PUFAs and their Impact on Atherogenesis and Inflammation

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Polyunsaturated Fatty Acids (PUFA)s

Overview of n-3 PUFAs

Polyunsaturated fatty acids have two or more double bonds within the hydrocarbon chain. Omega-3 polyunsaturated fatty acids (n-3 PUFAs) have the first double bond 3 carbons away from the end of the chain. Commonly mentioned n-3 PUFAs include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in fish, flax seed, canola oil, and pharmaceutical preparations (Sakamoto, et al, 2019). n-3 PUFAs have gained attention due to observed anti-inflammatory effects. These anti-inflammatory effects are important as inflammation is the primary cause of cardiovascular diseases (Willerson and Ridker, 2004). However, epidemiological research in humans has generated mixed results which may be due in part to the particular n-3 PUFA under study, as drug trials have provided clear evidence that EPA has benefits for those at risk of cardiovascular diseases (Bhatt *et al.*, 2019).

Mechanisms of Inflammation Resolution

The mechanisms by which n-3 PUFAs reduce inflammation are proposed to involve their conversion to other compounds. The enzymatic metabolism of EPA and DHA results in the production of lipid mediators known as specialized proresolving mediators (SPM), which promote inflammation resolution. SPMs include resolvins, marsins, and protectins (Bäck, 2017). D-series resolvins and protectins result from DHA, while E-series resolvins come from EPA. These molecules act on G-protein-coupled receptors (GPCR) to activate an inflammation resolution response. In the case of atherosclerosis, these molecules reduce the risk of immunosuppression that causes unresolved inflammation in this disease (Carracedo *et al.*, 2019). n-3 PUFAs can also modulate membrane fluidity so that dimerization and recruitment of toll-like receptor-4 (TLR-4) is disrupted, which reduces the activity of nuclear factor-kappaB (NF-κB), and thus reduces inflammation (Mozaffarian and Wu, 2011). Baker *et al.* (2018) found that EPA and DHA reduce expression of vascular cell adhesion molecule 1, resulting in a subsequent decrease in adhesion of leukocytes, as well as leukocyte infiltration, to the vasculature. EPA and DHA can regulate inflammatory gene expression in order to modulate cytokine, chemokine, and adhesion molecule activity to reduce inflammation as well (Darwesh *et al.*, 2019). Liu *et al.* (2016) evaluated the effect of DHA on inflammation through activation of free fatty acid receptor 4 (FFA4). This study examined human endothelial cell line EA.hy926 treated with 100μM DHA for 16 hours, followed by 1ng/mL TNF-α. TNF-α induces Egr-1 gene expression, as well as increases the interaction between TAK1 and TAB2 in order to induce inflammation. DHA reduces these effects, suggesting that DHA can resolve inflammation through its effect on TNF-α signaling pathway through the FFA4. Therefore, DHA-induced activation of FFA4 reduces inflammation (Liu *et al.*, 2016).

Clinical Studies on Inflammation Resolution through n-3 PUFAs

Makarewicz-Wujec *et al*. (2017) in a double-blind trial with 30 participants following a myocardial infarction (MI) found that n-3 PUFA supplementation did not experience significant reductions in inflammatory markers. Participants supplemented 1g/day n-3 PUFAs (fish oil with DHA and EPA) for 12 weeks. No significant difference was observed between placebo and treatment groups in regards to inflammatory markers such as C reactive peptide (CRP), monocyte chemoattractant protein - 1 (MCP-1), homocysteine, or CD-40 (Makarewicz-Wujec *et al*., 2017). Hu *et al.* (2017) in an analysis of randomized controlled trials involving 487 patients found that n-3 PUFA supplementation did not reduce inflammatory markers. The authors report serum levels of CRP (SMD, -0.20; 95% CI, -0.44 to 0.05; p=0.11), IL-6 (SMD, 0.00; 95% CI, -0.33 to 0.33; p=0.99) and TNF-α (SMD, 0.14; 95 % CI, -0.17 to 0.44; p=0.38) between the omega-3 fatty acids supplementation group and control (Hu *et al.*, 2017). The compiled studies supplemented 0.8-6g/day n-3 PUFAs, for 2-6 months. The ASCEND Study Collaborative Group (2018) examined the effect of 840 mg EPA and DHA supplementation per day for 7 years in type 2 diabetics to find that n-3 PUFAs do not reduce end point major cardiovascular events (3% difference between placebo and treatment groups). Major cardiovascular events were defined as fatal MI, nonfatal stroke, transient ischemic attacks, and fatal cardiovascular events. This study did find a 19% reduction in fatal cardiovascular events. (ASCEND Study Collaborative Group, 2018). Manson *et al.* (2019) in the VITAL study found similar results with 840 mg EPA and DHA per day with 2,000 IU vitamin D3 in healthy participants for 5 years. The VITAL study found a non-significant 8% decrease in major end point cardiovascular events from the treatment to the placebo group. Major end point cardiovascular events were defined as stroke, fatal cardiovascular disease, and myocardial infarction. However this study did report a 28% decrease in MI incidence with the treatment group (Manson *et al.*, 2019), (Kris-Etherton *et al*., 2019).

Contrary to the above studies, Bhatt *et al.* (2019) in the REDUCE-IT trial, with 3,600mg EPA supplementation by icosapent ethyl supplement (IPE) per day for 5 years in participants already on a statin, reported a 26% decrease in primary cardiovascular disease end point events. Primary cardiovascular disease end points were defined as fatal cardiovascular events, fatal and nonfatal MI, unstable angina, fatal and nonfatal stroke, and urgent revascularization, among other cardiovascular diseases (Bhatt *et al.*, 2019), (Kris-Etherton *et al*., 2019). IPE is approved by the FDA under the trade name Vascepa. Budoff *et al.* (2019) examined similar effects at 9 months with Vascepa in the EVAPORATE study, reporting a 20% decrease in the primary end point in the treatment group with interim results, compared to the control. EVAPORATE includes 80 participants with coronary atherosclerosis and elevated triglycerides prescribed a statin. The primary endpoint is defined as a change in low-attenuation plaque volume (Budoff *et al*., 2018; Budoff *et al*., 2019).

Summary

Notably, between null and significant studies examining the clinical significance of n-3 PUFA supplementation, the dose and duration of n-3 PUFA has been variable. Further, the specific n-3 PUFA under study has been variable. In order to better assert the clinical significance of the anti-inflammatory mechanisms of n-3 PUFAs, studies should better analyze the importance of supplementation duration, as well as clarify the minimum effective dose for clinical significance. Further, research examining the importance of the specific n-3 PUFA under study will provide insight into the degree of significance in the relationship between n-3 PUFA supplementation and anti-inflammatory effects.

Atherosclerosis

Atherogenesis:

Atherogenesis (the development of atherosclerotic plaques) is a complex process involving several factors all of which are driven by a chronic inflammatory response. Atherosclerosis is a multifocal disease that attacks sites with low or oscillatory shear stresses on the endothelium. These sites of low/oscillating shear occur where the laminar flow of blood is disturbed by a curvature or vessel branch. These inflamed regions develop due to the activation of the NF-Kß pathway in endothelial cells (Denis et al, 2017). NF-kß is an important inflammatory signaling molecule (Halade et al, 2018). Once activated, NF-kß travels to the nucleus to increase expression of the protein of inflammation. This increase in NF-kß signaling in the endothelium, along with the presence of oxidized LDL (oxLDL) particles circulating in the blood, causes the expression of adhesion proteins in the endothelium (VCAM, ICAM, selectin, etc) that recruit leukocytes to these regions (Baker et al, 2018). Once the monocyte are held by the endothelium in these inflamed regions, they migrate across the endothelium and enter the intima. Once on the other side of the endothelium, the monocytes transition to activate macrophages. They remain in this location (the vascular intima) and begin to absorb the oxLDL particles (Baker et al, 2018). These oxLDL particles enter the vessel intima due to the increase endothelial cell permeability. As macrophages are unable to degrade the oxLDL, the macrophages continue to absorb oxLDL until they turn into foam cells (oxLDL laden macrophages). Two phenotypes of macrophage (M1 and M2) have been identified in atherosclerotic plaques that both secreting cytokines. The M1 macrophage cytokines are more proinflammatory than M2 (Bentzon et al, 2014; Conti, 2015). The M1 macrophages release the cytokine tumor necrosis factor (TNF) which stimulated the apoptosis of foam cells (Conti, 2015). The death of these foam cells causes the formation of a necrotic core in the plaque as it enlarges (Bentzon et al, 2014). The intimal enlargement of the plaque derives from the cytokines released by the macrophages that thicken the intimal layer by enhancing endothelial permeability to allow more LDL and monocyte to enter the intimal layer (Baker et al, 2018). Smooth muscle cells migrate to the intima where are converted to a synthetic phenotype that secretes growth factors (eg., platelet derived growth factor) that stimulate more smooth muscle cell to migrate. In addition to the growth factors, they also secrete collagen that forms the basis for the plaque: the enlargement in the intimal layer (Bentzon et al, 2014).

Inflammatory cytokines

Inflammation is tightly connected to atherogenesis. During atherogenesis, macrophages releases cytokines that further the inflammatory response (Conti, 2015). M1 cells release many cytokines including IL-1 and TNF. IL-1 induces TNF which stimulates apoptosis in endothelial cells as well as reducing growth factor secretion. These growth factors are also considered cytokines. M2 cells also release important cytokines like IL-10. IL-10 can deactivates M1 macrophages which contributes to the progression of the plaque (Conti, 2015). TNF from M1 cells upregulates expression of ICAM's and VCAM's which recruits more monocytes.

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Abstract

Consumption of omega-3 fatty acids benefits the cardiovascular system by reducing blood vessel inflammatory responses. Our recent work showed omega-3 fatty acids decrease connexin 43 (Cx43) expression in cultured endothelial cells after 1 day. Our interest was Cx43as a marker of inflammation as omega-3 fatty acids are strongly implicated as anti-inflammatory agents. Localized inflammatory responses drive the growth of the atherosclerotic plaques responsible for a majority of cardiovascular diseases. Anything that affects the inflammatory response will affect the growth (and stability which is also important) of those plaques. This poster will summarize current knowledge of atherosclerosis and inflammation as well as the impact of omega-3 fatty acids on inflammation.

Summary

The original poster was to have presented evidence on the impact of plating density on culture endothelial cell expression of Cx43. This was based upon controls experiments in the lab that showed a decrease in Cx43 with increasing cell density The ultimate goal of this research was to better understand how inflammation affects endothelial cells (with Cx43 as a marker of inflammation in the endothelium) and more importantly the protective effects of PUFAs (omega-3 in particular) on inflammatory response (the original work showed omega-3 fatty acids decreased Cx43 expression at 24 and 48 hours of treatment). The student authors had collected proteins and were waiting to run the Western blots when the covid-19 hit. Thus, Madison (first column) and Kyle (second column) on short notice, wrote this quick review of PUFAs and atherogenesis.

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