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## The Role of $\omega$ -3 Polyunsaturated Fatty Acids in Programming Inflammation Resolution

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# The Role of $\omega$ -3 Polyunsaturated Fatty Acids in Programming Inflammation Resolution

## **Disciplines**

Pharmacy and Pharmaceutical Sciences

## **Comments**

Poster presented at Faculty Scholarship Celebration, St. John Fisher College, October 25, 2012.

## Abstract

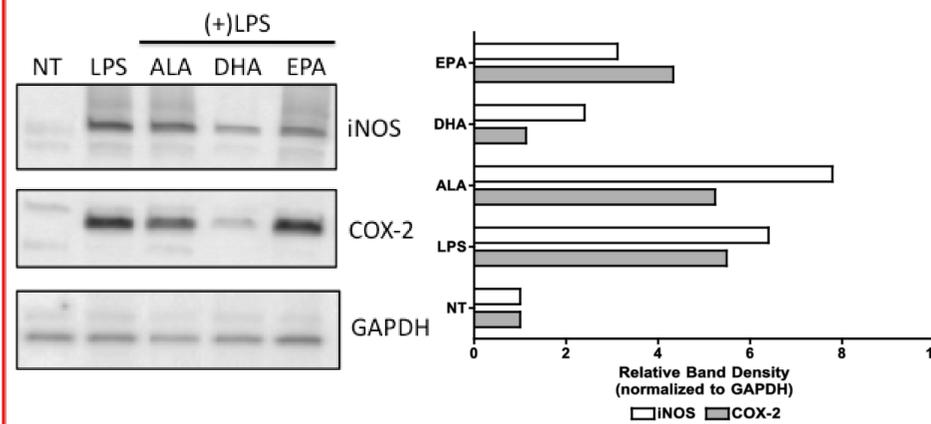
**Objective:** Macrophages are important to study in the context of inflammation given that their physiological locality situates these cells to respond to the initial inflammatory insult. Dietary supplementation with omega-3 polyunsaturated fatty acids ( $\omega$ -3 PUFAs) have been shown to decrease levels of inflammatory mediators produced by macrophages, yet little is known with regard to their therapeutic mechanism of action(s). This study is set to determine if the introduction of  $\omega$ -3 PUFAs promote inflammation-resolution as a result of modifying the functional phenotype and/or molecular pathway profile of activated macrophages during an inflammatory cascade.

**Methods:** As a model for macrophage immune cell function, RAW264.7 cells, a murine-derived macrophage cell line, was exposed to inflammatory stimuli following pre-treatment with n-3 PUFAs. Cells were treated with different doses/combinations of alpha linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) prior to macrophage activation to assess the combinatory impact of dietary n-3 PUFAs. To model an inflammatory event, RAW264.7 were stimulated with bacterial lipopolysaccharide (LPS). Cell culture supernatants and cell lysates were collected and analyzed for different biomarkers of inflammation. The tests performed included Griess reaction (to measure the production of nitrite) and western blots (to measure levels of inflammatory proteins).

**Results:** A decrease in the inflammatory markers cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were detected by western blot analyses with various concentrations and DHA and EPA, compared to control samples. The most significant decreases in nitrite levels were observed in cultures treated alone or in combination with DHA. Current studies are underway to evaluate changes in the functional phenotype of macrophages (i.e. phagocytic ability, TNF-alpha expression) following  $\omega$ -3 PUFA treatment and LPS activation.

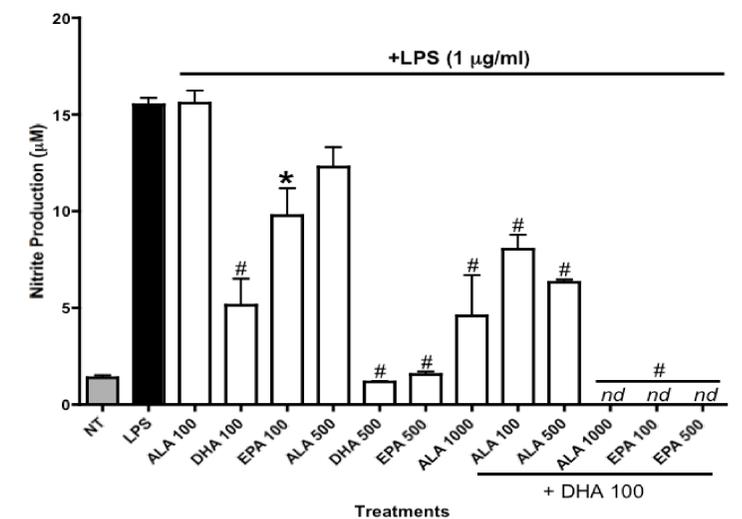
**Clinical Implications:** Understanding the biological role of n-3 PUFAs in inflammation will advance the knowledge-base into the therapeutic role it can play in reducing inflammation and developing novel therapies to treat chronic inflammatory diseases.

## Exposure to $\omega$ -3 PUFAs attenuates iNOS and COX-2 expression in LPS-stimulated RAW264.7 macrophages



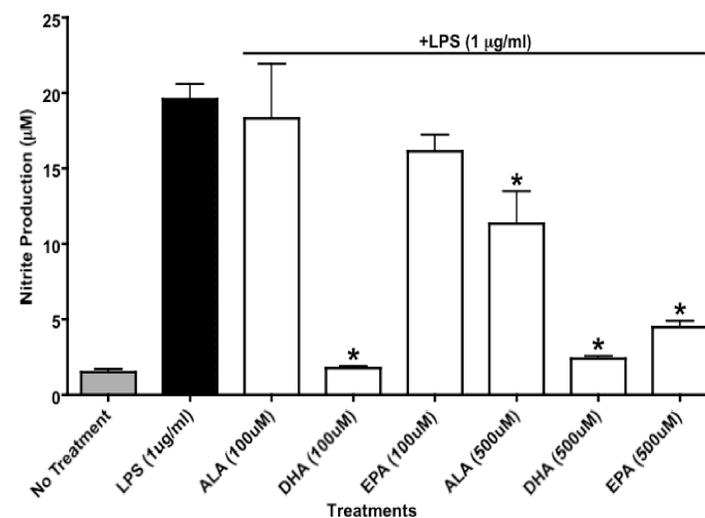
Western blot: RAW264.7 macrophages were seeded at a density of 200,000 cells/ml and pre-treated with  $\omega$ -3 PUFAs  $\sim$ 18 hours prior to a 24 hour LPS (1  $\mu$ g/ml) stimulation. All  $\omega$ -3 PUFAs were tested at a dose of 100  $\mu$ M. Non-treated (NT) cultures were treated with the same final concentration of ethanol as vehicle. Cell lysates were collected and subjected to western blot analysis using an anti-iNOS, anti-COX-2, or anti-GAPDH antibody. The antibody-specific bands were quantified by densitometry and were normalized to GAPDH. Blot shown is a representative image from 3 independent experiments.

## Addition of DHA in combination with another $\omega$ -3 PUFAs results in additive/synergistic attenuation of nitric oxide (nitrite) production in LPS-stimulated RAW264.7 macrophages



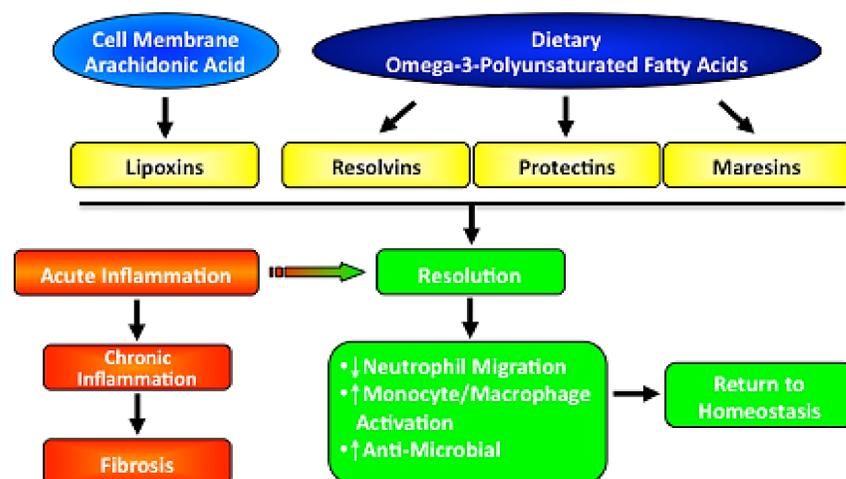
Nitric oxide (NO) production was indirectly assessed as previously described. RAW264.7 macrophages were seeded at a density of 200,000 cells/ml and pre-treated with the indicated concentrations (in  $\mu$ M) and combinations of  $\omega$ -3 PUFAs  $\sim$ 18 hours prior to a 24 hour LPS (1  $\mu$ g/ml) stimulation. Data is represented as mean  $\pm$  SEM for  $n=3$ . \* $p$ <0.01 versus LPS-treated cultures by one-way ANOVA; # $p$ <0.001 versus LPS-treated cultures by one-way ANOVA. (nd = not detected by sensitivity of assay.)

## Exposure to $\omega$ -3 PUFAs attenuates the levels of nitric oxide (nitrite) production in LPS-stimulated RAW264.7 macrophages



Nitrite Measurement: Nitric oxide (NO) production was indirectly assessed by measuring nitrite (a primary metabolite of NO) levels in cultured media (colorimetric-based Griess reaction). RAW264.7 macrophages were seeded at a density of 200,000 cells/ml and pre-treated with  $\omega$ -3 PUFAs  $\sim$ 18 hours prior to a 24 hour LPS (1  $\mu$ g/ml) stimulation. Data is represented as mean  $\pm$  SEM for  $n=3$ . \* $p$ <0.01 versus LPS-treated cultures by one-way ANOVA

## Background



>Resolution of inflammation is an active process mediated by lipid mediators, and return to homeostasis is maintained by a balance between pro-inflammatory and pro-resolving pathways  
 >Omega-3 PUFAs have anti-inflammatory and pro-resolving activity in pre-clinical disease models (e.g. peritonitis, colitis, asthma)  
 >Chronic inflammation is a key factor in the pathogenesis of numerous diseases (e.g. COPD, RA, IBD)

## Conclusions

>The action of  $\omega$ -3 PUFAs drives the decrease in proinflammatory mediators: COX-2, iNOS, and nitric oxide (nitrite)  
 >DHA is shown to have additive/synergistic effects when combined with other  $\omega$ -3 PUFAs  
 >The polarization of macrophages during inflammatory responses to functionally distinct phenotypes may play a role in both inflammation and resolution of inflammation following treatment with  $\omega$ -3 PUFAs.

