OMEGA-3 FATTY ACIDS IN THE EARLY ORIGINS OF METABOLIC SYNDROME

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ABSTRACT

The role of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA), in particular docosahexaenoic acid (DHA) in decreasing fat deposition, fat cell formation and improving insulin sensitivity *in vitro* and in adult animals had led to suggestions that increasing the supply of these fatty acids before birth could improve later metabolic health outcomes in the child. However, few studies had explored the role of DHA in programming of obesity and type 2 diabetes, and the results had been inconsistent. Furthermore, the mechanisms through which exposure to an increased supply of DHA during development impacted on later health outcomes in the infant were also unclear. While studies in animal models had highlighted the importance of epigenetics in the link between intrauterine nutrition and the subsequent risk of obesity and insulin resistance in the offspring, the role of whether epigenetics in the prenatal programming of obesity in humans was unknown. In addition, while there was evidence that placental alterations played a critical role in developmental programming, few studies had explored the effect of DHA on placental gene expression/function. The central aim of this thesis was to determine the effect of a specific nutritional intervention (maternal DHA supplementation) on (1) markers of metabolic health (BMI, percent body fat, insulin sensitivity) in the children at 5 years of age and (2) global and gene-specific DNA methylation profiles in children at birth and at 5 years of age. I also aimed to determine the effect of DHA on placental proliferation and gene expression *in vitro*.

This thesis studied children born to women who participated in a large randomised controlled trial of n-3 LCPUFA supplementation in pregnancy, the DOMInO trial, at birth and 5 years of age. Insulin sensitivity in these children was estimated from the

HOMA-IR index at 5 years of age. DNA was obtained from blood samples of the DOMInO children collected at birth (n=1012) and at 5 years of age (n=715) for the assessment of global and genome-wide methylation. A human placenta first trimester cell line (HTR8/SVneo) was treated with a DHA-rich emulsion in order to determine effects of DHA on placental proliferation and gene expression.

Maternal DHA supplementation was associated with a reduced insulin sensitivity (increased HOMA-IR index) and increased fasting insulin concentrations in the children at 5 years of age, particularly in males. There were also small but significant differences in methylation of 44 genomic regions at birth and 30 at 5 years of age, and more differentially methylated regions (DMRs) in males compared to females at both time points. DHA treatment increased proliferation rate of HTR8/SVneo cells, and 96 genes were differentially expressed between DHA and no treatment groups. Overall, the results of this thesis provide evidence that maternal DHA supplementation may reduce insulin sensitivity in the children, although whether this translates into differences in the incidence of type 2 diabetes later in life remains to be determined. I also demonstrated that DHA has small but significant effects on DNA methylation of specific genomic regions, and significantly altered proliferation and gene expression of a placental cell line *in vitro*, which suggests that both epigenetic and placental modifications may be involved in mediating the effects of increased DHA exposure before birth on health outcomes in the child.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree of diploma 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 wok will, in the future, be used in a submission for any other degree of 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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Jing Zhou

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LIST OF ABBREVIATIONS

5hmeU	5-Hydroxymethalted Uracil
AA	Arachidonic Acid
AID	Activation-Induced Cytidine Deaminase
ALA	Alpha-Linolenic Acid
ANGPTL4	Angiopoietin-Like 4
ANOVA	One-Way Analysis of Variance
AOX	Acyl-Coa Oxidase
APOBEC	Apolipoprotein B Mrna Editing Enzyme
ARHFAP35	Rho Gtpase Activating Protein 35
ASNS	Asparagine Synthetase
BHT	Butylated Hydroxyanisole
BIS	Bioelectrical Impendence Spectroscopy
BMI	Body Mass Index
C	Cytosine
CAST	Calpastatin
CCK	Cholecystokinin
CI	Confidence Interval
CpG	Cytosine Phosphate Guanine
CV	Coefficient of Variation
DHA	Docosahexaenoic Acid
DMAC	Data Management and Analysis Centre
DME	Demeter
DMRs	Differently Methylated Regions
DNA	Deoxyribonucleic Acid
DNMT	DNA Methyltransferase
DOMInO	DHA to Optimise Mother Infant Outcome
DPA	Docosapentaenoic Acid
EPA	Eicosapentaenoic Acid
ESPCR	End-Specific PCR
FA	Fatty Acid
FADS2	Fatty Acid Desaturase
FAME	Fatty Acid Methyl Esters
FDR	False Discovery Rate
FID	Flame Ionisation Detector
FMC	Flinders Medical Centre
FO	Fish Oil
Foligos	Facilitator Oligonucleotides
G	Guanine
GC	Gas Chromatograph
GEE	Generalised Estimating Equation
GR	Glucocorticoid Receptor
HOMA-IR	Homeostatic Model Assessment Of Insulin Resistance

IPA	Ingenuity Pathways Analysis
IQ	Inter-Quartile Range
IQL	Instrument Quantitation Limit
LA	Linoleic Acid
LINE-1	Long Interspersed Nucleotide Element 1
LOD	Limit of Detection
MAGEB3	Melanoma Antigen Family B3
MeCP2	Methyl Cpg Binding Protein-2
MGLL	Monoglyceride Lipase
miRISC	Microrna-Induced Silencing Complexes
MQL	Method Quantitation Limit
mRNA	Messenger RNA
N	Number
n-3 LCPUFA	Omega-3 Long Chain Polyunsaturated Fatty Acids
NFAT5	Nuclear Factor of Activated T-Cells 5
NSCs	Child's Newborn Screening Cards
OECD	Organisation for Economic Co-Operation and Development
PANTHER	Protein Analysis Through Evolutionary Relationships
PCR	Polymerase Chain Reaction
PEPCK	Phosphoenolpyruvate Carboxykinase
POMC	Pro-Opiomelanocortin
PPAR	Peroxisomal Proliferator-Activated Receptor
PPFIBP1	Protein Tyrosine Phosphatase Receptor Type F Polypeptide-Interacting Protein-Binding Protein 1
PTV	Programmed Temperature Vaporization Injector
RBC	Red Blood Cells
RCTs	Randomised Controlled Trials
RISC	RNA-Induced Silencing Complexes
RNA Pol	RNA Polymerase
ROS1	Glycosylases Repressor Of Silencing 1
RQI	Rna Quality Indicator
RSD	Relative Standard Deviation
RXRA	Retinoid X Receptor-A
SAH	S-Adenosyl Homocysteine
SAM	S-Adenosyl Methionine
SAMD4A	Sterile Alpha Motif Domain Containing 4A
SD	Standard Deviation
SNP	Single Nucleotide Polymorphism
SO	Soy Oil
SREBP	Sterol Regulatory Element Binding Protein
SRM	Serum Reduced Media
STON1	Stonin1
T	Thymine
TDG	Thymine DNA Glycosylase

TLC	Thin Layer Chromatography
VMRs	Variable Methylated Regions
WCH	Women's And Children's Hospital
WHO	World Health Organisation