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Original Article

Relationship between the omega-3 index and specialized pro-resolving lipid mediators in patients with peripheral arterial disease taking fish oil supplements

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KEYWORDS:
Peripheral artery disease;
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Abstract: **BACKGROUND:** Oral supplementation with n-3 polyunsaturated fatty acids (PUFA) increases the omega-3 index, a biomarker of red blood cell eicosapentaenoic acid and docosahexaenoic acid, and plasma levels of biosynthesis pathway markers and potent lipid mediators involved in the resolution of inflammation among patients with peripheral arterial disease (PAD).

OBJECTIVE: We aimed to quantify the association between an upstream change in the omega-3 index and downstream changes in lipid mediator production.

METHODS: We conducted a secondary analysis of the OMEGA-PAD I Trial, a randomized, placebo controlled trial investigating high-dose n-3 PUFA oral supplementation in PAD patients. Eighty subjects were randomized to either 4.4 g of fish oil or placebo for 1 month. Regression analyses using generalized estimating equation techniques were used to investigate the relationship between changes in the omega-3 index and changes in lipid mediators, pre- and post-intervention.

RESULTS: In the fish oil group, there was a significant increase in the omega-3 index (5 ± 1% to 9 ± 2%, $P < .001$) as well as in the plasma levels of several downstream lipid mediator pathway

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markers of resolution, which are involved with the regulation of leukocyte effector function and host defense. A doubling of the omega-3 index correlated with increases of 2.3-fold in 18-hydroxy-eicosapentaenoic acid (HEPE; $P < .0001$), 1.7-fold in 15-HEPE ($P = .03$), 1.9-fold in 5-HEPE ($P = .04$), and 3.6-fold in 4-hydroxy-docosahexaenoic acid ($P < .001$).

CONCLUSION: Among subjects with symptomatic PAD who took oral fish oil supplements for 1 month, observed changes in the omega-3 index were strongly associated with increases in downstream mediators in the biochemical pathways of resolution.

Introduction

The atherosclerotic lesion, the hallmark of the disease process in peripheral arterial disease (PAD), represents a series of cellular and molecular responses that are the result of excess inflammation.[1,2] In recent years, it has become appreciated that specific biochemical signals, rather than a passive decrescendo of inflammatory cytokines, leads to the resolution of inflammation. This work has demonstrated that a unique class of specialized pro-resolving lipid mediators (SPMs) drives the process of resolution.[3] SPMs are derived from the omega-3 polyunsaturated fatty acids (n-3 PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found in marine oils. It has long been recognized that fish oils have beneficial actions in cardiovascular disease,[4–6] and more recent work in the field of resolution biology has implicated SPMs as having a paramount role in a variety of inflammatory diseases,[7–12] many of which may be heralded by a resolution deficit.

The process by which n-3 PUFA impacts inflammation begins with the incorporation of EPA and DHA into cellular membranes. Once incorporated, n-3 PUFA can affect the activity of membrane proteins and physical membrane characteristics and, once released by intracellular phospholipases, they can be converted into a wide variety of bioactive lipid mediators.[13] Alternatively, circulating unesterified n-3 PUFA have been found to arrive at a site of inflammation where direct conversion to bioactive SPMs occurs.[14] Several families of structurally and functionally distinct SPMs have been identified including the E-series resolvins (RvE) derived from EPA via P450 metabolism or aspirin-acetylated cyclooxygenase (COX-2), and the D-series resolvins, protectins, and maresins derived from DHA via lipoxygenase or aspirin-acetylated COX-2.[3] More specifically, the formation of the RvE begins via the action of acetylated COX-2 or cytochrome P450 on EPA, which results in an intermediate known as 18-hydroperoxy eicosapentaenoic acid (HpEPE).[15] 18-Hydroperoxy eicosapentaenoic acid is subsequently reduced by a peroxidase to form 18-hydroxy eicosapentaenoic acid (HEPE); this is followed by lipoxygenation by the enzyme 5-lipoxygenase to form a hydroperoxide, which is then transformed to an epoxide that undergoes enzymatic hydrolysis to form RvE1.[15] The RvE have been found to act through G-protein coupled receptors to have potent anti-inflammatory and pro-resolving action.[16–19]

The omega-3 index is a validated biomarker used to define the red blood cell (RBC) content of EPA and DPA, thus it reflects the interplay between oral intake and metabolism of n-3 PUFA.[20] Erythrocyte fatty acid composition consists almost exclusively of phospholipids with little biologic variability between individuals.[21] Identifying the percentage contribution of EPA and DHA to total identified RBC fatty acids allows precise reflection of plasma and tissue levels of EPA and DHA.[22] However, the relationship between blood levels of these n-3 PUFA and the downstream biochemical pathways producing SPMs is poorly understood.

The physiological impact of oral supplementation of n-3 PUFA for each individual is variable depending on baseline characteristics such as prior dietary intake and hereditary metabolic factors.[20] In the OMEGA-PAD I Trial, we recently demonstrated the impact of n-3 PUFA supplementation on altering biochemical SPM pathways in PAD patients.[23] We aimed here to investigate if changes in lipid mediator pathways are associated with changes in the omega-3 index in a cohort of PAD subjects undergoing short-term (1 month) fish oil supplementation.

Methods

Study population, intervention, and protocol

This study was a secondary data analysis of the randomized, double-blinded, and placebo-controlled OMEGA-PAD I Trial; the methods of the OMEGA-PAD study have been previously published.[23,24] Briefly, the study included 80 patients aged ≥50 years with symptomatic lower extremity PAD, who presented to vascular surgery clinic at the Veterans Affairs Medical Center in San Francisco. Subjects were excluded from the trial if they were already taking a fish oil supplement. Subjects were randomized to 2.2 g oral n3-PUFA (Pro-Omega; Nordic Naturals, Watsonville, CA) twice daily (totaling 4.4 g/d) or a matched placebo for 1 month. Each ProOmega capsule contained 325 mg of EPA and 225 mg of DHA. Treatment corresponded to 4 capsules twice daily, totaling 2.6 g of EPA and 1.8 g of DHA daily.