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Review

Omega-3 fatty acids and inflammation: A perspective on the challenges of evaluating efficacy in clinical research

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ABSTRACT

Chronic inflammation is a common underpinning of many diseases. There is a strong pre-clinical evidence base demonstrating the efficacy of omega-3 fatty acids for ameliorating inflammation and thereby reducing disease burden. Clinically, C-reactive protein (CRP) serves as both a reliable marker for monitoring inflammation and a modifiable endpoint for studies of anti-inflammatory pharmaceuticals. However, clinical omega-3 fatty acid supplementation trials have not replicated pre-clinical findings in terms of consistent CRP reductions. Methodological differences present numerous challenges in translating pre-clinical evidence to clinical results. It is crucial that future clinical nutrition research clearly distinguish between the reversal of established inflammation and preventing the development of inflammation. Future clinical studies evaluating the ability of omega-3 fatty acids to attenuate an excessive inflammatory response, may be advanced by employing new statistical approaches and utilizing models of induced inflammation, such as low-dose human endotoxemia.

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1. Introduction

Inflammation is an essential biological process initiated by the immune system in response to injury, irritation, or infection. However, when inflammation becomes prolonged or chronic it is a key component in the etiology of several diseases, including cardiovascular disease (CVD), diabetes, rheumatoid arthritis, cancer, and neurodegenerative diseases such as Alzheimer's disease. Current standard-of-care medical therapies for such diseases are very costly and/or carry significant side effects. As such, there is a need

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; CVD, cardiovascular disease; AHA, American Heart Association; hs-CRP, high sensitivity C-reactive protein; DPA, docosapentaenoic acid; LPS, lipopolysaccharide.

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to identify dietary patterns, lifestyle factors, and alternative pharmacological strategies capable of ameliorating inflammation and thereby reducing the burden of disease.

The pre-clinical evidence base strongly supports the efficacy of the marine-derived omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), for preventing the development of inflammation. In observational studies for example, individuals who report greater dietary EPA and DHA intake experience less inflammation as they age [1–4]. This relationship is also present when biomarkers of intake are used [5–7]. For instance, in healthy adults with relatively low omega-3 intake, higher quartiles of red blood cell (RBC) DHA were inversely associated with plasma concentrations of the inflammatory cytokine TNF-α [8]. This association between omega-3 fatty acids and inflammation is also supported by investigations utilizing in vitro, ex vivo, and animal disease models, which have begun to illuminate the mechanisms by which omega-3 fatty acids may act to prevent excessive inflammation [9–14]. In particular, research utilizing the *fat-1* transgenic mouse [15–17]—which is able to endogenously convert omega-6 fatty acids to omega-3 fatty acids—has provided a system in which the effects of elevated tissue omega-3 fatty acids can be completely isolated from other confounding factors, such as diet. When exposed to inflammatory stimuli, these mice experience attenuated inflammatory responses and are more resilient than their omega-3 deficient littermates [18].

However, randomized controlled clinical supplementation trials have yielded mixed results for accepted markers of inflammation [19,20]. This creates much difficulty in evaluating whether these fatty acids are efficacious as anti-inflammatory therapies, and if so, determining critical details in terms of their delivery (e.g. optimal dose, duration, target condition, and relative efficacy of the individual fatty acid species).

This article will discuss many of the unique challenges regarding clinical research evaluating the efficacy of omega-3 fatty for reducing biomarkers of inflammation and suggest potential means of improvement for future investigations. Following a discussion of clinical biomarkers, the current clinical research evidence base will be presented. Further discussion of the methodological difficulties of evaluating therapeutic interventions for inflammatory states will be given, specifically with regard to achieving adequate signal-to-noise in clinical studies. Finally, models of human induced inflammation will be introduced as a potential strategy for progress in future clinical research.

2. C-reactive protein (CRP): a clinical marker of inflammation and outcome measure in research

The acute phase product C-reactive protein (CRP) is typically used to evaluate systemic levels of inflammation in the management of chronic inflammatory diseases and assessment of CVD risk. CRP is produced hepatically in response to elevated plasma inflammatory cytokines (TNF-α and IL-6), which spill over into the systemic circulation from sites of inflammation. The plasma concentration of these inflammatory cytokines can fluctuate rapidly (hour-to-hour), but CRP serves as a more stable reflection of their production during periods of inflammation. CRP concentrations are markedly elevated during periods of active pathology, such as rheumatoid arthritis and inflammatory bowel disease flares. The American Heart Association (AHA) has established evidence-based guidelines defining cardiovascular risk categories based on CRP levels, where values <1 mg/L represent low risk, values 1–3 mg/L represent intermediate risk, and values >3 mg/L represent high risk [21]. As values greater than 10 mg/L may represent acute infection, it is recommended that a re-evaluation be performed after at least 2 weeks to determine whether such elevations are acute or chronic. Persistently high CRP levels—such as those that occur with rheumatoid arthritis—signify radically increased risk of CVD events and mortality. Clinicians can order either a standard CRP to monitor active inflammation or a high sensitivity (also referred to as "cardio") CRP (hs-CRP) test that can quantify CRP values <10 mg/L (lower limit = 0.2 mg/L). Diagnostic labs also do not typically measure IL-6 and TNF-α.

Box 1: Common causes of acute CRP elevation that may occur in clinical research participants*

- Viral illness (Ex: cold or flu exposure)
- Bacterial infection (Ex: urinary tract infection)
- Dental cleanings or procedures
- Unusual strenuous physical activity (Ex: marathon race, downhill running, or implementation of a new weight lifting routine)
- Exacerbation or "flare" of inflammatory disease unrelated to study
- Change in medications (Ex: withdrawal of anti-inflammatory medication)
- Physical trauma (Ex: cuts, scrapes, contusions, sprains, strains, and broken bones)
- Significant changes in psychosocial stressors (Ex: becoming a caretaker, losing a job, or death of a loved one)
- Prolonged sleep deprivation
- Significant weight gain or dramatic dietary changes

*It is possible to minimize these inflammatory insults by counseling participants to avoid certain activities prior to measurements and rescheduling when such events are reported. However, bacterial and viral exposures can be asymptomatic, and many other events go unreported.

Although CRP concentrations are more stable than plasma cytokines, they can exhibit large day-to-day variations due to acute inflammation that is unrelated to either study procedures or the target inflammatory conditions. This means that significant treatment effects are contingent on a large magnitude of response to an intervention. Additionally, CRP elevations may be present even in the absence of symptoms because CRP remains elevated for several days following the resolution of acute inflammation. Exacerbations of acute inflammation can be caused by an assortment of factors and may not always be perceived and/or reported by research participants (Box 1).

In addition to reflecting active inflammation, CRP values can be reduced by anti-inflammatory therapies. Immunosuppressive therapies, including anti-TNF biologics, decrease CRP concentrations and CVD risk in people with rheumatoid arthritis [22–24]. However, these therapies carry a significant risk of side effects, which limits their use. In several large trials, including the JUPITER study, statin administration also reduced CRP values [25]. Such trials demonstrate that CRP can function as a modifiable endpoint for clinical research on anti-inflammatory interventions.

3. Evaluating existing clinical evidence for the efficacy of marine omega-3 fatty acids to reduce plasma CRP

Although many clinical trials have assessed whether supplemental EPA and/or DHA can reduce plasma CRP, it is not typically the primary endpoint. A systematic review by Rangel-Huerta et al. identified and evaluated the randomized placebo-controlled clinical trials investigating the potential anti-inflammatory effects of omega-3 fatty acid supplementation [19]. For the present discussion, additional recently published studies were added to those originally reviewed by Rangel-Huerta et al. (Table 1). Only randomized controlled trials in which CRP was measured are included.