

Enteral Docosahexaenoic Acid  
Supplementation To Attenuate Inflammation  
In The Preterm Infant

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## ABSTRACT

Preterm infants have an underdeveloped immune system and as such they are predisposed to developing unregulated inflammatory responses that are associated with disease in the postnatal period. Docosahexaenoic acid (DHA) is an omega-3 long-chain polyunsaturated fatty acid (LCPUFA) with known immunomodulatory properties, however the effect of dietary DHA on the regulation of immune responses in preterm infants is largely unknown. This thesis employs a multi-system approach to address questions related to the efficacy of omega-3 DHA to regulate inflammation in preterm infants and in human type II alveolar epithelial cells (AEC). The N3RO randomised controlled trial (RCT) provided the opportunity to carry out a single-centre nested study to examine the effect of supplemental DHA in preterm infants on pro-inflammatory and regulatory biomarkers in blood and levels of a common bacterial pathogen in the gastrointestinal tract. The aim of the N3RO RCT was to assess the efficacy of an enteral DHA emulsion to reduce bronchopulmonary dysplasia (BPD) in preterm infants < 29 weeks gestation compared to a standard soy emulsion without DHA.

Prior to analysis of biological samples from preterm infants, the immune response to enteral DHA and soy emulsions in human type II AECs, one of the primary cell types affected in respiratory disorders, was assessed *in vitro*. The enteral emulsions assessed in the N3RO RCT were tested in conjunction with other commercially available parenteral lipid emulsions. Omega-3 DHA in both enteral and parenteral emulsions significantly reduced pro-inflammatory cytokines (IL-1 $\beta$ , IL-8 and IFN $\gamma$ ) when compared to soy-based emulsions.

There are very few studies that have assessed what, if any, targets DHA interacts with to exert an immunomodulatory effect in preterm infants. Inflammatory cytokines are known to play a crucial role in the progression of airway inflammation, epithelial and vascular damage and subsequent development of BPD. Such inflammatory mediators are also involved in the

development of other neonatal inflammatory disorders such as sepsis, necrotising enterocolitis and retinopathy of prematurity. A total of 144 blood samples were collected from 51 preterm infants enrolled in the nested study. Supplemental DHA did not reduce pro-inflammatory cytokine levels in plasma or whole blood culture supernatants (after a 24 hour incubation with *E. coli* lipopolysaccharide).

Inflammatory mediators in the gut environment can influence initial colonisation and resulting abundance of both commensal and pathogenic bacteria. *Staphylococcus* is among the first colonisers of the respiratory and gastrointestinal tracts and it is one of the most important pathogens in the neonatal intensive care unit. Colonisation by methicillin-resistant bacteria including *Staphylococcus* in preterm infants also causes significant morbidity and mortality in the neonatal intensive care unit. In the neonatal period, diet has a significant effect on microbial colonisation of the gut, however the effect of supplemental omega-3 LCPUFA on *Staphylococcus* colonisation in preterm infants is unknown. A total of 220 stool samples were collected from 41 preterm infants enrolled in the nested study. Levels of *Staphylococcus* and bacteria carrying the gene coding for methicillin-resistance (*mecA*) decreased significantly over time in both groups, but DHA did not have an effect on abundance.

The original contribution this thesis makes to the knowledge base is that supplementing preterm infants < 29 weeks gestation enterally with 60 mg/kg/day of DHA does not affect circulating levels of pro-inflammatory or regulatory cytokines, the immune response to an infectious stimuli nor does it influence *Staphylococcus* and *mecA*<sup>+</sup> bacteria in the gut. This thesis contributes important information regarding the use of DHA at supplemental levels in nutrition regimens for preterm infants.

## DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Naomi H. Fink

Date: 17 February, 2017

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**LIST OF ABBREVIATIONS**

|                |  |
|----------------|--|
| AA             | Arachidonic acid                           |
| ACTRN          | Australian Clinical Trials Registry Number |
| AEC            | Alveolar epithelial cell                   |
| ALA            | Alpha linolenic acid                       |
| APC            | Antigen presenting cell                    |
| ATCC           | American Type Culture Collection           |
| BPD            | Bronchopulmonary dysplasia                 |
| CA             | Corrected age                              |
| CD             | Cluster of differentiation                 |
| CRF            | Case report form                           |
| CRP            | C-reactive protein                         |
| C <sub>t</sub> | Cycle threshold                            |
| DHA            | Docosahexaenoic acid                       |
| DMSO           | Dimethylsulfoxide                          |
| DPPE           | Dipalmitoylphosphatidylcholine             |
| EFA            | Essential fatty acid                       |
| EN             | Enteral nutrition                          |
| EPA            | Eicosapentaenoic acid                      |
| FACS           | Fluorescence-activated cell sorting        |
| FBS            | Fetal bovine serum                         |
| GA             | Gestational age                            |
| GC             | Gas chromatography                         |
| GPR            | G-protein coupled receptor                 |

**LIST OF ABBREVIATIONS (CONTINUED)**

|                                  |  |
|----------------------------------|--|
| GSH-PX                           | Glutathione peroxidase                 |
| LCPUFA                           | Long-chain polyunsaturated fatty acids |
| HCl                              | Hydrochloric acid                      |
| H <sub>2</sub> SO <sub>4</sub>   | Sulfuric acid                          |
| KCl                              | Potassium chloride                     |
| KH <sub>2</sub> PO <sub>4</sub>  | Potassium phosphate                    |
| IL                               | Interleukin                            |
| IVH                              | Interventricular haemorrhage           |
| ITT                              | Intention to treat                     |
| LA                               | Linoleic acid                          |
| LPS                              | Lipopolysaccharide                     |
| LxA4                             | Lipoxin A4                             |
| MIP                              | Macrophage inflammatory protein        |
| MinDC                            | Minimum detectable concentration       |
| MUFA                             | Monounsaturated fatty acid             |
| NaCl                             | Sodium chloride                        |
| NaOH                             | Sodium hydroxide                       |
| Na <sub>2</sub> HPO <sub>4</sub> | Sodium phosphate                       |
| NICU                             | Neonatal intensive care unit           |
| NEC                              | Necrotising enterocolitis              |
| PBS                              | Phosphate buffered saline              |
| PN                               | Parenteral nutrition                   |
| PMA                              | Postmenstrual age                      |

**LIST OF ABBREVIATIONS (CONTINUED)**

|       |  |
|-------|--|
| PP    | Per protocol                               |
| PPAR  | Peroxisome proliferator-activated receptor |
| PUFA  | Polyunsaturated fatty acid                 |
| RBC   | Red blood cell                             |
| RCT   | Randomised controlled trial                |
| ROP   | Retinopathy of prematurity                 |
| RvD1  | Resolvin D1                                |
| SCBU  | Special care baby unit                     |
| SFA   | Saturated fatty acid                       |
| SOD   | Superoxide dismutase                       |
| SOP   | Standard operating procedure               |
| SP    | Surfactant protein                         |
| TAE   | Tris base, acetic acid and EDTA buffer     |
| TAP   | Total antioxidant potential                |
| T-AOC | Total antioxidant capacity                 |
| TBL   | Total bacterial load                       |
| TGF   | Transforming growth factor                 |
| Th    | T helper                                   |
| TLR   | Toll-like receptor                         |
| TNF   | Tumor necrosis factor                      |
| TPN   | Total parenteral nutrition                 |
| T-reg | T regulatory                               |
| VLBW  | Very low birth weight                      |

**LIST OF ABBREVIATIONS (CONTINUED)**

|     |                                 |
|-----|---------------------------------|
| WCH | Women’s and Children’s Hospital |
| <   | Less than                       |
| >   | Greater than                    |