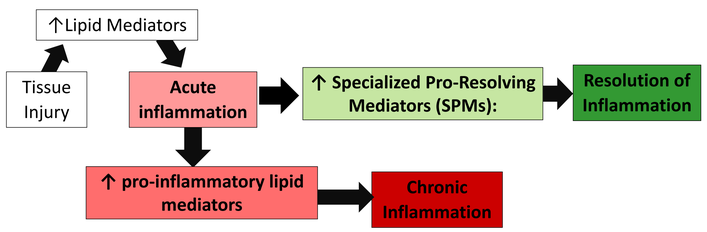
Dietary DHA Mitigates Ozone Induced Pulmonary Inflammation and Reductions in Specialized Pro-Resolving Mediators

Kymberly M. Gowdy[1], B. Kilburg-Basnyat[1], S.W. Reece[1], M. Hodge[1], E. Browder[1], C. Psaltis[1], J. Manke[2], M.L. Armstrong[2], N. Reisdorph[2], R. Tighe[3], S.R. Shaikh[4]

[1]Department of Pharmacology and Toxicology, East Carolina University; [2]School of Pharmacy, University of Colorado, Denver, Denver, CO; [3]Department of Medicine, Duke University Medical Center, Durham, NC; [4]Department of Nutrition, University of North Carolina Chapel Hill, Chapel Hill, NC.

Background

- Pulmonary exposure to air pollutants, such as ozone (O_3), leads to enhanced pulmonary and cardiovascular morbidity and mortality (Alfaro-Moreno *et al.,* 2007; Nawrot *et al*., 2006; Pope *et al.*, 2002).
- O_3 exposure has been reported to induce pulmonary inflammation and alter systemic lipid metabolism in rodent and human models (Robertson *et al.,* 2013; Peter *et al.,* 2004).
- During resolution of inflammation, the immune system switches from pro-inflammatory lipid mediator production to the production of specialized pro-resolving mediators (SPMs).
- Previously, we have shown that O_3 exposure reduces pulmonary SPM levels are decreased including docosahexaenoic acid (DHA)-derived 17-hydroxydocosahexaenoic acid (17-HDHA, resolvin D1 precursor) and protectin D1 (PD1). (Kilburg-Basnyat *et al,* 2018).

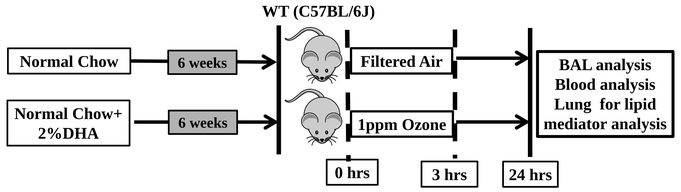


- Western diet (defined as a diet containing high intake of saturated fats) has been associated with increased respiratory symptoms and lower lung function in multiple lung diseases (Brigham et al., 2018).
- DHA is a n-3 polyunsaturated fatty acid (n-3 PUFA) that is poorly consumed in the western diet (Calder et al., 2012).
- Dietary DHA intake has been associated reduced inflammation in infectious and non-infectious lung diseases (Farjadian et al., 2016; Bates et al., 2018; Kosaraju et al., 2017).
- Recently, n-3 PUFA intake has been linked with decreased exacerbation of pollution driven lung diseases (Brigham et al., 2019).
- It is currently unknown if dietary intake of DHA decreases O_3-induced pulmonary inflammation and injury and mitigates reductions in DHA-derived SPMs.

Hypothesis

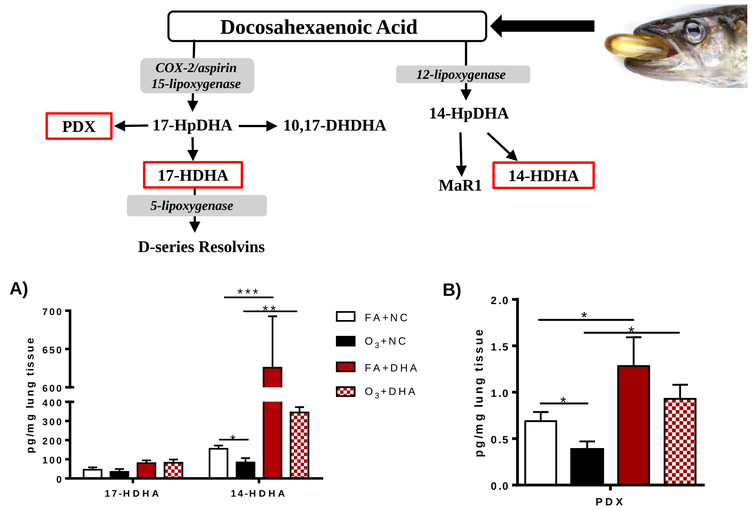
Dietary supplementation with the parental fatty acid DHA mitigates O_3-induced pulmonary inflammation by increasing pulmonary levels of DHA-derived SPMs.

Murine model of ozone exposure



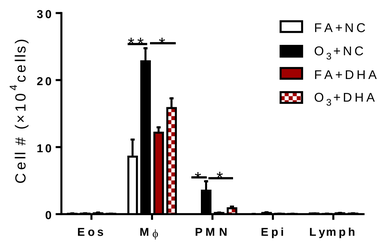
C57Bl/6J (WT) male mice were fed normal chow (NC) or NC+ 2%DHA diet (NC+DHA) for 6 weeks followed by a 1h exposure to 1ppm of O_3 or FA. 24h following exposure, mice were necropsied.

Figure 1. DHA supplementation increases pulmonary SPMs.



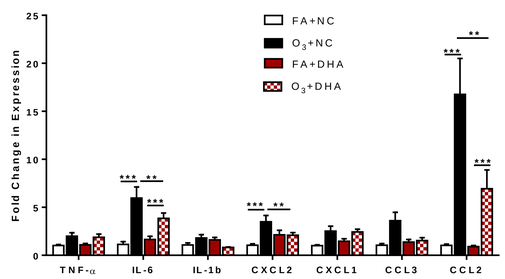
Male wildtype (WT) mice were fed normal chow (NC) or normal chow supplemented with 2% DHA (DHA) diets for 6 weeks. After 6 weeks of diet, mice were exposed to filtered air (FA) or 1ppm of O_3 for 3hrs. Mice were necropsied 24 hrs after exposure and lung tissue was used for LC-MS/MS analysis. **A)** Lung tissue levels of SPM precursors 17(R)-HDHA (17-HDHA) and 14(S)-HDHA (14-HDHA) and **B)** levels of the 17(S)-DiHDoHE (PDX). * p< 0.05; ** p< 0.01; *** p< 0.001.

Figure 2. Dietary DHA supplementation reduces O_3-induced cellular inflammation into the airspace.



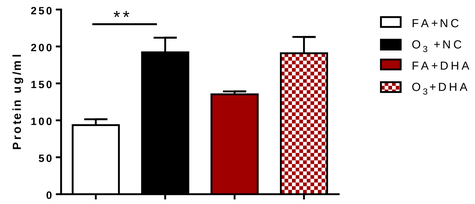
Male wildtype (WT) mice were fed normal chow (NC) or normal chow supplemented with 2% DHA (DHA) diets for 6 weeks. After 6 weeks of diet, mice were exposed to filtered air (FA) or 1ppm of O_3 for 3hrs. Mice were necropsied 24 hrs after exposure and bronchoalveolar fluid (BAL) was used for cellular differentials. * p< 0.05; ** p< 0.01.

Figure 3. DHA supplementation reduces select O_3-induced cyto/chemokine expression in the lung.



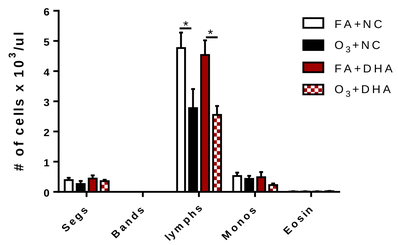
Male wildtype (WT) mice were fed normal chow (NC) or normal chow supplemented with 2% DHA (DHA) diets for 6 weeks. After 6 weeks of diet, mice were exposed to filtered air (FA) or 1ppm of O_3 for 3hrs. Mice were necropsied 24 hrs after exposure and lung tissue was harvested and analyzed for cytokines and chemokines expression by real time PCR. * p< 0.05; ** p< 0.01; *** p< 0.001.

Figure 4. Dietary DHA does not alter O_3-induced pulmonary injury.



C57Bl/6J (WT) male mice were fed normal chow (NC) or normal chow supplemented with 2% DHA (DHA) diets for 6 weeks followed by a 1h exposure to 1ppm of O_3 or FA. Lungs were lavaged and analyzed for total protein (n=10 per group). ** p< 0.01.

Figure 5. Dietary DHA does not alter circulating immune cells.



C57Bl/6J (WT) male mice were fed normal chow (NC) or normal chow supplemented with 2% DHA (DHA) diets for 6 weeks followed by a 1h exposure to 1ppm of O_3 or filtered air (FA). 24h following exposure, mice were necropsied and blood was analyzed for complete blood counts (n=10 per group). * p< 0.05.

Conclusions and Future Directions

- Ozone exposure reduces SPM levels in lung while increasing inflammation.
- Dietary DHA increase pulmonary SPM levels.
- Dietary DHA mitigates ozone induced lung inflammation by:
  - decreasing BAL macrophages and neutrophils.
  - decreasing pro-inflammatory cytokine IL-6, monocyte chemoattractant CCL2, and neutrophil chemoattractant CXCL2.
- Total protein was increased in BAL fluid in all diet groups exposed to O_3.
- Circulating immune cells were unaltered with dietary DHA supplementation.

- **Overall Conclusion: Supplementing a diet with DHA increases pulmonary SPMs and their precursors resulting in a decrease in O_3-induced cellular infiltration and subsequent cyto/chemokine production. This data supports the novel idea that a DHA supplemented diet may mitigate O_3-induced pulmonary responses by increasing pulmonary SPMs.**

- ***Future directions:***
  - *Determine if a DHA-supplemented diet alters TLR4 driven inflammation in the lung after O_3 exposure.*
  - *Examine if O_3 exposure decreases SPM concentrations in specific pulmonary immune cell subsets (alveolar, interstitial, exudative macrophages).*
  - *Evaluate whether or not SPM signaling is decreased in the lung during O_3 exposure, driving pulmonary inflammation and injury.*

Acknowledgements

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