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-1β, IL-8 and IFNγ) 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*+ 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

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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_t	Cycle threshold
DHA	Docosahexaenoic acid
DMSO	Dimethylsulfoxide
DPPC	Dipalmatoylphosphtidylcholine
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_2SO_4	Sulfuric acid
KCl	Potassium chloride
KH_2PO_4	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_2HPO_4	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