that, under equal conditions of oxidative stress, fatty acids oxidized at different rates and generated different oxidation products, in a manner that was unrelated to their degree of unsaturation. Lipid peroxide levels were in fact highest following oxidation of linoleic acid (18:2 ω6).10 Arachidonic acid (20:4 ω6) and DHA generated lower levels of lipid peroxides, with lowest levels arising from oxidation of EPA. Formation of conjugated dienes was also maximal for 18:2 ω6. In particular, the production of conjugated dienes from 20:4 ω6 and EPA was approximately 25% of that of 18:2 ω6. DHA oxidation yielded only 10% of the conjugated dienes relative to that of 18:2 ω6.10

The data relating to the effects of ω3 fatty acids on lipid peroxidation and oxidative stress in vivo are contradictory.7 These inconsistencies may be related to differences in the populations studied, the quantity and presentation of the...
ω3 fatty acids (fish versus encapsulated fish oils), the duration of the study, the study design, the antioxidant content of the supplement, or the composition of the background diet. It also has also been suggested that the total concentration of polyunsaturates, rather than the unsaturation index, may be more important in determining lipid peroxidation.11 However, the most plausible explanation for the inconsistency between studies is differences in the methodologies employed to assess lipid peroxidation.

Much of the literature regarding the effect of ω3 fatty acids on lipid peroxidation is based on indirect and/or non-specific assays. Studies utilizing the oxidizability of LDL have shown either enhanced,16–17 or reduced, or no effect,18–22 of ω3 fatty acids. In this assay, LDL is isolated from plasma and then subjected to oxidative conditions. It is feasible that in patients on various medications, LDL oxidizability could be affected by partition of drugs into the LDL. Indeed, a divergence has been shown between the measurement of LDL oxidative susceptibility and urinary F2-isoprostanes as a measure of oxidative stress.23

One of the most common methods for assessment of lipid peroxidation measures thiobarbituric acid reactive substances (TBARS) by a colorimetric assay. This assay has been widely criticized on the basis of its lack of specificity and results need to be interpreted with care.24 Studies of ω3 fatty acids using this methodology have shown either elevated levels16,17,25–28 or no change29,30 in TBARS. The limitation of this assay was exemplified by Higdon et al.25 who showed that, in post-menopausal women given ω3 fatty acids, plasma TBARS were 10-fold higher than malondialdehyde (MDA) measured by gas chromatography mass spectrometry. In addition, plasma TBARS were significantly elevated following ω3 fatty acids, whereas MDA was reduced. When plasma MDA levels were normalized to plasma polyunsaturated fatty acid concentrations, significant differences were eliminated.25

Other measures of oxidative stress include electron spin resonance detecting free radical species,24 measurement of antibodies to oxidized LDL31 and breath excretion of ethane and pentane.24 The latter assay, however, has yielded variable results in animals32 and humans27 supplemented with ω3 fatty acids. Other literature reports have shown that ω3 fatty acids had no adverse effects on plasma protein oxidation28 and rendered erythrocytes more resistant to hemolysis following oxidative challenge.33

On the basis of studies reporting adverse effects on lipid peroxidation, some researchers have suggested that ω3 fatty acids may affect the antioxidant status and, therefore, should be taken in conjunction with vitamin supplementation. The data from such studies, however, are inconclusive.16,27,34–37

Although most of the above-mentioned methods represent different aspects of lipid oxidation and collectively provide some knowledge of oxidative damage, none is considered a reliable measure of oxidative stress. In this regard, the F2-isoprostanes are prostaglandin-like metabolites of free radical peroxidation of arachidonic acid38 and there is now good evidence that they provide a reliable measure of in vivo oxidative stress.39,40 In support of this, elevated F2-isoprostanes have been reported in animal models of free radical injury, in human conditions associated with increased oxidative stress, and in vitro experimental models.38–40

Using F2-isoprostanes, measured by gas chromatography–mass spectrometry, we have demonstrated that these metabolites are significantly reduced following consumption of ω3 fatty acids taken as fish oils or in fish meals. We showed that fish meals providing approximately 3.6 g/day of ω3 fatty acids for 8 weeks to Type 2 diabetic patients, significantly (P = 0.013) reduced urinary F2-isoprostanes by 20% (Fig. 1).41 Relative to a control group, urinary F2-isoprostanes were reduced by 0.83 nmol/24-h. This effect was independent of age, gender, body weight change and the increase in ω3 fatty acids or the fall in ω6 fatty acids in plasma, platelets and red blood cells.

We recently have shown that cord plasma and urinary F2-isoprostanes were reduced in infants whose mother received fish oil during pregnancy.42 Pregnant atopic women received 4 g daily fish oil or olive oil from 20 weeks’ gestation. Cord plasma F2-isoprostanes were significantly lower (P < 0.001) in the offspring of women who had taken fish oil during pregnancy compared with those who took olive oil (Fig. 2). These differences were
independent of red cell 20:4 \( \omega_6 \) levels. Urinary F2-isoprostanes corrected for creatinine excretion tended to be lower in infants whose mother had taken fish oil (\( P = 0.06 \)).

Our findings are in accordance with several other studies in which \( \omega_3 \) fatty acids have been supplemented. Quaggiotto et al.\(^43\) showed that, compared to beef tallow, high doses of \( \omega_3 \) fatty acids reduced plasma F2-isoprostanes after coronary occlusion in pigs. Similarly, Higdon et al.\(^25\) demonstrated a fall in plasma F2-isoprostanes in post-menopausal women given \( \omega_3 \) fatty acids compared with diets enriched in oleate or linoleate. In the latter study, however, significant differences were eliminated when F2-isoprostanes were adjusted for plasma arachidonic acid concentrations.\(^25\)

We have demonstrated in two trials that both EPA and DHA equally reduced F2-isoprostanes.\(^44,45\) In overweight, mildly hyperlipidaemic men, supplementation with 4 g daily of purified EPA or DHA for 6 weeks decreased post-intervention urinary F2-isoprostane levels by 27% following EPA (1.24 nmol/24-h, \( P < 0.0001 \)) and 26% following DHA (1.20 pmol/24-h, \( P < 0.0001 \)), relative to an olive oil control group, after adjusting for baseline values (Fig. 3A).\(^44\) In a study of similar design in treated hypertensive Type 2 diabetic patients, we showed that post-intervention urinary F2-isoprostanes were reduced 19% by EPA (\( P = 0.017 \)) and 20% by DHA (\( P = 0.014 \)), relative to an olive oil group (Fig. 3B).\(^45\)

In each of these studies,\(^41,42,44,45\) the changes in F2-isoprostanes were unrelated to changes in EPA, DHA, 20:4 \( \omega_6 \), total \( \omega_3 \) or \( \omega_6 \) fatty acids. This lack of association with changes in fatty acids is noteworthy, in view of the fact that F2-isoprostanes are derived from free radical oxidation of arachidonic acid, which is significantly reduced following \( \omega_3 \) fatty acids. Therefore, the changes in F2-isoprostanes most likely reflect a true reduction in oxidative stress, rather than as result of a reduction in the supply of substrate.

How F2-isoprostanes are reduced following \( \omega_3 \) fatty acid supplementation remains unresolved. We suggested this might be explained, at least in part, by the anti-inflammatory effects of \( \omega_3 \) fatty acids and a reduction in leukocyte free radical formation. Activated leukocytes generate powerful oxidants during phagocytosis\(^46\) and cytokines such as TNF-\( \alpha \) and IL-6 stimulate leukocytes and endothelial cells to generate free radicals, further propagating the pro-oxidant condition. In support of this hypothesis, we showed that the changes in urinary F2-isoprostanes were significantly positively associated with changes in TNF-\( \alpha \) concentration.\(^45\) Numerous studies have demonstrated anti-inflammatory actions of \( \omega_3 \) fatty acids, with falls in cytokines most often observed following leukocyte stimulation.\(^47\) \( \omega_3 \)-Fatty acids also
have been shown to suppress production of reactive oxygen species (superoxide and hydrogen peroxide) in stimulated leukocytes. Superoxide production was reduced in isolated human\textsuperscript{52} and rat\textsuperscript{44} polymorphonuclear leukocytes, as well as in human monocytes.\textsuperscript{45,43} Other potential mechanisms may relate to the assembly of ω3 fatty acids in membrane lipids and lipoproteins making the double bonds less susceptible to free radical attack.\textsuperscript{53} inhibition of the pro-oxidant enzyme phospholipase A\textsubscript{2},\textsuperscript{56} and stimulation of antioxidant enzymes.\textsuperscript{57,58} In this regard, there is evidence that ω3 fatty acids up-regulate gene expression of antioxidant enzymes and down-regulate genes associated with production of reactive oxygen species.\textsuperscript{59}

CONCLUSIONS

There is no evidence for a pro-oxidant effect of ω3 fatty acids. Our findings and the recent literature demonstrate that ω3 fatty acids do not adversely affect, and indeed may attenuate, oxidative stress. The results clearly highlight the need for caution in choosing methodologies for the assessment of oxidative stress. Further studies are also required to explore potential mechanisms for the observation of an association between oxidative stress, markers of inflammation and atherosclerosis following ω3 fatty acids. Nonetheless, there appears no reason why ω3 fatty acids should not be taken either as fish meals or fish oil capsules, in view of their overall favourable effects on cardiovascular risk reduction.

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