A Randomised Controlled Trial of DHA-Rich Fish Oil Supplementation During Pregnancy and Subsequent Development of Language in Early Childhood

NICOLA R GAWLIK

School of Paediatrics and Reproductive Health

Discipline of Paediatrics

University of Adelaide

June 2016

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ABSTRACT

There is no more important period in human development than conception through early childhood in maximizing developmental potential. It is during the last trimester of pregnancy when brain development accelerates (1, 2) and where accumulation of docosahexaenoic acid (DHA) in neural tissues occurs most rapidly (1, 3). Dietary intake and maternal stores of DHA during pregnancy and lactation have important implications for the developing brain. Uncertainty surrounding the ability of Westernised diets to fulfill requirements of DHA during pregnancy has raised concern for the developmental outcome of children raised in this dietary context (4).

Some children in Australia have very limited language ability, impacting both the individual and society. Intervention for language development during the early years should be a primary focus for research. The role that DHA might play presents as a compelling area of investigation undertaken in this thesis.

This thesis contains a literature review, including a systematic review and meta-analysis, and also proposes a theoretical framework from which to understand the potential variation in language development as a function not only of DHA but also of interacting biological and social variables **(Chapter 1)**. The methods used in the current study are detailed **(Chapter 2)**. Within a randomised controlled trial design **(Chapter 3)** the current study investigates whether DHA supplementation during the prenatal period has an

effect on language development at 4 years of age. Interactions between DHA and other individually contributing factors posed by the bio-ecological model **(Chapter 4)** and relationships between markers of DHA and language development **(Chapter 5)** are examined**.** A model proposed to provide a broader or more comprehensive conceptualization of the role of DHA within the larger system of influences on language development was tested **(Chapter 6)**.

The current study found no significant effect of DHA supplementation during pregnancy on children's language development at 4 years of age as measured by the primary outcome of the current study: mean Core Language Scores, assessed using the second edition of the Clinical Evaluation of Language Fundamentals Preschool. There were no significant interactions between treatment group and child sex, maternal age, in utero exposure to maternal cigarette smoking or alcohol consumption, or maternal depression. There was, however, a significant interaction for maternal education. There was also no significant relationship between markers of DHA status and language development for the whole group, and no significant difference in language development between those with cord blood DHA in the 25th and 75th percentile. There were, however, both significant positive and negative relationships between the number of fish meals and DHA foods (respectively) the child consumed in the month prior to the 4-year assessment and language development at 4 years of age. Findings from structural equation modelling analyses provided no support for understanding the relationship between DHA and children's language

development through focusing on the relationships proposed by the bio-ecological model.

Overall, findings suggest that prenatal DHA supplementation does not benefit children's language development. Longer-term follow-up of early DHA supplementation is required to determine whether delayed effects emerge.

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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Nicola Gawlik

21 June 2016

ACKNOWLEDGEMENTS

First and foremost, thank you to the children and their parents who so generously volunteered their time and support. Without you, the completion of this research would not have been possible.

I offer my sincerest gratitude to my supervisors, Prof Maria Makrides and Dr Lisa Kettler, who have supported me throughout my thesis with their patience and knowledge whilst allowing me the scope to work in my own way. Their advice, supervision, and crucial contribution made them a backbone of this research and so too this thesis.

To Dr Julie Robinson for perusing my thesis proposal with a critical eye and providing many helpful suggestions and ideas, I thank you for your initial input and attention to detail.

Many thanks to all the staff at the Child Nutrition Research Centre who helped me collect my data. In particular, I would like to thank Helen Loudis for adding a smile, laugh, and shot of inspiration to my days.

I also thank those who have offered personal support during this study: Jessica Collins, Georgia Lawrence, Lauren Frensham, Lenka Malek, Jacqueline Gould, Ashlee Davies, Anna Seamark, Helen Thorne, Suzy Stiles, and Natalie Fuller. Thank you for listening to my stream of consciousness, for the uncontrollable laughter, and for the free flowing wine and countless cups of tea!

More recently but by no means less importantly, I have greatly valued the support and wisdom of Ms Rosemary Hambledon and Dr Claire Ralfs. Together they have not only helped me across the line but have triggered and nourished my intellectual maturity that I will benefit from for a long time to come.

To my Mum and Dad, Mary and Konrad, thank you for your unflagging love and unconditional support. You have always taught me to do things well, pursue what interests me, and work with pride on whatever is in front of me. Without you (tolerating me), what I have achieved might not have been possible!

I would also like to thank everybody who was important to the successful realisation of this thesis, as well as expressing my apologies that I could not mention each individual personally.

Finally, it is with my deepest gratitude and love that I dedicate this thesis to my grandparents who have been a constant source of knowledge and inspiration. And also for the myriad of ways in which, throughout my life, they have actively supported me in my determination to find and realize my potential, and to make a positive contribution to our world.

GLOSSARY

AA	Arachidonic Acid
AEDC	Australian Early Development Census
AEDI	Australian Early Development Index
AI	Adequate Intake/s
ALA	Alpha linolenic Acid
ASQ	Ages and Stages Questionnaire
Bayley-II	Bayley Scales of Infant Development, Second Edition
Bayley-III	Bayley Scales of Infant Development, Third Edition
BRIEF-P	Behaviour Rating Inventory of Executive Function–Preschool
BW	Birth Weight
C	Capsule
CA	Corrected Age
CBCL	Child Behaviour Checklist
CELF P-2	Clinical Evaluation of Language Fundamentals Preschool, Second Edition
CFI	Comparative Fit Index
CI	Confidence Interval
CLAMS	Clinical Linguistic and Auditory Milestone Scale
CLS	Core Language Score
CNS	Central Nervous System
Ctrl	Control
d	Day/s

DAS-II	Differential Abilities Scales, Second Edition
DHA	Docosahexaenoic Acid
Diff	Difference
DNBC	Danish National Birth Cohort
DNS	Day Night Stroop
DOMInO	DHA to Optimise Mother Infant Outcomes
DPA	Docosapentaenoic Acid
Egg-DTG	Egg-Derived Triglyceride
ELVS	Early Language in Victoria Study
EP	Egg Phospholipid
EPA	Eicosapentaenoic Acid
EV	Expressive Vocabulary
F	Formula
FA	Fatty Acid/s
FAD GF	Family Assessment Device – General Functioning subscale
FAS	Fetal Alcohol Syndrome
FASD	Fetal Alcohol Spectrum Disorders
FMC	Flinders Medical Centre
FO	Fish Oil
g	Grams
GA	Gestational Age
GMDS	Griffiths Mental Development Scales
GP	General Practitioner

H	Hypothesis
HM	Human Milk
HSQ	Home Screening Questionnaire
ICU	Intensive Care Unit
IQ	Intelligence Quotient
Kg	Kilograms
KPS	Knobloch, Passamanick, & Sherrard's Developmental Screening Inventory
LA	Linolenic acid
LCPUFA	Long-Chain Polyunsaturated Fatty Acid/s
M	Mean
MRI	Magnetic Resonance Imaging
MCDI	MacArthur-Bates Communicative Development Inventories
mg	Milligrams
MLU	Mean Length of Utterance
N	Number
n-3	Omega-3
ND	None Detected
NEPSY	NEuroPSYchological Assessment
NHMRC	National Health and Medical Research Council
NR	Not Reported
PPCT	Person Process Context Time
PPVT	Peabody Picture Vocabulary Test
PPVT-R	Peabody Picture Vocabulary Test, Revised

PPVT-III	Peabody Picture Vocabulary Test, Third Edition
Preg	Pregnancy
RBC	Red Blood Cell
RCT	Randomised controlled trial/s
RLE	Recent Life Events
SD	Standard Deviation
SDQ	Strengths and Difficulties Questionnaire
SS	Sentence Structure
SSRI	Selective Serotonin Reuptake Inhibitor
TLI	Tucker and Lewis Index
Trt	Treatment
UK	United Kingdom
USA	United States of America
Veg	Vegetable
VIQ	Verbal IQ
VLBW	Very Low Birth Weight
WASI	Wechsler Abbreviated Scale of Intelligence
WCH	Women's and Children's Hospital
WIAT-II	Wechsler Individual Achievement Test, Second Edition
WISC-III	Wechsler Intelligence Scale for Children, Third Edition
Wk	Week/s
WMD	Weighted Mean Difference
WPPSI-R	Wechsler Preschool and Primary Scale of Intelligence – Revised

WPPSI-III	Wechsler Preschool and Primary Scale of Intelligence, Third Edition
WS	Word Structure
y	Year/s

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CHAPTER 1

1 LITERATURE REVIEW

1.1 INTRODUCTORY REMARKS

The prenatal period is an important time for human brain development (1, 2), and research directed at understanding how experiences unfold later in life cannot only shed light on mechanisms of major significance to scientific knowledge about brain development and function, but may also have implications for public health (5). This creates important opportunities for informed investments in young children - for their well-being and also for building participation, productivity and, in particular, human capital (6-8).

Brain development is a complex process in which the availability of particular nutrients can have lasting effects on function (9-12). The role of long-chain polyunsaturated fatty acids (LCPUFAs) and in particular the omega-3, or n-3, fatty acid DHA as a biochemical agent in brain development is appearing as an area of extensive scientific investigation and of substantial relevance to public health (3, 13-18). Because DHA accretion is greatest during fetal development and early infancy this is thought to be an important period for which not getting enough DHA may have consequences for brain function in the longer term (19, 20). The uncertainty surrounding the ability of Westernized diets to fulfill requirements of

DHA to meet the physiological requirements and maintain optimum health highlights these periods of increased need as an opportunity to optimize children's cognitive abilities through supplementation (21, 22).

Results from the Australian Early Development Census (AEDC) illustrate that a considerable number of children in Australia are 'vulnerable' in language and communication skills, suggesting that their abilities are considerably lower than average (23, 24). This is concerning as these abilities are an important part of human capability formation with difficulties having implications for the individual and society (25). Thus, for children growing up in Australia, intervention for language development during the early years should be a primary focus for research and the role that DHA might play presents as a compelling area of investigation undertaken in the current study.

Evidence for the effect of DHA on children's language as a specific aspect of cognition is particularly controversial (26-30), and its potential to optimize language development warrants further investigation. Findings from observational studies suggest that DHA does bear some influence on children's language development although this evidence reveals nothing about causation (31-34). A thorough systematic review and meta-analyses of the available Randomised Controlled Trials (RCTs) of prenatal maternal, postnatal maternal and direct infant DHA supplementation with language outcomes was conducted, and concluded that there was no conclusive evidence of an effect, either positive or negative, on children's language outcomes. Trial quality was an issue but more to

the point there were a multitude of issues related to the lack of uniform measurement approaches to assessing language skills. Furthermore, findings with regard to subgroup analyses warranted further attention.

As a result, this thesis (the current study) proposes a new RCT of high quality in which a broad range of receptive and expressive language abilities are assessed in order to examine the effect of maternal DHA supplementation during pregnancy on linguistic acquisition. A theoretical framework from which to understand the relationship between DHA and language development with particular consideration of interacting biological and social variables is also offered.

1.2 THE SCIENCE OF EARLY CHILDHOOD DEVELOPMENT

1.2.1 THE IMPORTANCE OF THE EARLY YEARS

The World Health Organization emphasizes the importance of the first two years of life as a "window of opportunity" for promoting optimum child development, and highlights the potentialities for intervention during the prenatal period and early life to circumvent suboptimal child neurodevelopment (5). Similarly, the Council of Australian Governments recently explicitly acknowledged the value of "investing" in the early years, not only for the well-being of the individual child but also for building participation, productivity and, in particular, human capital (6, 7). This important shift in the focus

toward intervention in the early years is considered to be a human capital investment because of the relatively high rates of return in early childhood, where benefits of intervention outweigh the costs and also because the return from investment decreases as children grow up (35) **(Figure 1)**. Potential benefits of early intervention and investment in the early years include reduced expenses related to special education, criminal justice and welfare and also increases in income, taxes, national productivity and the Gross Domestic Product (36). Not surprisingly, underpinning the emergence of interest in early intervention is a growing body of evidence that optimal brain development is important for and interrelates with all aspects of children's lives, including their ongoing development and long-term outcomes (37).

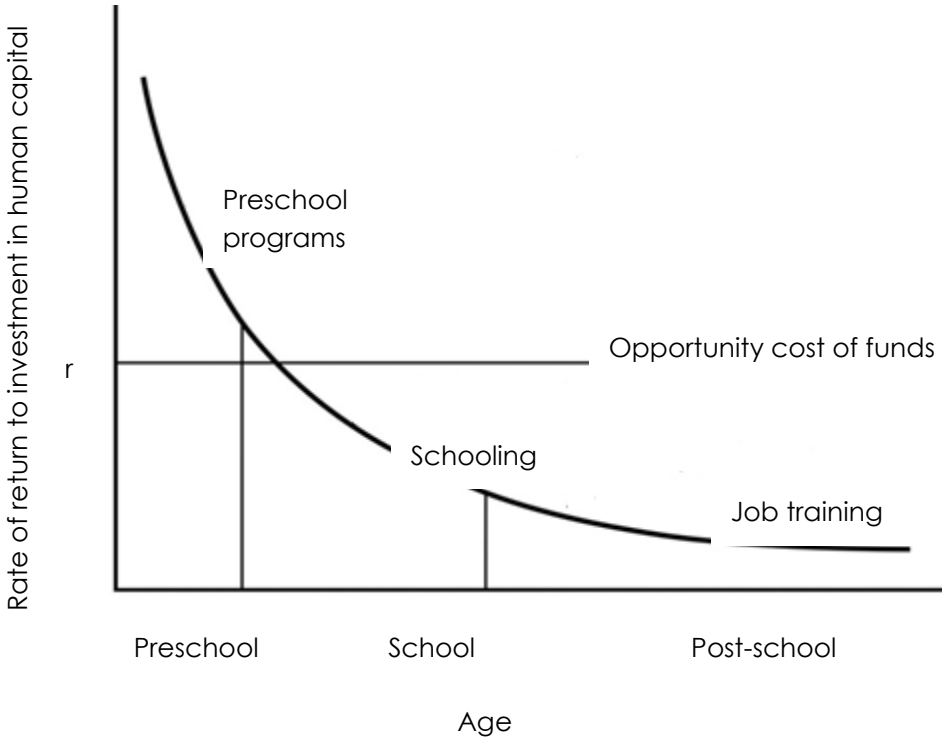


Figure 1. The returns of investment in human capital as product of the age at which it commenced, reproduced from (35)

1.2.2 AN OVERVIEW OF BRAIN DEVELOPMENT

Beginning before birth and continuing into adulthood, brain development is an ongoing process that is frequently likened to the construction of a building, including the wiring of its electrical system (38). First, its "basic architecture" is established. Later, more complex circuits (such as those underlying cognitive functions) are built upon earlier, simpler circuits (such as those for vision and hearing) (38). At the organ level the series of events in which the human brain develops can be explained in stages, or processes, starting with the formation of the neural tube, followed by neuronal proliferation, migration and neuritic differentiation, myelination, and synapse formation **(Figure 2)** (39, 40).

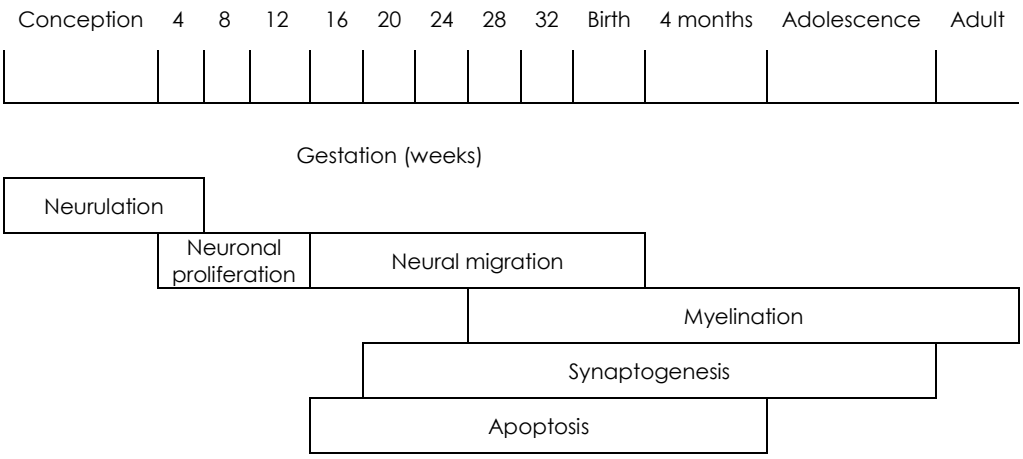


Figure 2. Overview of important events in brain development, reproduced from (39)

Beginning at gestational age (GA) weeks 2-3, neurulation is the process by which the neural plate forms into a neural tube. Cells are

proliferating by GA weeks 5-6 and diversify into neurons by GA week 8. Neural migration is an important mechanism by which neurons move their functional location in the brain, from lower to higher brain structures, and is most active during GA weeks 12 and 20 and complete by GA weeks 26-29. Thereafter, neurons grow and differentiate into various adult-like brain structures. This process encompasses the outgrowth of axons and dendrites **(Figure 3)**. (39-43)

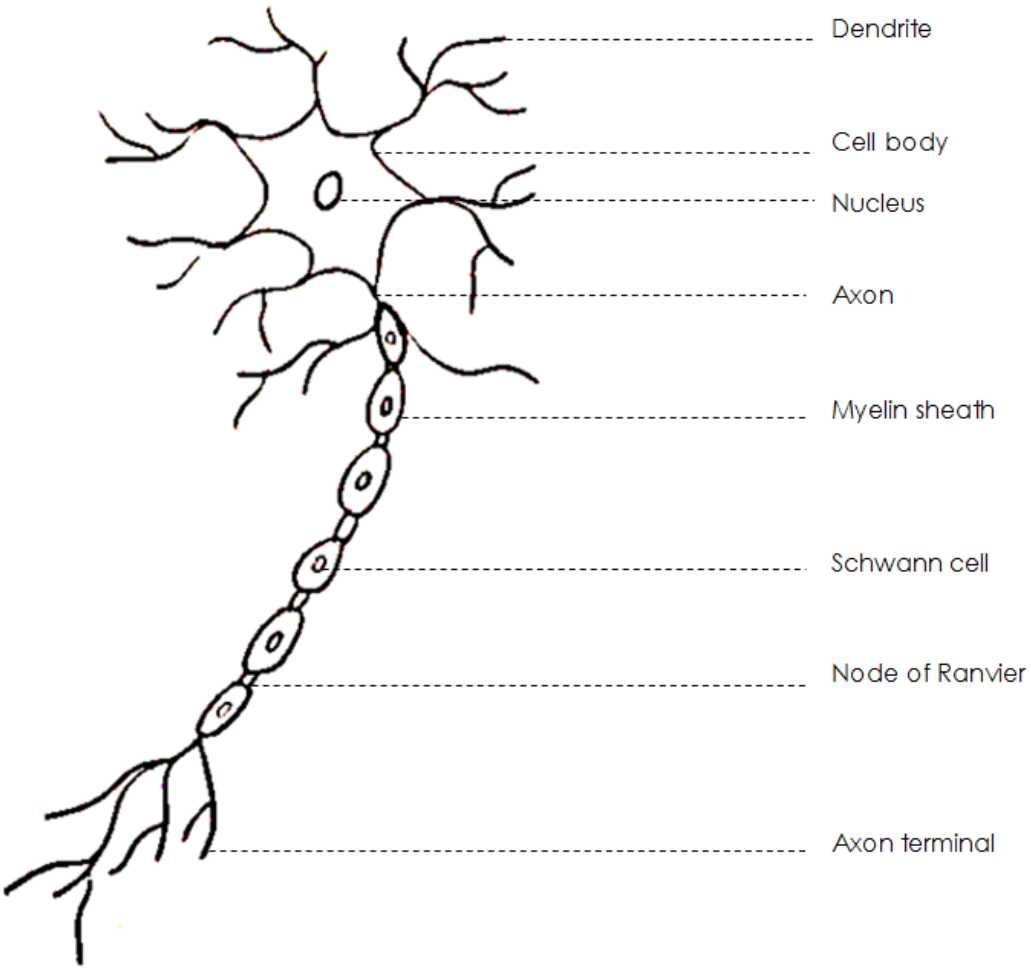


Figure 3. Simplified anatomy of a neuron, reproduced from (44)

Myelination commences during the third trimester, between GA weeks 20 and 28, and extends into adulthood. Myelin is a fatty substance that, like insulation on a telephone wire, coats and protects the axon and speeds up transmission of electrochemical signals, or communication, between neurons. It is white in appearance, which explains why fiber pathways of the brain are often referred to as "white matter". Neurons involved in basic processes are myelinated first and myelination of those in the frontal lobe of the cortex is not completed until adulthood. (39, 40, 45)

Synaptogenesis also occurs during the third trimester. Particularly at GA week 34 the number of connections between neurons increases substantially to form more sophisticated neural networks that play a role in various functions. The infant brain has more synapses than the adult brain. In time, connections that are used bloom in that they become more strengthened and survive. Those that are not used or are not used that often stay weak and tend to be pruned away (**Figure 4)**. (39, 40, 46, 47)

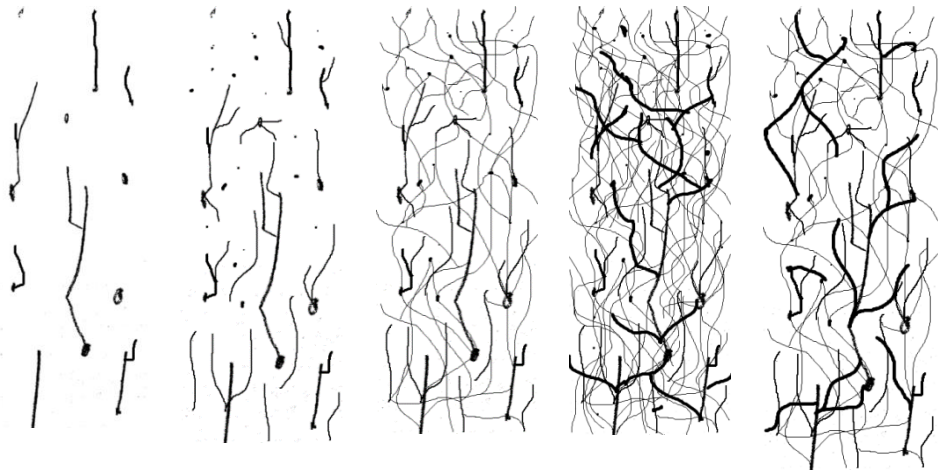


Figure 4. Synapse density over time, reproduced from (48).

The period lasting from the last trimester of pregnancy up to 2 years of age is a time of rapid change in brain structure and function (1, 2). The brain grows to approximately 80% of its adult size by 2 years of age (49). This period is known as the "brain growth spurt" and is illustrated by a sigmoid curve when its weight is plotted against its age (50).

1.2.3 EXPERIENCE AND BRAIN DEVELOPMENT

The brain develops through ongoing interaction between genes, the environment and experience (51, 52). Genes provide a pattern for brain structure and function. In particular, they encode proteins which contribute to the structural integrity of cells which in turn connect to other cells and circuits of the brain to influence function (39). Experience refers to the interaction of a child with his or her environment. The environment makes available the materials out of which the brain is constructed. Neural circuits and cells change in response to the environment. An unhealthy environment can prompt neurons to acquire atypical properties and connections and ultimately prevent the best possible function (53).

Experience can affect brain development in at least two ways. Experience-expectant processes are more likely to occur in early life wherein the brain relies on the processes happening for normal development (54). This includes the typical experience for instance, of sounds, sights and movement. The brain also develops in response to experience-dependent processes that are unique to the individual throughout life (54). Over time this sort of experience has an increasingly more prominent role in brain development by

way of fostering new neuronal connections and reorganizing, or shaping, existing structures (39). The result is a progressively more unified neural network with corresponding functional capabilities accounting for learning and behaviour (51).

Basically, while early experience influences maturation of low-level circuit architecture, experience after birth increasingly influences that of higher-level, individually tailored, circuits. With the absence of such experience the brain's architecture will not develop normally which can lead to disparities in function. Experience early on in life must be continued into life later on, in order for the brain to develop to its full potential. (36, 55-57)

Notably, there is ambiguity surrounding the level above which the effects of enriched environments do not occur, or may possibly be counter-productive. That is, it is not known what, if any, the upper limits may be on the positive neurodevelopmental impacts of environmental stimulation and experience. This leaves open the possibility that overly stimulating environments may have adverse consequences for brain development. Though challenging, it is pertinent that these concerns are not only acknowledged but also addressed in order to advance understanding of the effects of early experience with a fair and holistic approach. (58)

Notions of the brain's "plasticity" in response to various experiences early in life suggest that the early years of a child's life thus present not just as an important period of opportunity, but also vulnerability, and that in order to invest it is first necessary to direct resources aimed at understanding what factors, or experiences, throughout

the pregnancy and postnatal period have an influence on the brain and neurodevelopment. Nutrition is a key, environmentally variable experience and understanding its impact on early brain development may offer important environmental interventions that serve to maximize brain development. (38, 59, 60)

1.2.3.1 THE CONTRIBUTION OF NUTRITION TO EARLY BRAIN DEVELOPMENT

In humans, experience that influences brain development begins before birth, as the fetus experiences the environment of the womb and develops and organizes its function accordingly (55, 61). There is substantial literature indicating the contribution that nutrition makes to the structure and function of the developing brain cannot be overstated (9-12). Nutrients can affect the developing brain as they are required for the formation and function of specific metabolic pathways and structural components. All nutrients are important but some seem to be particularly influential during the last trimester of pregnancy and early life (11). As previously noted, it is during this time period that the brain undergoes remarkable structural and functional changes to more closely resemble the adult brain (62). An inadequate supply of micronutrients during this period, as well as throughout life, can compromise brain function. This includes iron (63), zinc (64), folate (65), and LCPUFAs (66) Notably, our 'Western diet' contributes to poor nutrition and has been put forward as a key risk factor for compromised brain development. If a causal relation exists between such nutrient deficiencies or insufficiencies and suboptimal brain function, then it could have important consequences for public health. Thus, in order to provide children with the optimal beginning in life it is

crucial to consider the contribution that particular nutrients during this period might make to children's brain development.

1.2.4 SUMMARY

There have been important advances in scientific knowledge about early brain development and corresponding interest in its applicability to enhance early brain development. Establishing a strong foundation for the brain in the early years increases the probability of optimal functional outcomes. The potential for experiences to influence the production of a weak or sturdy foundation for brain development and success later on in life underscores the importance of research related to how the pre and postnatal environment, particularly nutrition, can influence the long-term development of the child. In doing this it is important to communicate findings to policy makers in order to accurately inform decisions aimed at optimising children's development.

1.3 LONG CHAIN POLYUNSATURATED FATTY ACIDS IN EARLY LIFE

1.3.1 ESSENTIALITY, STRUCTURE AND FUNCTION

Fatty acids (FAs) are a major class of lipids (11). More specifically, they are chains of carbon atoms with a carboxylic acid at the beginning of the chain, or tail, (-COOH), and a methyl group at the opposite end (-CH3) (67). It is the carboxyl group that gives them their acidic character. The number of carbons varies in that there can be one or multiple double bonds between the carbons. As illustrated in **Figure 5**, those FAs classified as saturated have only carbon-carbon single bonds. Also, those FAs that are unsaturated contain either one carbon-carbon double bond in their chain as is the case with monounsaturated FAs, or at least two carbon-carbon double bonds as is the case with polyunsaturated FAs (PUFAs) (68). The double bond keeps the fatty acid chains from packing together side-to-side which provides the structure with flexibility.

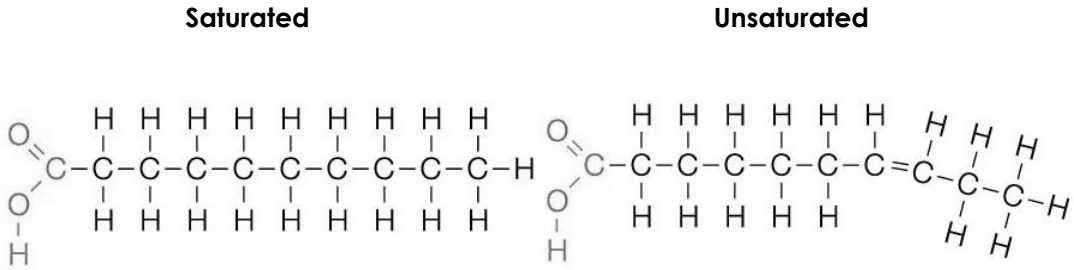


Figure 5. The difference in the molecular structure of saturated and unsaturated fatty acids.

PUFAs with 20 or more carbons on their carbon chain are known as LCPUFAs. The last carbon in the carbon chain of a LCPUFA is known as an omega, and different categories of LCPUFA are signaled by the location of the first double bond from the methyl end. Alpha linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) are precursors of the omega-3 and omega-6 LCPUFAs and are vital for the maintenance of optimal health. This is achieved by insertion of additional double bonds into, and elongation of, the acyl chain **(Figure 6)**. Specifically, this involves microsomal desaturation (involving delta-6 and delta-5 enzymes), microsomal chain elongation, and peroxisomal chain shortening (69).

n-3 PUFA	Enzymes	n-6 PUFA
18:3 n-3 ALA		18:2 n-6 LA
↓	Δ-6-desaturase	↓
18:4 n-3		18:3 n-6
↓	Elongase	↓
20:4 n-3		20:3 n-6
↓	Δ-5-desaturase	↓
20:5 n-3 EPA		20:4 n-6 AA
↓	Elongase	↓
22:5 n-3		22:4 n-6
↓	Elongase	↓
24:5 n-3		24:4 n-6
↓	Δ-6-desaturase	↓
24:6 n-3		24:5 n-6
↓	B-oxidation	↓
22:6 n-3 DHA		22:5 n-6 DPA

Figure 6. Essential fatty acids and their derivatives. AA, arachidonic acid; ALA, alpha linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LA, linolenic acid

Docosahexaenoic acid (DHA) is an omega-3, or n-3, FA. As illustrated in **Figure 6**, the total number of carbons, total number of double bonds, and the location of the first double bond from the methyl group are represented by the 22, 6 and number following n respectively. Converting ALA into DHA is a slow process in humans and it has been estimated that less than 10% is actually converted to DHA (70). Conversion also varies according to intake of other FAs (71) as ALA and LA compete for common desaturation and elongation enzymes (72, 73). It has been suggested that this process might be made more efficient by decreasing LA intake or increasing ALA intake (74-76). However, this presents as a considerable challenge for those consuming a contemporary Western diet wherein LA intakes (such as vegetable oils, nuts, seeds and animal products) are typically high (77). This suggests that any ALA intake may not be able to meet physiological requirements for DHA. While it has recently been proposed that intake of more direct forms of DHA may be the surest way to achieve this (78) how possible this might be is questionable as DHA is not widely available in the diet of Western nations (79).

1.3.2 DOCOSAHEXAENOIC ACID

1.3.2.1 LOCATION AND IMPORTANCE

DHA is an important nutrient for human health throughout life. It is required for every organ of the body for normal function to occur (80). The proportions of DHA found in various organs of adults are depicted in **Figure 7**. Compared with other organs, the brain grey matter and retina have an unusually high content of DHA (81, 82).

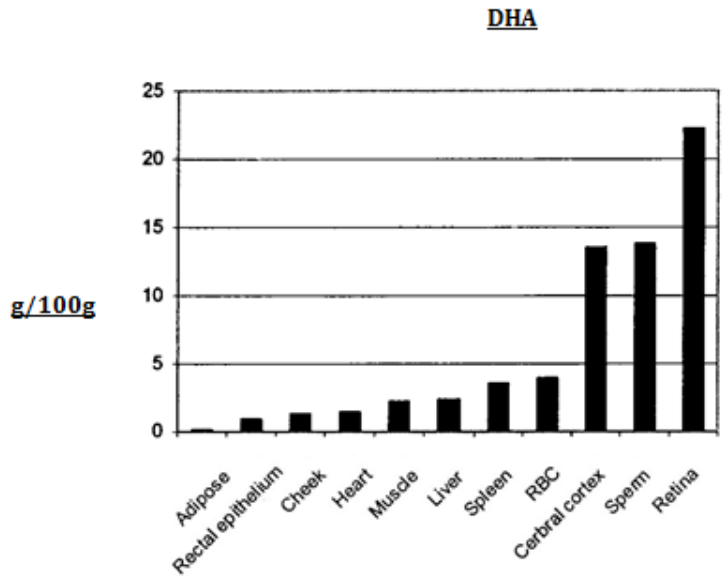


Figure 7. g/100g DHA in different parts of the human body. RBC, red blood cell.

DHA has the potential to modulate brain functions on many levels. It is one of the basic structural components of the membrane phospholipids of all cells (83, 84). The amount of DHA within the brain can influence neuronal membrane fluidity and the physical structure of neurons (85), affect neural functioning through its impact on gene expression, protect neural cells from apoptotic death (86) and, in particular, facilitate synaptic plasticity that underlies learning and memory. Notably, the hippocampus is a significant brain region for forming memories (87, 88) and it is here that DHA can enhance the process of neurite outgrowth (13) and promote growth of pre- and post-synaptic proteins in neurons which support synaptic transmission and long-term potentiation (89).

1.3.2.2 DOCOSAHEXAENOIC ACID IN THE CURRENT HUMAN DIET

DHA is mostly present in seafood and fish. Oily fish such as salmon and sardines have particularly high concentrations of DHA. Other fish sources include tuna, bluefish, and mackerel. DHA is also found in some animal products (although in much lower concentrations) including eggs and meats such as lamb and chicken (16). In Westernized countries, some supermarket products such as bread are now fortified with DHA.

The uncertainty surrounding the ability of contemporary diets to fulfill requirements for DHA to maintain optimum health highlights periods of increased need such as pregnancy and lactation as particularly vulnerable.

1.3.3 DOCOSAHEXAENOIC ACID IN EARLY LIFE

1.3.3.1 THE IMPORTANCE OF DOCOSAHEXAENOIC ACID FOR EARLY BRAIN DEVELOPMENT

DHA accumulates in neural tissues throughout fetal, neonatal, infant and child development. The period during the last trimester of pregnancy and through into the second year of life is one of maximum deposition of DHA in the brain (3, 19, 20). The human fetus acquires approximately 70 mg of omega-3 per day of which most is DHA (18). Unfortunately, the fetus has limited ability to synthesize DHA and therefore is almost entirely dependent upon placental

transfer (90). This is thought to take place through a number of mechanisms (see (91, 92)).

In utero, fatty acids are released from maternal triglycerides by an enzyme called lipoprotein lipase. This is produced in fat cells (adipocytes) and is attached to the walls of capillaries. This activity occurs on the maternal surface of the placenta, where fatty acids are specifically bound to plasma membrane fatty acid binding protein and cytoplasmatic transport proteins. Placental transport specifically enriches DHA in fetal blood which results in higher levels of DHA in cord blood compared to maternal blood (64).

Multiple studies have suggested that the maternal diet may be the best source of DHA available to the developing fetus. They have found a strong link between human dietary intake of DHA during pregnancy and maternal circulating DHA (15, 93) and maternal dietary and circulating DHA is a predictor of fetal blood concentrations of DHA (94, 95). After birth, infants are reliant on maternal breast milk as the sole source of DHA (96) which is also particularly influenced by the mother's diet in a dose dependent manner (97). Furthermore, doubt surrounding infants' ability to synthesize enough DHA endogenously for their optimal development postnatally has led to recent commercial availability of DHA-fortified infant formula.

Studies of developing animals have repeatedly demonstrated the long term effects of restricting n-3 fatty acids in the diet on the brain. These include reduced levels of DHA and increased levels of n-6 fatty acids in brain tissue and, from a functional standpoint,

behavioural deficits (16, 94, 98). Furthermore, infants born with low levels of DHA in their blood tend to have lower neural maturation than children born with normal DHA levels (99). These findings have been supported by post mortem human infant analyses which revealed that infants fed formula not supplemented with DHA had approximately 15% less brain cortex DHA compared to those who were breast-fed and had a dietary source of DHA (20).

1.3.3.2 RECOMMENDATIONS FOR DOCOSAHEXAENOIC ACID DURING PREGNANCY AND THE NEONATAL PERIOD

The World Health Organization (100) and various expert authorities worldwide (including the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation) (101) agree upon the increased need for dietary DHA during pregnancy and lactation and recommend women take at least 200mg per day during this period in order to provide the fetus with the estimated requirement of 70mg.

In Australia, the National Health and Medical Research Council (NHMRC) delineates Australian Dietary Guidelines based on the "best available scientific evidence" and is used by health professionals, policy makers, educators, food manufacturers, food retailers and researchers. This includes information regarding nutritional requirements and dietary advice intended for pregnant and breastfeeding women in order to give children the "best possible start in life" (102). The NHMRC currently acknowledges that there is a lack of evidence about the requirements of DHA during pregnancy and lactation and has consequently established

adequate intakes (AIs) for total n-3 LCPUFA (i.e. DHA, EPA and DPA) which range from 110-145 mg per day **(Table 1)**. At present, such recommendations for pregnancy are derived from that of women who are not pregnant as well as the increased average body weight in pregnancy (i.e. x 1.25). For those who are lactating the recommendation was based on that for women who are not pregnant or lactating in addition to that of the infant. Also notably, no upper level of DHA intake has been set. (102)

TABLE 1. NHMRC Adequate Intake recommendations for total n-3 LCPUFA (DHA+EPA+DPA) during pregnancy and lactation

Maternal age	Pregnancy	Lactation
14-18 years	110 mg/day	140 mg/day
19-50 years	115 mg/day	145 mg/day

AI, adequate intakes; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; LCPUFA, long-chain polyunsaturated fatty acid; mg, milligrams; n-3, omega-3; NHMRC, National Health and Medical Research Council

There is current evidence to suggest that a substantial proportion of women in Western nations do not consume the suggested amount of DHA during pregnancy and lactation (103). Prevalence figures show that this includes 91% of women in Australia, wherein the median intake is ~96 mg of DHA per day (ranging 8 to 632mg) (103).

1.3.3.3 CHALLENGES

Although meeting the amount of DHA recommended by expert guidelines can be reached by eating 1-2 portions of fish per week (17) various government advisory bodies have specific

recommendations for including fish in the diet during pregnancy while avoiding the presence of environmental contaminants such as methylmercury that can have adverse consequences on neurodevelopment **(Figure 8)**. While on the one hand informative, on the other it is possible that these nuanced recommendations may overwhelm many pregnant women in their dietary decisions, which may in turn explain why they are further reducing their fish intake or eliminating fish from their diets (33). Studies assessing the potential advantages and threats to a child's neurodevelopmental outcomes with different levels of maternal seafood intake during pregnancy have found not only that higher intake was more favorable but that lower seafood consumption did not protect children from negative outcomes (104). Overall, this suggests that the potential consequences of not getting enough DHA may actually be worse than those of exposure to trace contaminants.

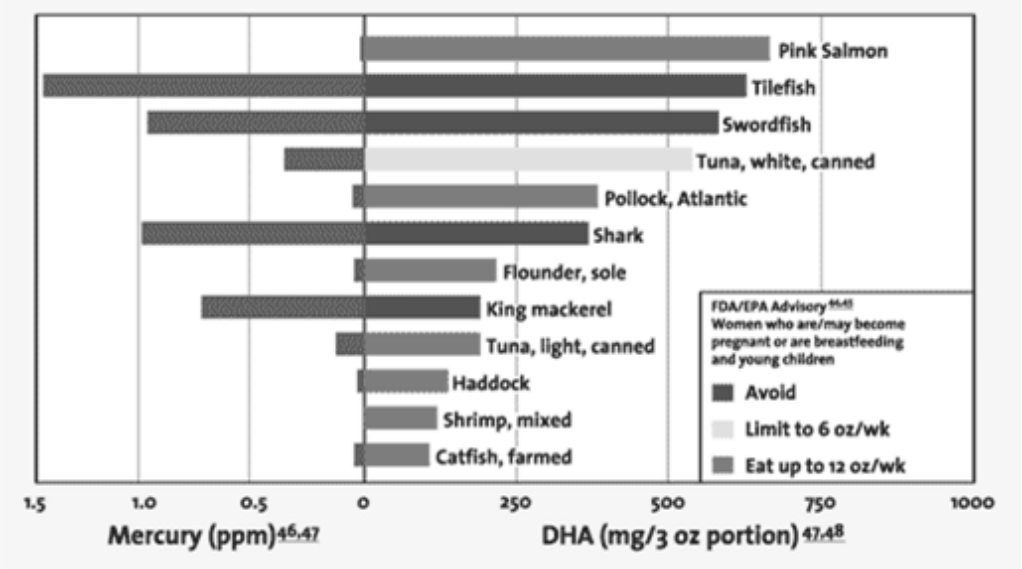


Figure 8. Recommendations for fish consumption during pregnancy and lactation, reproduced from (105).

Also, in order to maximize DHA intake via lactation, recommendations by such authorities also advise breastfeeding as the only kind of nutrition for the first 6 months after birth (106). Preterm infants also have significantly lower DHA statuses than do full term infants and the potential for dietary DHA insufficiency presents as a challenge because they do not receive the intrauterine supply in the third trimester (107).

1.3.4 ASSESSING THE FUNCTIONAL EFFECTS OF DOCOSAHEXAENOIC ACID

Considering the important biochemical roles that DHA plays in supporting all aspects of the brain's structural development it is plausible to suggest that an insufficiency may have quite specific effects on functional development, including cognitive abilities (21, 22). On the other hand, given the ability of the brain to adapt in early life (due to brain plasticity) it may be that reductions in DHA availability may not have adverse consequences. To date the specific functional roles for DHA in the brain are unclear (108). The concept of cognition is broad, essentially pertaining to the mental processes involved in acquiring knowledge and understanding (i.e. thinking, concentrating, remembering, learning, imagining, problem solving, using logic, organizing information, and using symbols) and, encompasses communication skills including language (109). One major difficulty in interpreting and comparing results across different studies investigating the relationship between DHA and cognition is that different aspects of cognition were measured. There is some evidence suggesting that different cognitive capacities involve different neural systems and that experimental manipulations can have different implications for performance depending on the task

(110). This suggests that global tests of cognitive development may not be sensitive enough to distinguish an effect of DHA availability, and that it might be more useful to consider the functional domain that is being targeted and assess the outcome before drawing any conclusions about the effect of DHA. The results of future more specific cognitive tests should prove more informative. The assessment of language proficiency which is reliant on integrated neural networks could be a more suitable domain in which to evaluate the effects of DHA.

1.3.5SUMMARY AND IMPLICATIONS

Brain development is a complex process in which the availability of particular nutrients can have lasting effects on function. At present while the basis for supplementing pregnant women with DHA to optimize infants' cognitive development is logical and theoretically strong, evidence for its efficacy is in doubt and so caution should be taken in identifying it as a factor that impacts on the development of cognitive capacities. Still, the contradictory results of the studies reviewed do not necessarily exclude the potential benefits of DHA supply for the cognitive development of infants. Before trustworthy recommendations for dietary, supplemental or formula enriched DHA can be made functional benefits need to be clearly demonstrated. Future studies should consider taking a more sensitive approach to assessing cognitive development by identifying and measuring specific aspects of cognition as an investment in early child development and human capital formation. The following sections will provide a better understanding of language and what language development entails and also thoroughly explore current research on the effect of DHA on language outcomes.

1.4 LANGUAGE DEVELOPMENT

1.4.1 DEFINING AND DISENTANGLING KEY TERMS

Communication is a process which involves giving and receiving information to and from others (111). Language refers to a shared set of rules that facilitate this process, allowing individuals to communicate meaningfully (111, 112). This occurs through writing, signing, reading, listening, and talking. Speech specifically concerns the verbal expression of language (111). Language has traditionally been broken down into two domains: receptive language (the information that someone receives from another person), and expressive language (the information that someone sends to another person)(113). Although these are often the headings used on formal reports there is a more detailed way of looking at an individual's language profile. (112, 114, 115)

1.4.2 THE STRUCTURAL COMPONENTS OF LANGUAGE

Language involves five structural components including speech sounds (phonetics and phonology), morphology, syntax, semantics and pragmatics. Although these components can be identified separately they are interrelated elements in communication. The term language development can be thought of as the acquisition of these components that enables individuals to communicate effectively. Phonetics concerns the production of speech sounds and phonology concerns how they may be combined. A phoneme is the basic unit of sound in a language. For instance, in English the

sound represented by the letter h (e.g. in the words hot and shot) is a phoneme. Morphology concerns the smallest meaningful units involved in a word or portion of a word that cannot be fragmented into smaller portions with any other meaning. For instance, some words such as help consist of a single morpheme (i.e. help), whereas others such as helper consist of two morphemes (i.e. help + er). The lexicon component pertains to vocabulary and associated knowledge. Syntax involves the ways words can be combined to form conventional phrases and sentences to convey meaning. It deals with how to interpret the meaning of a sentence depending on word order. For instance, in the sentence Jack hit Jill or Jill was hit by Jack, one can infer who did the hitting and who was hit. Semantics refers to what words and sentences mean. Pragmatics concerns contextual appropriateness of language use. For instance, this involves using polite language when talking with one's teacher. (112, 115)

To effectively understand language acquisition and, when necessary, inform approaches to intervention, the components of language have been cut down into three critical facets of communication: Form, Content, and Use (116) **(Figure 9)**. *Form* includes phonology, morphology, and syntax. *Content* pertains to semantics. *Use* has to do with pragmatics. Language competence is achieved when a speaker can manage all of these components so that he or she is able to communicate an intended message effectively. Difficulties related to any one of these aspects or in their assimilation may result in language problems. For instance, what an individual says might be structurally correct (form) but not really convey meaning effectively (content) or for the right reasons (use).

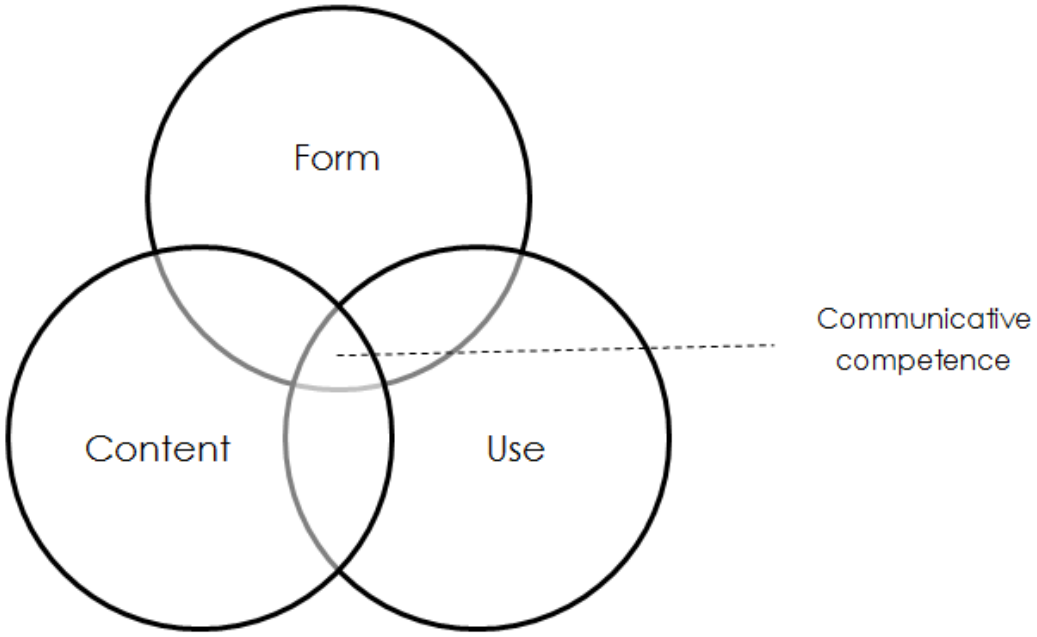


Figure 9. Language is made up of three components – form, content, and use, reproduced from (117).

1.4.3 AN OVERVIEW OF LANGUAGE DEVELOPMENT

1.4.3.1 CONTENT

During infancy (≤12 months) children can understand anywhere from 3 to 50 words, including names of individuals who are significant in their lives (*Daddy*), pets (*doggy*), automobiles (*bus*), toys (*ball*), food (*milk*), body parts (*toe*), clothes (*shoe*), furniture (*chair*), communicative games (*peek-a-boo*), and routines (*bye-bye*).

As toddlers (13 – 24 months) children's expressive vocabulary ranges from 50 to 100 words, and meaning, or semantics, are expressed in

one-word utterances, including agent (*Nicola*), action (*walk*), object (*ball*), location (*there*), possession (*yours*), rejection (*no*), disappearance (*no-more*), nonexistence (*no*) and denial (*no*). By the end of toddlerhood, children's average expressive vocabulary grows to 200 to 300 words.

During the preschool years (>24 months - ≤ 4 years) children's network of interrelated words and concepts expands. They understand color, shape (*circle, square, triangle*) and size (*big, little*) words as well as spatial (*in, on, under*) and kinship terms. They understand and use sentences and begin to understand and use questions about *What?, Who?, Where?, Why?*, *When?* and *How?*. They also begin to use some conjunctions (*and*, *because*) to link sentences. Before children begin schooling they have also developed some knowledge of how to name letters and numbers.

During the early school years (5 – 7 years) children's average expressive vocabulary size grows to 5000 words.

See (112, 115) for an overview of the development of language content in children.

1.4.3.2 FORM

The developing fetus can perceive many sounds produced both inside and outside of the womb and, consequently, can distinguish many sounds that are used in their native language from birth (115, 118). Before children actually produce speech there are a number

of approximate 'stages' through which vocal productions develop to increasingly have the phonetic features of adult speech.

During infancy (≤12 months) children make vegetative sounds, including gurgling, burping, coughing, bubble blowing and, cooing. Thereafter, canonical babbling appears wherein the characteristics of infants' vocalizations become true syllables (e.g. *da*, or *ba-ba-ba*). After this, jargon babble emerges where they combine different vowel and consonant syllables (e.g. *do-ba-di*) which are the building blocks for the production of words.

During the toddler period (13 – 24 months) children produce approximately 50 words, including two-word utterances and two-syllable words. Children also learn to use *-ing* endings on verbs, *in*, *on*, and *-s* plurals. They begin to use negation (e.g. *no*, *not*, *can't*, and *don't*) and their sentences more frequently contain semi-auxiliaries (*gonna*, *wanna*, *gotta* and *hafta*). Soon after they begin to use present-tense auxiliaries (*can*), *be* verbs (*I am happy*), and past-tense forms that are overgeneralised (*I ranned* instead of *I ran*).

During the preschool years (>24 months - ≤ 4 years) embedded sentences and complex sentence forms appear. Also, by this stage children are able to accurately place verbs in questions and negatives and have acquired irregular past tense, articles (*a, the*) and possessive *'s*.

As children enter into their school years complex sentence forms including full prepositional clauses (*The dog is on the table*), *wh-*

clauses (*I went where the other children were sitting*) and simple infinitives (*I want to see*) emerge and mistakes in expressing *s*, *r*, *l*, and *th* may persist.

During the early school years (5 to 7 years) children begin to use and understand passive sentences (*The dog was taken for a walk*), and divide words into syllables.

See (112, 115, 119, 120) for an overview of the development of language form in children.

1.4.3.3 USE

During infancy (≤12 months) children express intent through their actions such as smiling when hearing a voice or through different cries for when they are tired, hungry, or in pain. Later on they use gestures and vocalizations. From the first year onwards words increasingly replace these preverbal expressions.

During the toddler years (13 – 24 months) the frequency of children's communicative acts increases and they begin to use *Please* for polite requests. New intents at this age include pretend play, talking about objects that are not present and fibbing and mocking. Also, children's narratives, or stories, are characterized mostly by labels and descriptions (i.e. they use a series of unrelated ideas and express theme but no plot). Soon after, their use of language in play increases. As children approach the preschool years there is more flexibility in requesting, including direct requests (Can I have a

cookie?) and indirect requests (I am hungry). Also, at this stage their narratives begin to express some organization of events in time.

During the preschool years (>24 months - ≤ 4 years) new abilities develop including commenting on past events, reasoning, foreseeing, conveying empathy and playing make believe. Thereafter children increasingly respond to requests for explanation and their narratives begin to contain a plot, although no protagonist or resolution.

During the early school years (5 to 7 years) children's stories are true, containing a focus, high point and resolution, and they correctly use terms that identify time or context from the standpoint of the narrator (*this, that, here and there*).

See (112, 115, 121) for an overview of the development of language use in children.

1.4.4 LANGUAGE ASSESSMENT

Different types of measures can be employed in order to evaluate children's language skills.

Examination of spontaneous language samples provides an understanding of children's functional use of language skills for communication, that is, their ability to use each language domain to communicate competently in their day-to-day experiences. The

mean length of utterance (MLU) is an example of a common spontaneous language analysis. It measures syntax in terms of morpheme utterance length. See (122) for a description of analyses of samples of language as well as normative data and interpretation of results.

Norm referenced tests provide an indicator of how a particular child performs in a developmental outcome compared to how a sample of children considered to be developing typically performs. Normative scoring systems include the standard score and the percentile. Standard scores have a mean, or average, score and a standard deviation from that score. The mean score of most tests is 100 and the standard deviation is 15 points respectively. Accordingly, a score that lies between 85 – 100 or 100 – 115 is thought to be within one standard deviation of the mean and a score that falls within either 70 – 85 or 115 – 130 is thought to be within two standard deviations of the mean, etc. Up to date clinical standards suggest scores falling between 85-115 are considered to be typical developmental performance and those that fall below one standard deviation of the mean (i.e. a standard score below 85) are indicative of a language delay. Percentile scores are based on all of the children sampled and are considered to be a child's performance on a developmental measure in relation to how many children perform better and worse than him or her. For example, if a child's raw score puts them at the 90th percentile then 10% of the children at that same age performed better and 89% performed more poorly. Children falling under the 10th percentile are mostly recognized as having a developmental delay in the area being tested. (123, 124)

Criterion referenced tests determine the number skills a child has at a particular age. The abilities assessed are established by the series of developmental markers understood to be normal. (123, 124)

Assessments of language development can sample different language domains or a particular domain in depth. The former provide a general, or global, language score. For example, the second edition of the Clinical Evaluation of Language Fundamentals Preschool, Second Edition (CELF P-2; (125)) tests the child's reception and expression of a variety of language domains. The CELF P-2 can provide a Core Language Score of which three subtests are a part: Sentence Structure, Word Structure, and Expressive Vocabulary **(Chapter 3)**. The latter arguably provide a domain specific score. For example, the Peabody Picture Vocabulary Test, Fourth Edition, (PPVT-IV; (126)) is a test of receptive vocabulary.

Tests can be administered to children by clinicians and in this case the clinician is making direct observations of the language skills being tested. Alternatively, clinicians may seek parent report through interviews or questionnaires. Tests that are clinician administered to children are standardized whereas those that are parent report are typically not, although there are some exceptions.

1.4.5THE NEURAL CORRELATES OF LANGUAGE PROCESSING

1.4.5.1 A CLASSICAL VIEW: BROCA'S AND WERNICKE'S

The way in which language is organized in the brain is commonly thought of in terms of the 'classical model' developed in the 19th century by neurologists including Broca (127), Wernicke (128) and Lichtheim (129). The Wernicke-Lichtheim model proposes a frontal "expressive" area (specifically, the left inferior frontal cortex) for planning, talking and writing (aka Broca's area), and a posterior "receptive" area (specifically, part of the left temporal cortex) for detecting and making sense of linguistic sensory stimuli (Wernicke's area) **(Figure 10)**. The arcuate fasciculus is a white matter pathway thought to unite and coordinate these two language relevant regions.

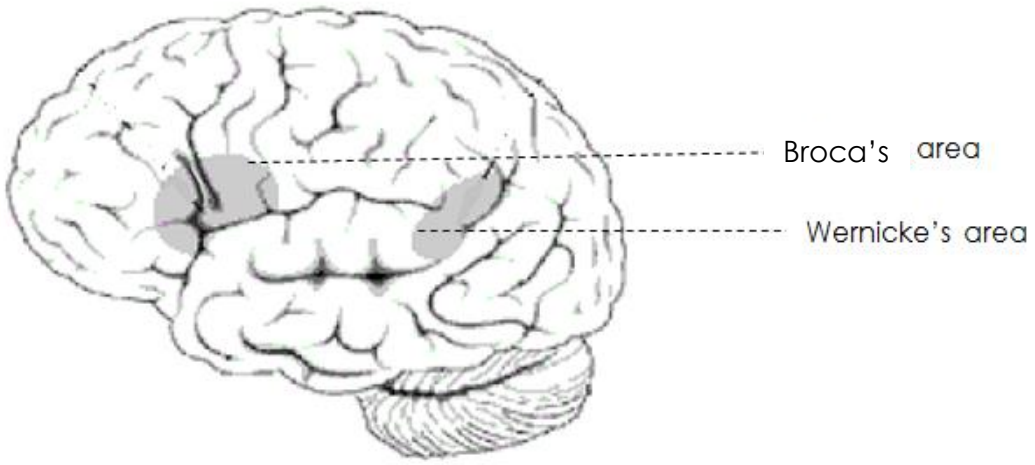


Figure 10. Classical brain regions associated with language processing, reproduced from (130).

Wernicke's area is enclosed by a part of the brain called Geschwind's territory. To enable comprehension, Wernicke's area pairs the sounds of spoken words to their meaning, and the neurons in Geschwind's territory integrate their properties (sound, sight, and meaning). To enable expression, Wernicke's area pairs the thought to the correct words which then pass through Broca's area through the arcuate fasciculus (or, possibly through Geschwind's territory) and then into sounds. This is achieved through moving the tongue, mouth, and jaw into the necessary place and setting the larynx in motion. See (131, 132) for further explanation.

1.4.5.2 BEYOND THE CLASSICAL VIEW

Importantly, the classical model underlying this conceptualization has a multitude of shortcomings from a biological, linguistic and psychological perspective (133). It is now acknowledged that surrounding areas play an important role in language (134-138). In addition, evidence for language processes being distributed across the two hemispheres is growing (137). Recently, tools for functional imaging of the brain provide a new sort of evidence about the localization of language processing (139). A recently published compendium of functional magnetic resonance imaging (fMRI) data illustrates many brain areas that are active during language use (140). These areas were dispersed in the left frontal lobe, left temporal/parietal lobes, right temporal lobe, and cerebellum. These areas can represent a functional network, or a coalition of brain areas that work together to perform a particular task related to language processing.

Despite strong curiosity in the neural correlates of early language development (141) the brain areas involved are still not yet clear , suggesting that this is an important topic for continued research (142). One study investigated brain structure in the first year of life in order to understand what predicts early language acquisition by examining the link between early concentration of gray and white matter in the brain at 7 months and infants expressive and receptive language abilities at 12 months (143). Utilizing voxel based morphometry magnetic resonance imaging (MRI) data results suggested that the cerebellum, posterior limb of the cerebral peduncle, and the hippocampus may be associated.

While children have the biological potential to develop language, their experience of their environment plays a role in the extent to which these sorts of abilities are mastered (144). Some studies even suggest that the neurobiological mechanisms underlying language development rely on cues available only in the environment. A primary mode of learning in the nervous system takes place as a function of blooming and pruning, when the synapse is formed or modified, as a function of such experience (also referred to as Hebbian learning).

1.4.6 DOCOSAHEXAENOIC ACID AND CHILDREN'S LANGUAGE DEVELOPMENT

1.4.6.1 EVIDENCE FROM EPIDEMIOLOGICAL STUDIES

Epidemiological studies exploring the relationship between maternal n-3 LCPUFA intake and cognitive outcomes, including

those for language and communication, in their children in later life have been able to shed light on the potential value of DHA. These studies have commonly determined maternal fish intake during pregnancy and lactation using food frequency questionnaires and various measures of cognitive development. Two studies used data from the Avon Longitudinal Study of Parents and Children (31, 32). One study revealed that fish intake by the mother prenatally (recorded at 32 weeks' gestation) and, additionally, the infant postnatally (recorded at 6 and 12 months of age), was positively associated with verbal comprehension as measured by the MacArthur Communicative Development Inventories (MCDI) at 15 months (n = 7421) (31). Specifically, there was a large association for the vocabulary comprehension scores which were close to 5 points higher among children whose mothers ate fish at least once per week during pregnancy compared with those whose mothers did not eat fish (p = 0.03) (31). The other notably larger study found a significant association between low maternal seafood intake (i.e. specifically <340 grams per week, recorded at 32 weeks gestation) and suboptimal Verbal Intelligence Quotient (VIQ), measured by the Wechsler Intelligence Scale for Children (WISC-III) at 8 years of age (n = 5407; no seafood consumption, odds ratio 1.48, 95% CI 1.16 – 1.90; some seafood consumption, 1.09, 0.92 – 1.29; overall trend, p = 0.0041) and also poorer communication skills as measured by the Denver Developmental Screening Test at 6 months (n = 8745; no seafood consumption, odds ratio 1.30, 95% CI 1.04 – 1.63; some seafood consumption, 1.15, 0.98 – 1.35; overall trend, p = 0.0184) and 18 months of age (n = 8237; no seafood consumption, odds ratio 1.26, 95% CI 1.03 – 1.53; some seafood consumption, 1.02, 0.90 – 1.17; overall trend, p = 0.0485) (32). Research in Project Viva found that maternal fish intake during the second half of pregnancy was associated with better receptive language abilities as assessed with

the Peabody Picture Vocabulary Test (PPVT) at 3 years of age (33). Specifically, this study noted that mothers who reported eating canned tuna at least two times a week had children with higher scores (n = 28; 3.7, 95% CI -0.9 – 8.3, p value not reported) compared with women who reported never eating tuna fish (n = 130). However, this study also noted that mothers who reported eating more than two servings of fish other than canned tuna did not have children with higher scores on the PPVT (n = 11; -1.4, 95% CI -8.9 – 6.1, p value not reported) compared with those who reported eating no fish excluding tuna (n = 97)(33). A smaller study assessed the relationship between maternal fish intake at both the early (15 weeks' gestation) and later (32 weeks' gestation) stages of the pregnancy and Verbal IQ, measured by the Wechsler Abbreviated Scale of Intelligence (WASI) at 9 years of age. Compared to children whose mothers did not eat fish in early pregnancy those whose mothers ate fish less than once a week, once or twice a week, and greater than three times a week had a Verbal IQ that was 7.66 points (n not reported, 95% CI 0.10 – 15.4), 7.32 points (n not reported, 95% CI 0.26 – 14.40), and 8.07 points (n not reported, 95% CI 0.28 – 15.90) higher respectively (34). Compared to mothers who ate no fish in late pregnancy, the children of those whose mothers ate fish in late pregnancy had a Verbal IQ that was 7.55 points (n not reported, 95% CI 0.75 – 14.40) higher, regardless of frequency (34). It is important to note, however that there were no significant associations between fish intake in early or late pregnancy and Verbal IQ (34).

While the results of observational studies imply that dietary exposure to DHA can have a positive impact on language and communication it is important to take into account a number of

issues before drawing any definitive conclusions. To begin with, seafood includes DHA in combination with other fatty acids and so it is difficult to ascertain from these studies whether DHA alone has a beneficial influence on cognitive outcome (56). Secondly, the questions of what type of seafood, how much of it and how often it should be consumed remain largely unanswered (145). These are important points of contention as there is, as mentioned earlier, concern regarding the potential risks of seafood intake during pregnancy (146). This is especially with regard to the presence of environmental contaminants such as methyl mercury which can have adverse consequences on the central nervous system of the developing infant (147, 148). Another concern is that it is difficult to accurately quantify intake via food frequency questionnaires as energy and food, including fats and fatty acids may be under reported (149). The major limitation, however, is that it is impossible to assign causality (8). Inadequate adjustment for confounders such as diet, education and age are potential confounding factors that threaten the validity of the results of observational studies (150). The easiest way to deal with these complexities is to consider evidence from studies using a RCT design.

1.4.6.2 EVIDENCE FROM INTERVENTION STUDIES

RCTs are considered to be the 'gold standard' for evaluating the efficacy of treatments, that is, whether they do more good than harm (151). A sample population is selected and randomly assigned either to the intervention (or experimental) group where they receive the treatment, or to the control group where they receive a placebo (or, in some cases, a comparison treatment) (152). Groups are then followed up at a later time point wherein outcomes of

interest are measured (152). Importantly, random assignment establishes equality between the groups with respect to any confounding factors such that any differences can be attributed, that is, said to be caused by, the only factor in which they differ – the treatment (153, 154) **(Figure 11)**. Systematic reviews and meta-analyses are important tools for bringing together data from numerous RCTs to examine the applicability of evidence to different populations and settings (155).

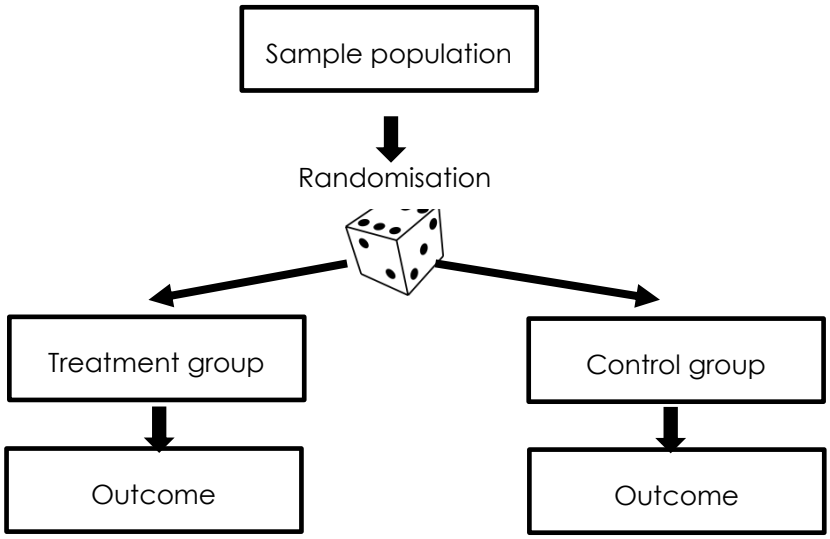


Figure 11. The randomised controlled trial design, reproduced from (152).

1.4.7 SUMMARY

The classical model underlying conceptualization of how the brain sub-serves language is hopelessly underspecified. Contemporary research has revealed that a number of regions outside of the classical language areas are now known to be recruited for various language functions, and it is more accurate to think of language development as an interactive process involving many functions governed by widespread neural areas that do not develop in isolation. In understanding the relationship between DHA and language development it is important to understand that DHA may influence brain development at or before birth when language learning begins and also the types of neurodevelopmental events that take place after birth and across the period in which language is acquired. Results from observational studies suggest that DHA does bear some influence on children's language development although this evidence reveals nothing about causation. While this in turn suggests that it is important to conduct a study on this area using a RCT design it is first and foremost important to ascertain what is already known from existing RCTS. This involves undertaking a systematic review and meta-analysis.

1.5 THE INFLUENCE OF DOCOSAHEXAENOIC ACID ON LANGUAGE DEVELOPMENT: A SYSTEMATIC REVIEW OF RANDOMISED CONTROLLED TRIALS

1.5.1 STATEMENT OF AUTHORSHIP

Statement of Authorship

Title of Paper	The influence of docosahexaenoic acid on language development: a systematic review of randomised controlled trials
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	N/A

Principal Author

Name of Principal Author (Candidate)	Nicola Gawlik
Contribution to the Paper	Conducted the initial search, undertook primary extraction of data, summarized the trials, assessed the risk of bias for each trial, performed the subsequent meta-analysis and prepared the manuscript.
Overall percentage (%)	90%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date: 2/2/16.

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Amanda Anderson
Contribution to the Paper	Secondary extraction of data, summarized the trials, assessed the risk of bias and assisted in interpretation and critical review of the manuscript.
Signature	Date: 2/2/16

Name of Co-Author	Maria Makrides
Contribution to the Paper	Provided primary supervision of the work, resolved discrepancies as part of the risk of bias assessment and interpreted and critically reviewed the manuscript.
Signature	Date: 2/2/16

Name of Co-Author	Jacqueline Gould		
Contribution to the Paper	Interpreted and critically reviewed the manuscript.		
Signature		Date	2·2·2016

Name of Co-Author	Lisa Kettler		
Contribution to the Paper	Provided secondary supervision of the work, and interpreted and critically reviewed the manuscript.		
Signature		Date	2/2/16

1.5.2 ABSTRACT

1.5.2.1 BACKGROUND

Pregnancy and infancy are important periods during which DHA can influence infant brain development, potentially laying the foundation for language skills. Evidence for an effect of DHA supplementation on language outcomes is conflicting. As children's language skills contribute to their subsequent success in life it is important to evaluate the potential for DHA supplementation to influence early language development.

1.5.2.2 OBJECTIVES

Our aim was to examine existing RCTs of DHA supplementation during pregnancy, or in the first 12 months postpartum, with assessments of language development in infants and children.

1.5.2.3 DESIGN

Seven databases were searched. To be eligible for inclusion in this review, studies had to be in English, be conducted in humans, have an RCT design, include supplementation with DHA during pregnancy or the postpartum period, include a placebo group without DHA supplementation, and report language outcomes. Trial quality was assessed.

1.5.2.4 RESULTS

Eighteen RCTs with 16 assessments of language, with one global language outcome, 10 domain specific language outcomes (i.e. those that were either receptive or expressive) and five outcomes for subscales of language domains in 6,277 participants were included in the review. Three RCTs intervened during pregnancy while two supplemented mothers and 13 supplemented infants after birth. Most trials had methodological issues, particularly low power.

This review revealed no conclusive evidence that DHA supplementation during pregnancy or between birth and 12 months of age enhances language development in the child, however, trial quality is an issue

1.5.2.5 CONCLUSIONS

The evidence from available RCTs is currently not robust enough to draw any strong inferences. Opportunities exist for future studies to make progress in investigating whether DHA supplementation influences language development, particularly in subgroups thought to be low/deficient in DHA

1.5.3 INTRODUCTION

1.5.3.1 RATIONALE

Pregnancy and infancy are important periods during which nutrition can affect the development of the infant brain, laying the foundation for language skills. The n-6 fatty acid arachidonic acid (AA) and the n-3 fatty acid docosahexaenoic acid (DHA) are long-chain polyunsaturated fatty acids (LCPUFAs) important for brain development and function (156). They are rapidly incorporated into the brain during the "brain growth spurt" which takes place during the last trimester of pregnancy and continues up to 2 years of age (1, 2). Unlike AA, very little DHA can be synthesized by the body and it must be obtained through the diet (66). There is concern that there is not enough DHA in the diet of Western nations (157), especially during periods of high demand such as pregnancy and lactation (158).

Evidence from randomised controlled trials (RCTs) for an effect of DHA supplementation on cognitive development is not consistent. While some RCTs have indicated benefits (26, 27) others have not (28, 29) and reviews of RCTs (30, 159) overall do not provide a compelling result, raising the possibility that DHA may have an effect on some cognitive capacities but not others. Effects on language outcomes are particularly conflicting, with negative (160, 161) as well as positive (162, 163) and null (164-167) results all reported. As children's language skills contribute to their subsequent success in life (168, 169) it is important to evaluate the potential of DHA supplementation to optimize early language development so

that appropriate recommendations can be formulated and disseminated.

1.5.3.2 OBJECTIVES

To date there have been no systematic reviews of literature investigating the effect of DHA supplementation on children's language development. Our aim is to examine existing evidence from RCTs on this topic and evaluate whether supplementation with DHA during pregnancy or postnatally, either to the mother or directly to the infant, in the first 12 months postpartum, effects language development in infants and children.

1.5.4 METHODS

1.5.4.1 INFORMATION SOURCES AND SEARCH STRATEGY

One author (NRG) searched the Cumulative Index to Nursing and Allied Health Literature (CINAHL), Cochrane Central Register of Controlled Trials (CENTRAL) Current Contents Connect, Excerpta Medica database (EMBASE), PsychInfo, PubMed, and Web of Science databases using strategies tailored to each database based on the PubMed search; "DHA OR Docosahex*enoic acid OR docosahex*enoate OR omega 3 OR LCPUFA OR long chain polyunsaturated fatty acid OR fish oil OR marine oil OR algal oil" AND "Language OR linguistics OR verbal OR vocabulary OR literacy OR reading OR communication OR language test OR neurodevelopment OR cognitive development" AND "RCT OR

randomi*e* OR intervention OR placebo OR control". The search strategy for other databases is available in **Appendix 1**. No date restrictions were set although studies had to be published in a journal and in English, and searches were limited to trials on humans. The titles and abstracts of all articles retrieved by the search were screened to assess eligibility. The reference lists of eligible articles identified by the search were also checked for other articles that may have been relevant. Search engines used were set up to email new publications identified by the search on a fortnightly basis; with the last update received December 2014.

1.5.4.2 STUDY SELECTION

To be eligible for inclusion in this review, a study needed to fulfill particular criteria. That is, it had to be in English, be conducted in humans, have a RCT design, include supplementation with DHA during pregnancy or the postpartum period (although trials that included LCPUFAs in conjunction with DHA were also considered), include a placebo group without DHA and report language outcomes.

In order to capture the language development of young children, measures derived from multiple sources were included. This involved 1) direct standardized assessment of language abilities in the child; 2) parent report of the child's language capabilities, and 3) observational measures of spontaneous speech derived from natural language samples. Our primary outcomes were global language scores. Secondary outcomes were domain specific language scores such as those for receptive language (i.e.

Vocabulary Comprehension scores as assessed by MacArthur-Bates Communicative Development Inventories (MCDI)) and scores from subscales that were components of domains (i.e. Information subscale scores which form part of the Verbal Intelligence Quotient of the Wechsler Preschool and Primary Scale of Intelligence – Revised (WPPSI-R)).

1.5.4.3 DATA COLLECTION PROCESS

Two review authors (NRG and AJA) independently used a standardized data extraction form to summarize the trials and assess the risk of bias for each trial, using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (170). Any disagreements were resolved through discussion and, if required, consultation with a third reviewer (MM). Clarification of trial details were requested via e-mail from the authors of 12 studies (160, 161, 163-165, 167, 171-176) and obtained from all but three (164, 165, 173).

1.5.4.4 SUMMARY MEASURES AND SYNTHESIS OF RESULTS

Potential differential effects of DHA supplementation according to the period of supplementation; during pregnancy (to the mother) or postnatally (to the mother or directly to the infant)) on children's subsequent language development were investigated. As what is measureable as language varies across age groups and as this may be a reflection of differing patterns of neural activity as children develop (177) language outcomes were considered separately for infants (≤12 months), toddlers (13-24 months), preschool (>24 months

- ≤ four years, 11 months) and school age children (five -12 years). The principal summary measures were difference in means.

Review Manager Version 5.2 software was used in order to undertake the meta-analysis. Treatment effects were determined based on combined data from individual trials that were considered to be homogenous. Specifically, trials had to be from the same supplementation period (i.e. prenatal or postnatal), assess language in the same age group (i.e. infant or toddler or preschool or school-aged) and also use the same language outcome measure. Data were pooled for trials that included more than one treatment group, that is, participant numbers were summed and mean language score and standard deviations averaged. Continuous outcome measures were expressed as a weighted mean difference (WMD) with 95% confidence intervals (CI) using the fixed effects model. Statistical heterogeneity was assessed by the I^2 test (a descriptor for the percentage of variance among effect estimates beyond that likely to be by chance), with considerable heterogeneity considered to be >50%. Overall estimates of differences in treatment are illustrated in forest plots. Due to impracticalities related to data synthesis from every study, a summary of individual trial results is provided.

1.5.5 RESULTS

1.5.5.1 TRIAL SELECTION

Searches of the databases yielded 1304 references, 634 of which remained after duplicates were removed **(Figure 12)**. Article

abstracts were reviewed and 593 were excluded for the following reasons: not human trials (n=63), did not involve omega-3 LCPUFA supplementation (n=127), were not RCTS (n=302) or did not have language outcome data (n=101). The full text of 41 citations was then examined in detail and further exclusions were made primarily due to a focus on the effects of supplementation on outcomes in adults (n=8), or supplementation occurring/commencing >12 months postpartum (n=8). Additionally, one trial could not be included as neither the abstract or the full text could be found (178); one conference abstract (179) was excluded as the full data was available in a published paper (172); one paper was excluded as the analysis was by feeding mode (180) one was excluded as the focus was on the effect of DHA on breast milk fatty acid composition and infant outcomes (181). One conference abstract that was referenced in another paper (27) and two papers received via email notification were eligible for inclusion (162, 182).

A total of 25 individual papers were included in the review (27, 160-167, 171-176, 182-190), three of which were included in the meta-analysis for the primary outcome of language development (171, 174, 191).

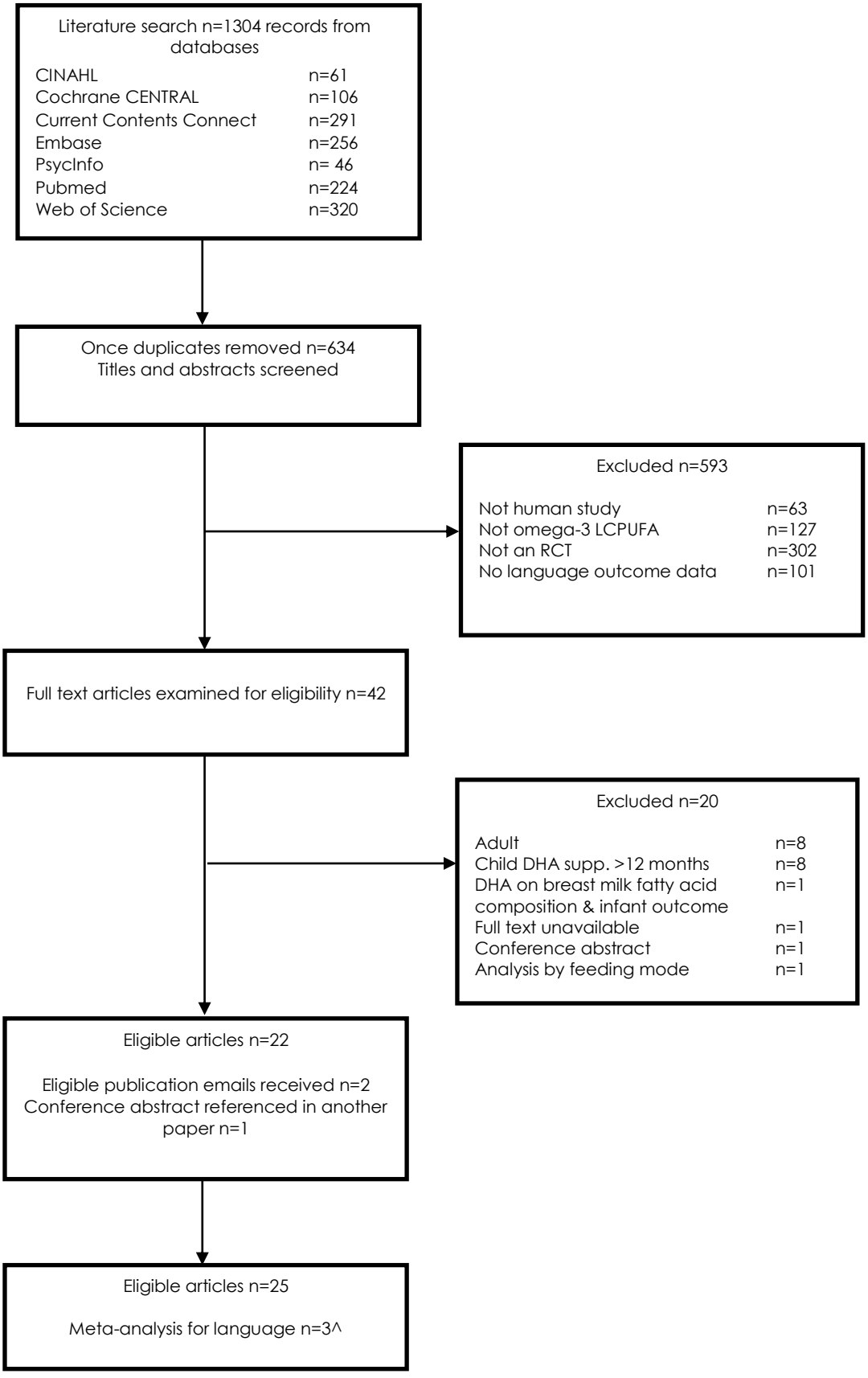


Figure 12. Progress of randomised controlled trials identified and included in the systematic review and meta-analysis. LCPUFA, long-chain polyunsaturated fatty acid; RCT, randomised controlled trial; sup., supplemented. ^ Few trials were included in the meta-analysis due to heterogeneity of language assessments used.

1.5.5.2 TRIAL CHARACTERISTICS

1.5.5.2.1 PARTICIPANTS

The 25 papers belonged to 18 individual RCTs investigating the effects of DHA supplementation implemented either during pregnancy or postnatally (up until 12 months after birth) on language development. The trials were published between 1998 and 2014 and were conducted in high income countries (North America, Australia, Sweden, Denmark, the United Kingdom, the Netherlands, Belgium and Italy) with English (161-167, 171-176, 182-185, 187, 188, 190), Swedish (27), Danish (160), Norwegian (186), Dutch (189), Flemish (182) and Italian (182) as the primary languages. Most children participating in the trials were from singleton pregnancies although some included twin (164-166, 174, 175, 187, 188) and triplet (175, 187, 188) pregnancies. Four trials did not report pregnancy type (163, 171, 172, 174, 186). Three trials involved women with a history of allergic disease (27, 163, 167).

1.5.5.2.2 PRENATAL DHA SUPPLEMENTATION INTERVENTIONS

Three RCTs have investigated the effect of maternal prenatal supplementation (27, 167, 183) in 2,642 infants. Mothers took capsules with DHA doses of 800 (183), 1,100 (27) and 2200 (167) mg per day. All trials examined the effect of DHA in combination with eicosapentaenoic acid (EPA). The supplementation period commenced between <21 and 25 weeks of gestation and ended at birth in two trials (167, 183) whereas one trial supplemented

women for three to three-and-a half months after birth (27). Two trials only included infants born at term (27, 167) whereas the other included preterm as well as term infants (183). A summary of these three trials is shown in **Table 2**.

TABLE 2. Summary of maternal prenatal DHA supplementation interventions included in the review [1]

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
Dunstan 2008 (167)	Australia Hospitals, antenatal clinic	Singleton; term Mothers had allergic disease, excluded if eating >2 fish meals/wk & if infant born preterm (<36wk)	N=52 Fish oil; C[2] DHA 2.2g/d EPA 1.1g/d	N=46 Olive oil; C[3] No DHA	4 C/d From 20 wk gestation - birth	PPVT-III Form A GMDS Speech & hearing subscale CBCL mean length of phrases CBCL mean N words in vocabulary	30 mo 30 mo 30 mo 30 mo	No diff. No diff. No diff. No diff.
Makrides 2010 (183)	Australia 5 hospitals/ perinatal centers, routine antenatal clinic	Singleton; term & preterm Women excluded if already taking DHA supplements	N=1197 Fish oil; C DHA 800mg/d EPA 100mg/d	N=1202 Veg. oil blend (rapeseed, sunflower, palm oils); C No DHA	3 C/d From <21 wk gestation - birth	Bayley-III Language Composite	18 mo CA[4]	No diff. No diff. No diff.
Karlsson 2010 (27)	Sweden Antenatal clinic, local newspaper	Singleton; term Mothers had allergic disease, excluded if taking	N=70 Source NR; C DHA 1.1g/d EPA 1.6g/d	N=75 Soy oil; C No DHA	9 C/d From 25 wk gestation - 3 ½ mo	WPPSI-III Verbal IQ WPPSI-III General Language Composite	46 mo	No diff. No diff.

[1] Bayley-III, Bayley Scales of Infant Development, Third Edition; C, capsule; CA, corrected age; CBCL, Child Behaviour Checklist; Ctrl, control group; d, day/s; DHA, docosahexaenoic acid; diff, difference; EPA, eicosapentaenoic acid; g, grams; GA, gestational age; GMDS, Griffiths Mental Development Scales; IQ, Intelligence Quotient; mg, milligrams; mo, month/s; N, number; NR, not reported; PPVT-III, Peabody Picture Vocabulary Test, Third Edition; RCT, randomised controlled trial/s; Trt, treatment group; Veg., vegetable; WPPSI-R, Wechsler Preschool and Primary Scale of Intelligence – Revised; WPPSI-III, Wechsler Preschool and Primary Scale of Intelligence, Third Edition; wk, week/s; y, year/s

[2] Trt capsules had a-tocopherol (vitamin E), 3-4mg/g oil, as an antioxidant

[3] Ctrl capsules had a-tocopherol (vitamin E), 3-4mg/g oil, as an antioxidant

[4] 726 infants (96 preterm & 630 randomly selected term) chosen from Adelaide for 18 mo assessment. N=333 infants (Trt) & N=361 (Ctrl) completed the 18 mo Bayley-III. Multiple imputation was used to deal with missing data. Results of the imputed analysis are reported.

1.5.5.2.3 POSTNATAL MATERNAL DHA SUPPLEMENTATION INTERVENTIONS

Two RCTs have assessed the effect of postnatal maternal supplementation (160, 164, 165) in 352 infants. Mothers were supplemented with capsules, muesli bars or cookies (and thus provided DHA to the infant via lactation) (160, 164, 165). In these trials mothers were provided with 200-900mg of DHA per day (160, 164). These RCTs examined the effect of DHA alone (164, 165) or in combination with EPA (160). The supplementation period commenced within five days of birth and lasted up until four months of age. These trials included exclusively term infants (160, 164, 165). A summary of these two trials is shown in **Table 3**.

TABLE 3. Summary of maternal postnatal DHA supplementation interventions included in the review[5]

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
Jensen 2005, 2010 (164, 165)	USA Local newspaper, advertisement at GP, childbirth class	Singleton & twin; term GA: >37wk BW: 2500 - 4200g	N=115 Source NR; C DHA ≈200mg/d	N=115 Veg. oil blend (soy, corn oil); C No DHA	1 C/d From ≤5d - 1st 4 mo of lactation	CLAMS CLAMS WPPSI-R Information subscale WPPSI-R Vocabulary subscale	12 mo 30 mo 5 y 5 y	No diff. No diff. No diff. No diff.
Lauritzen 2005 (160)	Denmark Selected from DNBC	Singleton; term Maternal fish intake <50th percentile of DNBC[7] GA: 37-43 wk BW: appropriate for GA	N=62 Tuna oil; musli bars, C, cookies DHA 22.8% AA 1.7% EPA 10%	N=60 Olive oil; musli bars, C, cookies No DHA	2 musli bars or cookies/d OR 6 C/d (trt), 4 C /d (ctrl) From 9±3d for 1st 4 mo lactation	MCDI[6] Vocabulary Comprehension MCDI Vocabulary Production MCDI Starting to talk MCDI Early gestures MCDI Late gestures MCDI Phrases understood MCDI Vocabulary Production MCDI Talk about "abstract" MCDI Use grammar MCDI Irregular words MCDI Overregularised Words MCDI Length of longest sentences	1 y 1y 1 y 1 y 1 y 1 y 2 y 2 y 2 y 2 y 2 y 2 y	Trt < Ctrl No diff. No diff. No diff. No diff. No diff. No diff. No diff. No diff. No diff. No diff. No diff.

[5] % reported are % of total fatty acids unless otherwise indicated; ≈, approximately; AA, arachidonic acid; BW, birth weight; C, capsule; CLAMS, Clinical Linguistic and Auditory Milestone Scale; Ctrl, control group; d, day/s; DHA, docosahexaenoic acid; diff, difference; DNBC, Danish National Birth Cohort; EPA, eicosapentaenoic acid; g, grams; GA, gestational age; GP, general practitioner; MCDI, MacArthur-Bates Communicative Development Inventories; mg, milligrams; mo, month/s; N, number; RCT, randomised controlled trial/s; Trt, treatment group; USA, United States of America; Veg., vegetable; wk, week/s; y, year/s

[6] For this trial, MCDI was translated into Danish

[7] <0.4g/d n-3 LCPUFA

1.5.5.2.4 INFANT DHA SUPPLEMENTATION INTERVENTIONS

Eight RCTs have assessed the effect of direct infant supplementation (161-163, 171-174, 176, 182, 184, 185, 189) in 2,139 infants. Trials supplemented infants via the addition of DHA to infant formula (161, 162, 171-174, 176, 182, 184, 185, 189) or straight into the mouth (163). In these trials infants received doses of DHA, ranging from 0.12 to 0.96% of total fatty acids (161, 162, 171-174, 176, 182, 184, 185, 189) and in one trial were reported as receiving either 260 or 280 mg per day (163). These RCTs examined the effect of DHA alone (184), in combination with AA (161, 162, 171, 172, 176, 182, 184, 185, 189), or EPA (163, 171, 172), or both AA and EPA (173, 174). Four of the trials involved more than one treatment group (161, 162, 171, 172, 174, 176, 184, 185) with differences between groups being source and dosage of DHA as well as combination with other fatty acids. The supplementation period commenced within the first week of life and lasted up until a maximum of 12 months of age. A summary of these eight trials is shown in **Table 4**.

TABLE 4. Summary of infant DHA supplementation interventions included in the review[8]

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
Scott 1998 **Auestad 2003 (171, 172)**	USA Children's hospitals, universities	Preg. Type NR; term[9] GA: ≥37 wk BW: appropriate for GA	N=65 Fish (tuna) oil; F DHA 0.23% DHA:EPA ≈4:1 N=68 Egg phospholipid; F DHA 0.12% AA 0.43%	N=65 F; DHA ND	Median 2d after birth – 12 mo[10]	MCDI Vocabulary Comprehension MCDI Vocabulary Production MCDI Early gestures MCDI Late gestures PPVT-R Receptive Vocabulary MLU Morphemes	14 mo 14 mo 14 mo 14 mo 39 mo 39 mo	No diff No diff. (Trt: EP) Trt FO < Ctrl No diff. No diff. No diff. No diff.
Lucas 1999 (173)	UK	Singleton; term BW: appropriate for GA	N=154 DHA 0.32% AA 0.30% EPA 0.01%	N=155	From 1st wk	KPS Language	9 mo	No diff.
Auestad 2001 (174)	USA Hospitals, practices	Preg. type NR; term[11] BW: ≥2500g, included small & large for GA infants[12]	N=82 Fish/fungal oil; F AA 0.46% EPA ≤0.04% N=80 Egg-DTG; F	N=77 F; DHA ND	From ≤ 9 d after birth –	MCDI Vocabulary Comprehension Expression	9 mo 14 mo	No diff No diff

[8] % reported are % of total fatty acids unless otherwise indicated; ≈, approximately; AA, arachidonic acid; Bayley-II, Bayley Scales of Infant Development, Second Edition; Bayley-III, Bayley Scales of Infant Development, Third Edition; BW, birth weight; C, capsule; CA, corrected age; CBCL, Child Behaviour Checklist; Ctrl, control group; d, day/s; DHA, docosahexaenoic acid; diff, difference; egg-DTG, egg-derived triglyceride; EP, egg phospholipid trt group; EPA, eicosapentaenoic acid; F, formula; FO, fish oil trt group; g, grams; GA, gestational age; IQ, Intelligence Quotient; MCDI, MacArthur-Bates Communicative Development Inventories; mg, milligrams; MLU, Mean Length of Utterance; mo, month/s; N, number; ND, none detected; NEPSY, NEuroPSYchological Assessment; NR, not reported; Preg., pregnancy; RCT, randomised controlled trial/s; Trt, treatment group; UK, United Kingdom; USA, United States of America; WASI, Wechsler Abbreviated Scale of Intelligence; WPPSI-R, Wechsler Preschool and Primary Scale of Intelligence – Revised; wk, week/s; y, year/s

[9] This trial included a non-randomised breastfed reference group (N=76)

[10] For a minimum of 4 mo as the sole source of nutrition

[11] This trial included a non-randomised breastfed reference group, weaned to formulas (Trt N=83; Ctrl N=82)

[12] i.e. <10th percentile and >90th percentile

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
			DHA 0.14% AA 0.45%					
Birch 2000, 2007 (184, 185)	USA Hospitals	Singleton; term[13] GA: 37 – 40 wk BW: appropriate for GA	N=26 Single cell oil; F DHA 0.35% Iron N=27 Single cell oil; F DHA 0.36% AA 0.72% Iron	N=26 F[14]; Iron	From ≤5d – 17 wk[15]	Bayley-II 'Language' WPPSI-R Verbal IQ	18 mo 4 y	No diff. No diff.
Meldrum 2012 (163)	Australia Routine antenatal clinics	Preg. type NR; term Mothers had allergic disease, excluded if fish eaten >3x/wk or fish oil supplement >100mg/d consumed during pregnancy GA: ≥36 wk BW: ≈3000-4000g	N=218 Fish oil; C DHA 250 – 280 mg EPA 60-110 mg	N=202 Olive oil; C DHA ND	From birth - 6 mo[16]	MCDI Phrases understood MCDI Words understood MCDI Words spoken MCDI Early gestures MCDI Late gestures MCDI Total gestures Bayley-III Language Composite Bayley-III Receptive language Bayley-III Expressive language CBCL Language Development Survey MCDI Phrases understood	12 mo 12 mo 12 mo 12 mo 12 mo 12 mo 18 mo 18 mo 18 mo 18 mo 18 mo	No diff. No diff. No diff. No diff. Trt > Ctrl Trt > Ctrl No diff. No diff. No diff. NR.[17] No diff.

[13] This trial included a non-randomised breastfed reference group (N=40)
[14] Fatty acid composition of formula NR
[15] No solids ≤17 wk, but commercial formula >17 wk
[16] It was recommended that capsules be given to infants in the morning before breastfeeding, or in formula during the first daily feed.
[17] Unclear if Language Development Survey was used as part of CBCL

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
						MCDI Words understood	18 mo	No diff.
						MCDI Words spoken	18 mo	No diff.
						MCDI Late gestures	18 mo	Trt > Ctrl
						MCDI Total gestures	18 mo	Trt > Ctrl
DeJong 2012 (189)	Netherlands University, hospitals, midwives	Singleton; term[18] GA: 37-42 wk BW: ≈3000-4000g	N=145 Egg DTG, tuna oil, fungal oil; F DHA 0.30% AA 0.45%	N=169 F; DHA ND	From birth - 2 mo	WASI Verbal IQ	9 y	NR.[19]
						NEPSY Language domain subscale	9 y	NR.
						CBCL Language development survey	9 y	NR.
Birch 2010 Drover 2011, 2012 Colombo 2013 (176, 190) (161, 162)	USA Hospitals	Singleton; term GA: 37-42 wk BW: 2490-4200g	N=84 Algal/fungal oil; F DHA 0.32% AA 0.43%	N=86 F[20]	From 1-9 d - 12 mo	MCDI Total Word Production[21]	18 mo	No diff.
						Bayley-II 'Language'[22]	18 mo	No diff.
						PPVT-III Receptive Vocabulary[23]	2 y	No diff. Trt 0.64%; Trt 0.32% & Trt 0.96% < Ctrl
			N=85 Algal/fungal oil; F DHA 0.64% AA 0.43%			PPVT-III Receptive Vocabulary[24]	3.5 y	No diff
						PPVT-III Receptive Vocabulary[25]	5 y	No diff. Trt 0.96%; Trt 0.32% & Trt 0.64% > Ctrl
			N=88 Algal/fungal oil; F DHA 0.96%			WPPSI-R Verbal IQ[26]	5 y	No diff.

[18] This trial included a non-randomised group of breastfed infants (N=160)
[19] Results only reported for smoking and non-smoking subgroups
[20] Fatty acid composition of formula NR
[21] Results reported for Kansas site participants only-quite odd they only reported for specific hospitals. If this is the case the numbers involved in the comparison need to be detailed and the issue needs to be adequately addressed in the results text of the manuscript
[22] Results reported for Dallas site participants only
[23] Results reported for Dallas site participants only- have as note 34
[24] Results reported for Dallas site participants only- have as note 34
[25] Results reported for Kansas site participants only- have as note 33
[26] Results reported for Kansas site participants only- have as note 33

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
			AA 0.43%					
Willatts 2013 (182)	UK, Belgium, Italy Hospitals	Singleton; term[27] GA: 37-42wk BW: 2500-4000g	N=111 Egg-DTG; F DHA 0.21% AA 0.35%	N=126 F; DHA ND AA <0.1%	From 1st wk life – 4 mo	WPPSI-R Verbal IQ[28]	6 y	No diff.

[27] Included a non-randomised breastfed reference group (N=139)
[28] WPPSI-R was available in an appropriate language for each centre (English in Dundee and Birmingham, Flemish in Leuven, & Italian in Milan)

Five RCTs have assessed the effect of direct infant supplementation (166, 174, 175, 186-188) in 1,144 preterm infants. Trials supplemented infants via the addition of DHA to infant formula (166, 174, 175, 187, 188)[29] or to human milk (166, 186). In these trials infants received doses of DHA, ranging from 0.17 to 29.5% of total fatty acids. These RCTs examined the effect of DHA in combination with AA (186), or both AA and EPA (166, 174, 175, 187, 188). One trial involved more than one treatment group (174) with differences between groups being source and dosage of DHA. The supplementation period commenced within the first week of life and lasted up until a maximum of 12 months of age. A summary of these 5 trials is shown in **Table 5**.

[29] For those who could not provide human milk for the entire intervention period infants received formula matching their group allocation (Smithers, 2010).

TABLE 5. Summary of trials involving supplementation to preterm infants[30]

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
Smithers 2010 (166)	Australia Hospitals	Singleton & twin; preterm GA: <33wk BW: randomised < by strata < 1200g or ≥ 1250g[32]	N=69 Tuna oil; C DHA 29.5% ≈900mg/d AA 1.8% EPA 6.5%	N=74 Soy oil; C No DHA	6 C/d From ≤5d of starting enteral feeds -term EDD[31]	MCDI Vocabulary Production	26 mo CA	No diff.
						MCDI Sentence complexity	26 mo CA	No diff.
						MCDI Irregular words	26 mo CA	No diff.
						MCDI MLU	26 mo CA	No diff.
						MCDI Combining Words	26 mo CA	No diff.
O'Connor 2001 (174)	USA, UK, South America Neonatal ICUs	Singleton & twin; preterm & small for GA[33] GA: <33 wk BW: randomised by strata 750-1250g or	N=140 Fish/fungal; F DHA 0.27/0.16%[34] AA 0.43%/0.43% EPA 0.08%/None	N=144 F; DHA ND	Pre discharge From enteral feeding (initiated by 28th day of life) – term CA	MCDI Vocabulary Comprehension	9 mo 14 mo	No diff. No diff.
						MCDI Vocabulary Production	14 mo	No diff.

[30] % reported are % of total fatty acids unless otherwise indicated; ≈, approximately; AA, arachidonic acid; ASQ, Ages and Stages Questionnaire; BW, birth weight; C, capsule; CA, corrected age; Ctrl, control group; d, day/s; DHA, docosahexaenoic acid; diff, difference; EDD, expected delivery date; egg-DTG, egg-derived triglyceride; EPA, eicosapentaenoic acid; F, formula; g, grams; GA, gestational age; HM, human milk; ICU, intensive care unit; IQ, Intelligence Quotient; kg, kilograms; KPS, Knobloch, Passamanick, & Sherrard's Developmental Screening Inventory; MCDI, MacArthur-Bates Communicative Development Inventories; mg, milligrams; MLU, Mean Length of Utterance; mo, month/s; N, number; ND, none detected; Trt, treatment group; UK, United Kingdom; USA, United States of America; VLBW, very low birth weight; WASI, Wechsler Abbreviated Scale of Intelligence; WIAT-II, Wechsler Individual Achievement Test, Second Edition; wk, week/s; y, year/s

[31] Women were encouraged to provide human milk for as long as possible, but formula with matching group allocation was provided if required

[32] Actual birth weights were Trt: 1312±439g (N=60); Ctrl: 1358±433g (N=61) (mean±SD)

[33] This trial included a reference group fed exclusively human milk (N=43)

[34] % of total fatty acids are presented as pre/post discharge

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
		1251-1800g[35]	N=143 Egg-DTG/fish oil; F DHA 0.24/0.15% AA 0.41%/0.41% EPA ND/None		Post discharge From term CA – 12 mo CA			
Fewtrell 2002 (175)	UK Neonatal ICUs	Singleton, twin & triplet; preterm GA: <37 wk BW: randomised by strata "<1200g or >1200g"[37]	N=95 Egg-DTG; F[36] DHA 0.17% AA 0.31% EPA 0.04%	N=100 F; DHA ND	From start of enteral feeds & if formula fed at 10 d - discharge from neonatal unit	KPS Language subscale	9 mo	No diff.
Henriksen 2008 (186)	Norway Neonatal ICUs	Preg. type NR; Preterm, VLBW GA: 26.6 – 30.9 wk BW: <1500g	N=68 Source NR; HM[38] DHA 6.9% AA 6.7%	N=73 HM[39]	From when Infant received >100 ml HM/kg/d until discharge or 100 ml oil used	ASQ Communication subscale	6 mo CA	No diff

[35] Actual birth weights were Trt: 1305±293g (N=138) (Fish/fungal), 1309±286g (N=140) (Egg-DTG); Ctrl: 1287±272g (N=142) (mean±SD)
[36] Trt formula contained more phosphorus, zinc, vitamin K and less sodium, potassium and iron than Ctrl formula
[37] Actual birth weights were Trt: 1336±284g (N=138); Ctrl: 1353±274g (N=100), (mean±SD)
[38] From either mother or donor
[39] From either mother or donor

Author/ reference	Recruitment	Participants	Treatment	Control	Duration	Language outcome	Age	Result
Fewtrell 2004 **Isaacs 2011** **(187)** **(188)**	UK Neonatal ICUs	Singleton, twin & triplet; preterm[40] GA: <35 wk BW: randomised by strata ≤1200g or >1200g[41]	N=122 Borage, tuna oil; F DHA 0.50% AA 0.04% EPA 0.10%	N=116 F; DHA ND	From birth - discharge or 2 kg, then nutrient enriched F used until 9 mo after term	KPS Language subscale WASI Verbal IQ NEPSY Language domain subscale WIAT-II Word reading WIAT-II Pseudo word decoding WIAT-II Spelling	9 mo CA 10 y 10 y 10 y 10 y 10 y	NR. No diff. No diff. No diff. No diff. No diff.

[40] Some infants received human milk during their hospital stay (Trt N=68, Ctrl N=60)
[41] Actual birth weights were Trt: 1487±342g (N=122); Ctrl: 1510±326g (N=116) (mean±SD)

1.5.5.2.5 LANGUAGE OUTCOMES

Overall, the assessed children ranged from six months to 10 years of age. In total these trials utilized 16 different assessments to report language outcomes.

Five were specifically designed to assess language abilities, including the Clinical Linguistic and Auditory Milestone Scale (CLAMS), MacArthur-Bates Communicative Development Inventories (MCDI), Peabody Picture Vocabulary Test, Revised (PPVT-R), Peabody Picture Vocabulary Test, Third Edition (PPVT-III), Mean Length of Utterance (MLU). Eleven were designed to measure global/cognitive development, including the revised Wechsler Preschool and Primary Scale of Intelligence (WPPSI-R), Wechsler Preschool and Primary Scale of Intelligence, Third Edition (WPPSI-III), Wechsler Abbreviated Scale of Intelligence (WASI), Bayley Scales of Infant Development, Third Edition (Bayley-III), Knobloch, Passamanick, & Sherrard's Developmental Screening Inventory (KPS), Bayley Scales of Infant Development, Second Edition (Bayley-II), Ages and Stages Questionnaire (ASQ), NEuroPSYchological Assessment (NEPSY), Griffiths Mental Development Scales (GMDS). One was designed to measure academic abilities Wechsler Individual Achievement Test, Second Edition (WIAT-II) and one was designed to measure behaviour, the Child Behaviour Checklist (CBCL).

One of these assessments of global/cognitive development provided scores of global language abilities (Bayley-III) and 10 provided scores of domain specific language abilities (CLAMS,

MCDI, PPVT-R, PPVT-III, MLU, KPS, ASQ, NEPSY, GMDS, CBCL) and five provided scores for subscales of specific language domains (WPPSI-R, WPPSI-III, WASI, WIAT-II, Bayley-II). Most assessments were clinician administered although some were parent report (ASQ, CBCL, CLAMS, and MCDI). While language results included for the Bayley-II were those reported by authors, it should be noted that this assessment does not formally include an individual language subscale.

1.5.5.2.6 RISK OF BIAS IN INCLUDED TRIALS

The risk of bias associated with each trial is shown in **Table 6**. Most trials had adequate sequence generation, treatment allocation and blinding of participant processes. Two trials lacked sufficient information to permit proper judgment of sequence generation (27, 167) and three lacked sufficient information to assess treatment concealment (160, 174, 182). All but two trials (163, 188) were assessed as having adequately blinded participants and personnel. While the former did not provide sufficient information in the follow-up study to permit proper judgment (i.e. (188)), the latter was judged as having high risk of bias for their blinding processes as 92.9% of participants correctly guessed their infant's group allocation (i.e.(163)). Three trials (164, 165, 173) were found to lack sufficient information for accurate judgment of blinding of outcome assessors. Only five trials (166, 174, 175, 183, 187) had a follow-up rate of ≥80% which is the minimum follow-up considered acceptable for minimizing attrition bias (69). However, two of these trials did not attain this rate at all assessment points (174, 188). Four trials addressed incomplete outcome data adequately (166, 173, 175, 183, 186). Three trials were considered to have a low-risk of

selective reporting bias (166, 167, 183) and one was considered to have low risk at one point in time (185). Eight trials were judged to be free of other bias (164-166, 171, 172, 174, 175, 183-186).

TABLE 6. Summary of risk of bias assessment for each included trial

Author and Reference	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Follow up >80%	Free of selective reporting	Incomplete outcome data	Free of other bias
Dunstan, 2008(167)	?	+	+	+	-	+	-	?
Makrides, 2010(183)	+	+	+	+	+	+	+	+
Karlsson, 2010(27)	?	+	+	+	-	?		?
Jensen, 2005, 2010(164, 165)	+	+	+	?	-	-/?[2]	-	+
Lauritzen, 2005(160)	+	?	+	+	-	-	?	-
Smithers, 2010(166)	+	+	+	+	+	+	+	+
Scott, 1998, Auestad, 2003(171, 172)	+	+	+	+	-	?	-	?
Lucas, 1999(173)	+	+	+	?	-	?	+	?
Auestad, 2001(174)	+	?	+	+	-	-	-	+
O'Connor, 2001(174)	+	+	+	+	+/-[5]	-	?	+
Fewtrell, 2002(175)	+	+	+	+	+	?	+	+
Birch 2000, 2007(184, 185)	+	+	+	+	-	?/+[3]	-	+
Henriksen, 2008(186)	+	+	+	+	-	?	+	+
Fewtrell, 2004, Isaacs; 2011(187, 188)	+	+	+/?[7]	+	+/-[6]	?/-[4]	?/-[1]	?
Meldrum, 2012(163)	+	+	-[8]	+	-	-	-	?
DeJong, 2012(189)	+	+	+	+	-	-	-	?
Birch, 2010, Drover, 2011; 2012; Colombo, 2013 (161, 162, 176, 190)	+	+	+	+	-	?	-	?
Willatts, 2013(182)	+	?	+	+	-	?	?	?

[1] Risk of bias assigned to 2004 and 2011 studies respectively
[2] Risk of bias assigned to 2005 and 2010 studies respectively
[3] Risk of bias is that assigned to 2000 and 2007 studies respectively
[4] Risk of bias assigned to 2004 and 2011 studies respectively
[5] ≥80% follow-up achieved at 1-year assessment but not at 2-year assessment
[6] Risk of bias assigned to 2004 and 2011 studies respectively
[7] Risk of bias assigned to 2004 and 2011 studies respectively
[8] Authors contacted via email reported risk as L but were judged as H as also reported in paper 92.2% of participants in treatment group correctly guessed their allocation

1.5.5.2.7 RESULTS OF INDIVIDUAL STUDIES

1.5.5.2.7.1 Results from prenatal DHA supplementation

The three trials of maternal prenatal DHA supplementation (27, 167, 183) showed no overall significant differences between treatment and control group language development between 18 months and four years of age using seven different assessments of language abilities, grouped into primary (*Global*) and secondary (*Domain specific*, and *Subscale components of domain specific*) language outcomes **(see Section 1.5.4.2 Study Selection)**. **(Table 2)**

Global language outcomes

Global language outcomes were reported by one trial wherein null effects were found for toddlers' (13-24 months) Language Composite Scores using the Bayley-III (183).

Domain specific language outcomes

Results from assessments of specific language domains similarly yielded no significant effects. This was found for toddlers' (13-24 months) Speech and hearing scores using the GMDS (167), and for preschoolers' Receptive Vocabulary scores with the PPVT-III (167).

Subscale components of domain specific language outcomes

As part of the Language Development Survey of the CBCL a null effect was found for the mean length of phrases and mean number of words in toddlers' (13-24 months) vocabulary (167). Using the WPPSI-III no effect was reported for preschoolers (>24 months - ≤ four years, 11 months) Verbal IQ or General Language Composite scores (27).

1.5.5.2.7.2 RESULTS FROM POSTNATAL MATERNAL DHA SUPPLEMENTATION TRIALS

The two trials of postnatal maternal DHA supplementation (160, 164, 165) primarily revealed null but also some negative effects for language development from 12 months to five years of age using four different assessments of language abilities, grouped into primary (*Global*) and secondary (*Domain specific*, and *Subscale components of domain specific*) language outcomes **(see Section 1.5.4.2 Study Selection). (Table 3)**

Global language outcomes

None of these trials (160, 164, 165) reported global language outcomes.

Domain specific language outcomes

No effect has been found for infants (≤12 months) as measured with the CLAMS (164). While no significant effects were found for this age group using the majority of the MCDI domains including Vocabulary Production, 'Starting to talk', 'Early gestures', 'Later gestures' and 'Phrases understood' (160) a negative effect was reported for Vocabulary Comprehension (160). For toddlers (13-24 months), trials have reported null effects for Vocabulary Production, 'Talk about abstract', 'Use grammar', 'Irregular words' 'Overregulised words', 'Length of longest sentences' (160). For preschoolers (>24 months - ≤ four years, 11 months), trials have revealed null effects using the CLAMS (164).

Subscale components of domain specific language outcomes

Using the WPPSI-R no effect was found for preschool (>24 months - ≤ four years, 11 months) (165) aged children's Verbal IQ scores which assess some language related abilities.

1.5.5.2.7.3 RESULTS FROM INFANT DHA SUPPLEMENTATION TRIALS

The eight trials of infant DHA supplementation (161-163, 171-174, 176, 182, 184, 185, 189) primarily revealed null but also some negative as well as positive effects for language development from six months to 10 years of age using 13 different assessments of language

abilities, grouped into primary (*Global*) and secondary (*Domain specific*, and *Subscale components of domain specific*) language outcomes **(see Section 1.5.4.2 Study Selection)**. For trials that included more than one group receiving DHA the results presented are applicable to all groups in the instances where they do not differ and, where they do, are described in further detail. **(Table 4)**

Global language outcomes

One of these trials reported global language outcomes, revealing null effects for toddlers' (13-24 months) Language Composite Scores as measured by the Bayley-III (163).

Domain specific language outcomes

No effect has been found for infants (≤12 months) Language scores using the KPS (173). While no significant effects were found for this age group using the majority of the MCDI domains including Vocabulary Comprehension (174) 'Words understood' or 'Words spoken', 'Early gestures', and 'Phrases understood' (163), a positive effect was found for 'Later gestures' and 'Total gestures' (163).

For toddlers (13-24 months), some trials that included more than one treatment group have reported differing results for the same outcome. One trial reporting results for Receptive Vocabulary as measured with the PPVT-III found null effects for those supplemented with 0.64% DHA and a negative effect for those

supplemented with 0.32% and 0.96% DHA (161). Another trial reporting MCDI Vocabulary Production scores found null effects for the treatment group supplemented with egg phospholipid (0.12% DHA) and a negative effect for those supplemented with fish oil (0.23% DHA) (171). Other trials have reported null effects for Vocabulary Production (162, 174) Vocabulary Comprehension (171, 174) 'Early gestures' (163, 171), 'Phrases understood', 'Words understood' and 'Words spoken' (163). Furthermore, contrasting null (171) as well as positive (163) effects were found for 'Late gestures' and a positive effect was found for 'Total gestures'(163). Null effects have been reported for Receptive and Expressive Language or the Language Composite using the Bayley-III (163). One trial using the CBCL did not report any results for language abilities (163).

For preschoolers (>24 months - ≤ four years, 11 months), trials have consistently revealed null effects in mean length of utterances (MLU) (172) and Receptive Vocabulary scores of the PPVT-III (161) and PPVT-R (172).

For school aged (five - 12 years) children a null effect on language development was found using the CBCL (189) and also in NEPSY Language domain subscale scores (189) although actual scores for these assessments were not reported. One trial reporting PPVT-III Receptive Vocabulary scores revealed null effects for those supplemented with 0.96% DHA and positive effects for those supplemented with 0.32% and 0.64% DHA (162).

Subscale components of domain specific language outcomes

Null effects were found for toddlers' (13-24 months) Bayley-II 'Language' scores (176, 184). Using the WPPSI-R no effect was found for preschool (>24 months - ≤ four years, 11 months) (185) or school (five - 12 years) (162, 182) aged children's Verbal IQ scores which assess some language related abilities. One trial using the WASI Verbal IQ also reported null effects but no data was shown (189).

1.5.5.2.7.3.1 RESULTS FROM TRIALS INVOLVING PRETERM INFANTS

The five trials of preterm DHA supplementation (166, 174, 175, 186-188) similarly showed no overall significant differences between treatment and control group language development between nine months and 10 years of age using six different assessments of language abilities, grouped into primary (*Global*) and secondary (*Domain specific*, and *Subscale components of domain specific*) language outcomes **(see Section 1.5.4.2 Study Selection)**. **(Table 5)**

Global language outcomes

None of these trials (166, 174, 175, 186-188) reported global language outcomes.

Domain specific language outcomes

Results from assessment of specific language domains revealed DHA had a null effect on the language development of infants' (≤12 months) Vocabulary Comprehension scores using the MCDI (174), Communication scores of the ASQ (186) or Language scores using the KPS (175, 187)(although (187) did not report actual data/ language scores). No significant effect of DHA was found for the language development of toddlers (13-24 months) as indicated by Vocabulary Comprehension or Vocabulary Production scores using the MCDI (174), for preschoolers Vocabulary Production, Sentence Complexity, Irregular words, MLU or Combining words scores again using the MCDI (166) or, finally, for school aged (five - 12 years) children's Language domain scores of the NEPSY (188).

Subscale components of domain specific language outcomes

Using the WASI and WIAT-II no significant effect was found for school aged children's Verbal IQ scores which assess some language related abilities (188).

1.5.5.2.7.4 SYNTHESIS OF RESULTS

Based on the pre specified selection criteria (wherein trials had to be from the same supplementation period, assess language in the same age group and use the same language outcome measure) five trials of postnatal DHA supplementation that reported language outcomes for toddlers (13-24 months) using the MCDI were eligible

for the meta-analysis (160, 162, 171, 174). Only results from three trials were able to be readily combined as the remaining two (160, 162) did not provide standardized scores. Data for the included trials that involved more than one treatment group (171, 174) were pooled according to the aforementioned strategy. DHA supplementation resulted in no difference for Vocabulary comprehension scores (Mean difference (MD) -0.04; 95% Confidence Interval (CI) -3.04 to 2.95; n=423; p=0.98) and there was no heterogeneity (chi-square = 3.39, p=0.18, I^2=41%) **(Figure 13)**. There was also no difference for Vocabulary production scores (MD -2.63; 95% CI -6.09 to 0.83; n=425; p=0.14) and again there was no heterogeneity (chi-square = 0.63, p=0.73, I^2=0%) **(Figure 14)**.

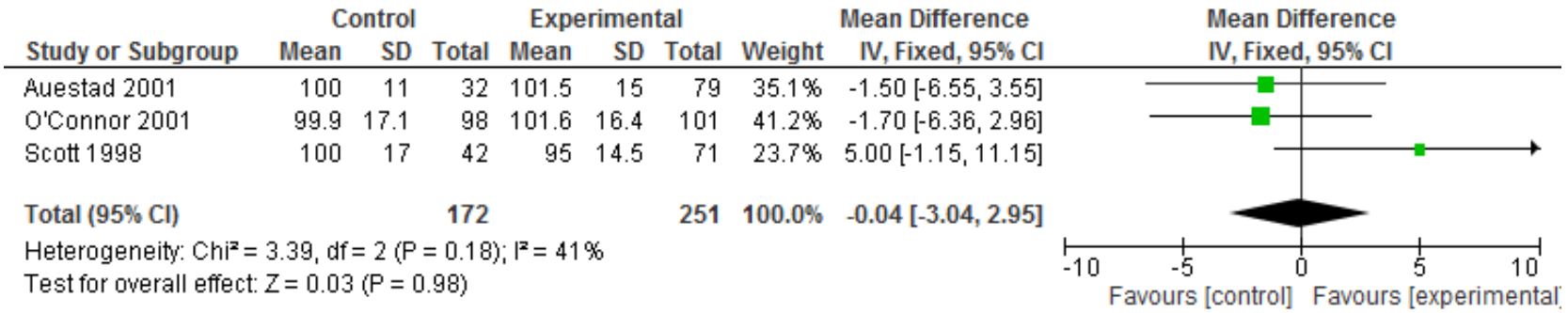


Figure 13. Meta-analysis forest plots of WMDs for language development at 14 months of age measured with the MCDI Vocabulary Comprehension scale - a standardized assessment instrument (mean ± SD: 100 ± 15) after supplementation with DHA during the postnatal period up until 12 months of age. DHA, docosahexaenoic acid; MCDI, MacArthur-Bates Communicative Development Inventories; WMDs, weighted mean differences

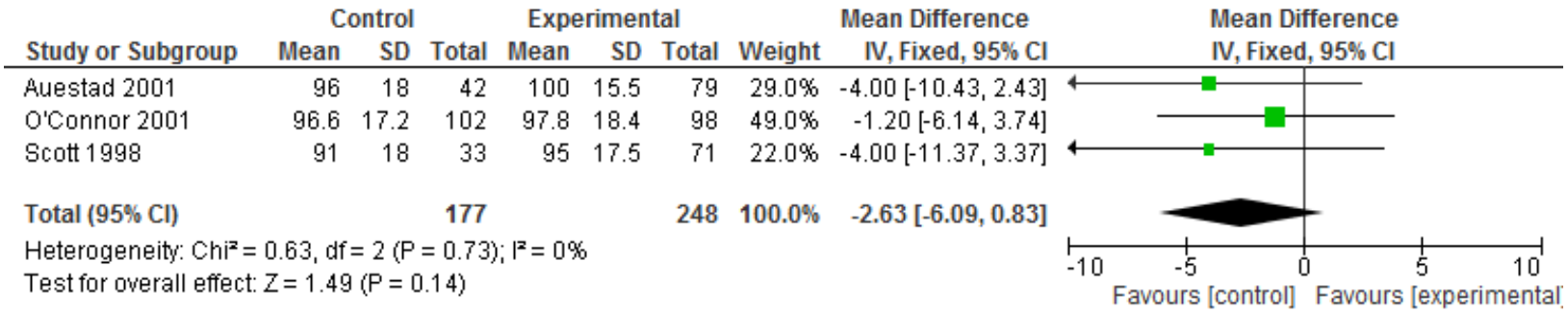


Figure 14. Meta-analysis forest plots of WMDs for language development at 14 months of age measured with the MCDI Vocabulary Production scale - a standardized assessment instrument (mean ± SD: 100 ± 15) after supplementation with DHA during the postnatal period up until 12 months of age. DHA, docosahexaenoic acid; MCDI, MacArthur-Bates Communicative Development Inventories; WMDs, weighted mean differences.

1.5.6 DISCUSSION

This systematic review is the first to evaluate objectively the emergent literature on the topic of DHA supplementation to improve child language development. The existing evidence does not conclusively support or refute the hypothesis that DHA supplementation improves children's language acquisition. Language abilities were assessed in a variety of ways, using tests of global developmental, linguistic, academic and behavioural abilities which provided scores of global language ability, domain specific language skills or even components of particular domains. Analyses revealed that in the majority of studies, null effects of DHA on language development were found, and in the few instances where DHA and control groups differed there was no indication of a clear effect (either positive or negative). The few trials that did find an effect on language development only involved postnatal supplementation of term infants, although the direction of the effect remained largely unclear with one trial suggesting bidirectional effects (161, 162). Despite the substantial number of trials available for review, clinical and methodological heterogeneity limited what data could be meaningfully combined for a meta-analysis. Nonetheless, results from the meta-analysis conducted suggested that postnatal DHA supplementation had no influence on toddlers' (13-24 months) production or comprehension of language. Notably, confidence in the results of the majority of the studies was constrained by methodological limitations and so it is important to exercise caution when interpreting intervention effects.

Positive aspects of the trials in this review related to consistent low risk of bias included randomisation generation involving a third party, allocation concealment by someone with no other study responsibilities and double-blinding. Beyond this, however, risk of bias in other study domains made it difficult to interpret results with certainty. One major limitation of many of the trials in this review concerns the high attrition rates (i.e. >20%) which could have reduced the power to detect statistically significant differences. Another major limitation concerned the lack of statistical power that was sufficient to detect a clinically meaningful effect of DHA on language development in particular, leaving many study results susceptible to a Type 1 error. Few trials addressed missing data with Intent To Treat analysis (173, 175, 183, 186) and the results of some language assessments were unavailable for inclusion in the review (163, 187, 189) which may have resulted in biased estimates of any effects of DHA. Remarkably, only one trial had a consistent low risk of bias (183). This trial reported no effect of DHA on language development at 18 months and was the only study to use multiple imputations to handle missing data. Notably, this trial also reported a negative effect of DHA on girls' language development. While it was beyond the scope of this review to investigate subgroup differences this nonetheless suggests that all paths have not been exhausted and ultimately points to the need to conduct future studies to investigate the DHA-language relationship, perhaps with a focus on theoretically justified subgroups.

Different tests can yield very different estimates of children's language capabilities. It was difficult to compare language outcomes between and within trials because of the inconsistent approaches to assessing language skills, including use of revised

versions of existing instruments (192). Without comparable measurements at different time points it is difficult to determine whether any change in language scores over time is due to DHA or simply a product of differing measurement approaches. Few trials provided scores for global language abilities with the majority using assessments that measured language domain specific abilities. Such assessments might not have been sensitive enough to detect subtle changes in brain function reflective of language abilities that might result from interventions of DHA supplementation (193) and also seldom provide the occasion to assess abilities aside from basic naming in children younger than 24 months which suggests that key aspects of language competence may have been missed. Use of the MCDI is particularly controversial. While some trials arguably did use the MCDI to measure language development (160, 162, 163, 166, 171, 174, 194) there is debate regarding its suitability for evaluating the effectiveness of interventions (195). In particular, the significant variability of scores, the inconsistent relationship between scores and sociodemographic variables, and the lack of strong correlations between scores at one and two years of age question the usefulness of this measure for such a purpose (195). Comparison was also difficult because of the lack of uniform terminology for describing language outcomes. As they move from the home and pre-school setting and enter into the school environment, children continue to acquire more complex linguistic abilities and the concept of language development itself develops to include reading and writing (196). Importantly, it has been suggested that the effects that early exposure to DHA has on brain structure and functional output might not be detectable until later on in life and so noticeable effects on language may only emerge in older individuals (21).

Importantly, the social environment provides children with the opportunity for language experience (197, 198). As brain development and its functions are compromised in an environment that is not appropriately stimulating (199) DHA may enhance the language abilities of children likely to be raised in an impoverished language environment or, in other words, an environment unlikely to provide them with appropriate stimulation for them to develop the neural pathways for acquiring language optimally. All but one trial included in this review reported accounting for maternal education (184, 185) and three trials (161, 163, 176, 182) also accounted for father's education. Additionally, one trial specifically accounted for maternal language (162). Few studies (164-166, 174, 183) accounted for environmental stimulation including that of the home environment so it remains unclear as to whether children received environmental support or experience for their language development which may have mitigated the true effect of the intervention.

Lastly, by only including published data (with the exception of one trial (27)) there was a risk of over estimating intervention effects; although all included trials were published despite minimal evidence for an effect of DHA on any outcome (i.e. in the case of (160-162, 171, 176)).

1.5.6.1 SUGGESTIONS FOR FUTURE RESEARCH

Variation between the studies in regards to the period, duration, and dosage of DHA as well as selection of the optimal test for language assessment made it difficult to identify what may have

contributed to results. Future studies would therefore benefit from adopting a uniform approach. Further research is needed in high-quality, well-powered RCTs of maternal supplementation during the prenatal period, when DHA is likely to have the most rapid deposition in the neural tissue and effect functional outcome, potentially that of language. In elucidating the DHA-language relationship it might be more informative for future research to move beyond focusing on statistically significant differences towards an understanding of how DHA contributes to the developmental trajectory of children's language abilities. Given researchers may come from a wide range of disciplines and expertise the goal for future studies should be to move beyond the ambiguous outcome of "language development" to a more uniform approach, using common measures and definitions that will facilitate comparison of outcomes across trials. Also, as the brain circuitry underlying language abilities continues to undergo neuroplastic changes, assessment at multiple time points is necessary. Finally, future trials should include a more focused investigation of the inter-individual, environmental and genetic influences which, importantly, would require large sample sizes in order to ensure a balance of influences between groups.

1.5.7 CONCLUSION

This review revealed no conclusive evidence that DHA supplementation to mothers during pregnancy or postnatally or directly to the infant up until 12 months of age enhances language development in the child. The evidence from available RCTs is currently not robust enough to draw any strong inferences. Before a high degree of confidence in results is ascertained, opportunities exist for future studies, or follow up of current existing high quality and methodologically sound studies, to make progress by investigating whether DHA supplementation influences language development. Of particular interest should be subgroups thought to be low/deficient in DHA.

1.6 RATIONALE FOR THE CURRENT STUDY

1.6.1 RESEARCH GAP

The brain's architecture develops prenatally and during infancy, laying the foundation on which the structure and function of the adult brain is based. It is possible that not getting enough DHA during critical periods of brain development may render the brain vulnerable to neurodevelopmental difficulties later on in life. Low DHA consumption by women eating Western diets, including those in Australia, has prompted some concern about the adequacy of maternal stores of DHA during pregnancy for the optimal cognitive development of their children (4). Research investigating the importance of DHA with respect to laying the foundation for abilities, particularly cognition, is ongoing and results so far have been largely inconsistent.

Early global measures of development commonly used in research of DHA supplementation (e.g. the Bayley Scales of Infant Development) are designed to identify atypical rather than typical development. As such, they may lack the sensitivity required to detect an effect on specific cognitive abilities. Evidence for the effect of DHA on children's language as a specific aspect of cognition is particularly controversial and its potential to optimize language development warrants further investigation.

1.6.2SIGNIFICANCE

The Australian Early Development Census (AEDC), formally the Australian Early Development Index (AEDI), is a population measure that provides a picture of how children from different communities across Australia are developing in five key areas, including: physical health and wellbeing; social competence; emotional maturity; language and cognitive skills, and; communication skills and general knowledge (174). Information is collected through a 96 question checklist completed by the child's teacher.

Assessment is undertaken in the first year of school in order to show how Australia's children are doing in the five different developmental domains that may, in time, be responsive to change through early intervention. This is an important mechanism to assist government policy and planning for better health, education and community services.

In Australia, results from the AEDC illustrated the variation in language abilities measured in children in the first year of school at five years of age. Notably, 23% of children were 'vulnerable' in language and cognitive skills and 25% were vulnerable in communication skills (23, 24). This means that they scored below the 10th percentile of the national AEDC population. Essentially these children demonstrate a lower than average competence. These findings are of concern as language is a major pathway that supports human capability formation (25) and problems with language accrue to the whole of society in terms of equality, productivity and intergenerational transmission of disadvantage.

1.6.3 SUMMARY

Applied nutrition is a rapidly emerging field that works from research to practice with the purpose of making new scientific knowledge available and actionable to society. This involves identifying research priorities by recognizing key scientific gaps in knowledge about how nutritional knowledge can be applied and then conducting research to address these gaps. For children growing up in Australia, intervention for language development during the early years should be a primary focus for research. As highlighted in the systematic review **(Chapter 1)** the role that DHA might play presents as a compelling area of investigation. Follow up of the largest and most robust trial, which suggested a possible negative effect, is imperative and presents as a valuable opportunity in this thesis.

1.7 THEORETICAL FRAMEWORK

In undertaking research in any scientific field the link between one's theory, methods, and analytic strategy should be closely aligned. The theory provides a conceptual framework from which to understand relationships among the variables under study, and to gain insight into others. The purpose of this section is to outline the theoretical context on which this thesis is based.

Unfortunately, research on language development does not conveniently stem from any particular theory but instead has various conceptual underpinnings, thereby making it difficult to know where to start from in building a cohesive understanding. That said, the mixed results and the limited practical value of much of the research conducted on the effect of DHA on language development to date may be due to a limited theoretical basis or the atheoretical nature of many interventions. The main goal of this section is to introduce the notion of Urie Bronfenbrenner's bio-ecological theory in its most recent form. A brief summary of the history, important features and propositions of the bio-ecological theory and outline how these are related to the current study is provided below.

1.7.1 A BRIEF OVERVIEW OF THE BIO-ECOLOGICAL THEORY OF HUMAN DEVELOPMENT

While aspects of the context in which a person develops (particularly the concepts of microsystem, mesosystem, exosystem,

and macrosystem) were central features of Bronfenbrenner's early thinking around his theory of human development, later evolutions incorporated the important role that the individual plays (200). An important distinction between his early and later theorizing was his focus on processes, particularly proximal processes, of human development, although this did not occur until the 1990s (201-204). Henceforth he discussed what has become the heart of his theory - the Process-Person-Context-Time (PPCT) model (205-207).

1.7.1.1.1 PROCESS

The concept of proximal processes plays a crucial role (i.e. they are considered to be a "primary mechanism") in development. They feature in two central "propositions" that Bronfenbrenner put forward as part of his later theorising:

"…human development takes place through processes of progressively more complex reciprocal interaction between an active, evolving biopsychological human organism and the persons, objects, and symbols in its immediate external environment. To be effective, the interaction must occur on a fairly regular basis over extended periods of *time*. Such enduring forms of interaction in the immediate environment are referred to as *proximal processes*"((204) p. 996)

"The form, power, content, and direction of the proximal processes effecting development vary systematically as a joint function of the characteristics of the developing person; of the environment – both

immediate and more remote – in which the processes are taking place; the nature of the developmental outcomes under consideration; and the social continuities and changes occurring over time through the life course and the historical period during which the person has lived" ((204) p. 996)

1.7.1.1.2 PERSON

Although Bronfenbrenner acknowledged the relevance of biological and genetic aspects of the person (201, 205), he devoted most attention to the personal characteristics that individuals bring with them into any social situation (202, 204, 208). These include those that act as an immediate stimulus to another person (e.g. age, sex, skin colour and physical appearance) which may influence initial interactions because of the expectations formed immediately; those characteristics that, by contrast, are not immediately apparent, including mental and emotional resources (e.g. skills and intelligence), and, finally; characteristics that have to do with differences of temperament (e.g. motivation and persistence). According to Bronfenbrenner, two children may have similar resource characteristics but their developmental trajectories will vary depending on their motivation to do well and persevere in tasks.

1.7.1.1.3 CONTEXT

The environment, or context, involves four hierarchically interrelated systems (200). The *microsystem* refers to the physical and social settings directly encountered by the child. The *mesosystem*

encompasses links between two or more microsystem settings. The *exosystem* involves settings that the child does not directly encounter but which influence microsystem characteristics. The *macrosystem* contains broader social (e.g. culture) and physical (resources and hazards) features which influence the characteristics and actions of aspects of the environment that are lower in the hierarchy.

1.7.1.1.4 TIME

Time as well as timing is equally important because all aspects of the PPCT model can be thought of in terms of relative constancy and change. Thus in understanding development it is important to take into consideration what is happening during the course of some specific activity or interaction, the extent, or consistency, to which activities and interactions occur in the developing person's environment, and also the notion that developmental processes are likely to vary according to the occurrence of historical events (204).

1.7.2 APPLICATION OF THEORY THE CURRENT STUDY

The current study seeks to use Bronfenbrenner's bio-ecological theoretical framework to provide a lens through which to investigate and elucidate the experiences of the pre and postnatal environments that shape children's continued language development in particular. This framework is similar to those used universally (209) wherein environmental variables understood to influence child development are grouped into key areas that researchers can select from to examine in relation to specific

developmental outcomes. The current study will involve an examination of prenatal DHA supplementation and also other factors related to the child, mother and family that have been identified as important to children's language development.

This current study is guided by Bronfenbrenner's bio-ecological theory which requires that all four elements of the PPCT model are present and discussed in subsequent sections.

Maternal nutrition during pregnancy is considered to be an important *proximal Process* influencing development. Specifically, provision of a higher amount of DHA during the prenatal period is thought to be important for language development. Other proximal processes proposed to interact with DHA supplementation include maternal smoking and alcohol consumption during pregnancy.

To understand how *Person* characteristics influence those proximal processes, the current study assesses the ways in which child age, sex, psychological well-being and behaviour influence the DHA-language development relationship.

As *Context* too influences proximal processes, the current study evaluates the differential influence of the microsystem in terms of characteristics of the child's mother (maternal age, education and depression) and family (family functioning and home environment) on the DHA- language development relationship.

Finally, regarding *Time*, the current study is longitudinal and considers what is taking place at various points of the children's lives in influencing language development.

1.7.3 SUMMARY

The current study has the overall aim of furthering our understanding of the potential role of DHA in children's developing language abilities by utilizing the bio-ecological theory of human development as the guiding framework. As part of the bio-ecological theory, early childhood intervention including that of prenatal DHA supplementation is considered a *proximal process* that would be expected to show complex relationships with other *person* variables, and which, together with different *context* variables, would have either favourable or unfavourable consequences for language development and functioning over *time*. Whereas a number of studies have maintained their focus on the efficacy of DHA interventions aimed to improve language outcomes, consideration of effectiveness is just as important. That is, whether DHA works in real life situations. Results of the current study thus have the potential to provide support for guidelines for recommendations for DHA during pregnancy and early life as they may indicate specific avenues for best supporting child language development.

CHAPTER 2

2 DESIGN AND IMPLEMENTATION OF DOUBLE BLINDED RANDOMISED CONTROLLED DOCOSAHEXAENOIC ACID INTERVENTION TRIAL

2.1 CONTEXT FOR THE CURRENT STUDY

The DOMInO trial (DHA to Optimize Mother Infant Outcome)(210) is the largest randomised controlled trial conducted to address the uncertainty surrounding the benefits of dietary docosahexaenoic acid (DHA) for the wellbeing of pregnant women and the neurodevelopment of their children. The trial has been conducted in three stages. The current study is part of the third stage of the trial, so an understanding of the first two stages of the trial provides important context for this study.

Stage One of the DOMInO trial explored whether increased intake of DHA decreased risk for and symptoms of postpartum depression for mothers. Mothers' depressive symptoms were measured at six weeks and six months postpartum by the Edinburgh Postnatal Depression Scale (211-213). Five Australian maternity hospitals were involved in the study. Recruitment is described in detail below.

Stage Two explored whether increased maternal intake of DHA during pregnancy had resulted in appreciable difference in

cognitive development, including language development, in their infants at 18 months of age. Participation was open to 726 children (96 preterm) and 630 randomly selected term) from two of the original five Australian maternity hospitals. Cognitive and language development were assessed using the third edition of the Bayley Scales of Infant and Toddler Development (Bayley-III) (214). Overall, during the 18-month follow-up at Stage Two the study found no difference in mean cognitive scores, or scores of any of the subscales of the Bayley-III. Surprisingly, subgroup analyses revealed that girls from the treatment group had poorer language standardized scores than those girls in the control group, a finding worthy of further investigation.

Stage Three is a four-year follow-up of cognitive and language development outcomes for the children that were assessed at Stage Two. I had primary responsibility for the design and conduct of the language outcomes in this Stage.

The structure of the DOMInO study overall, with participant numbers at each stage, is illustrated in **Figure 15**. Each stage of the trial is described in further detail below as the grounding for the current study.

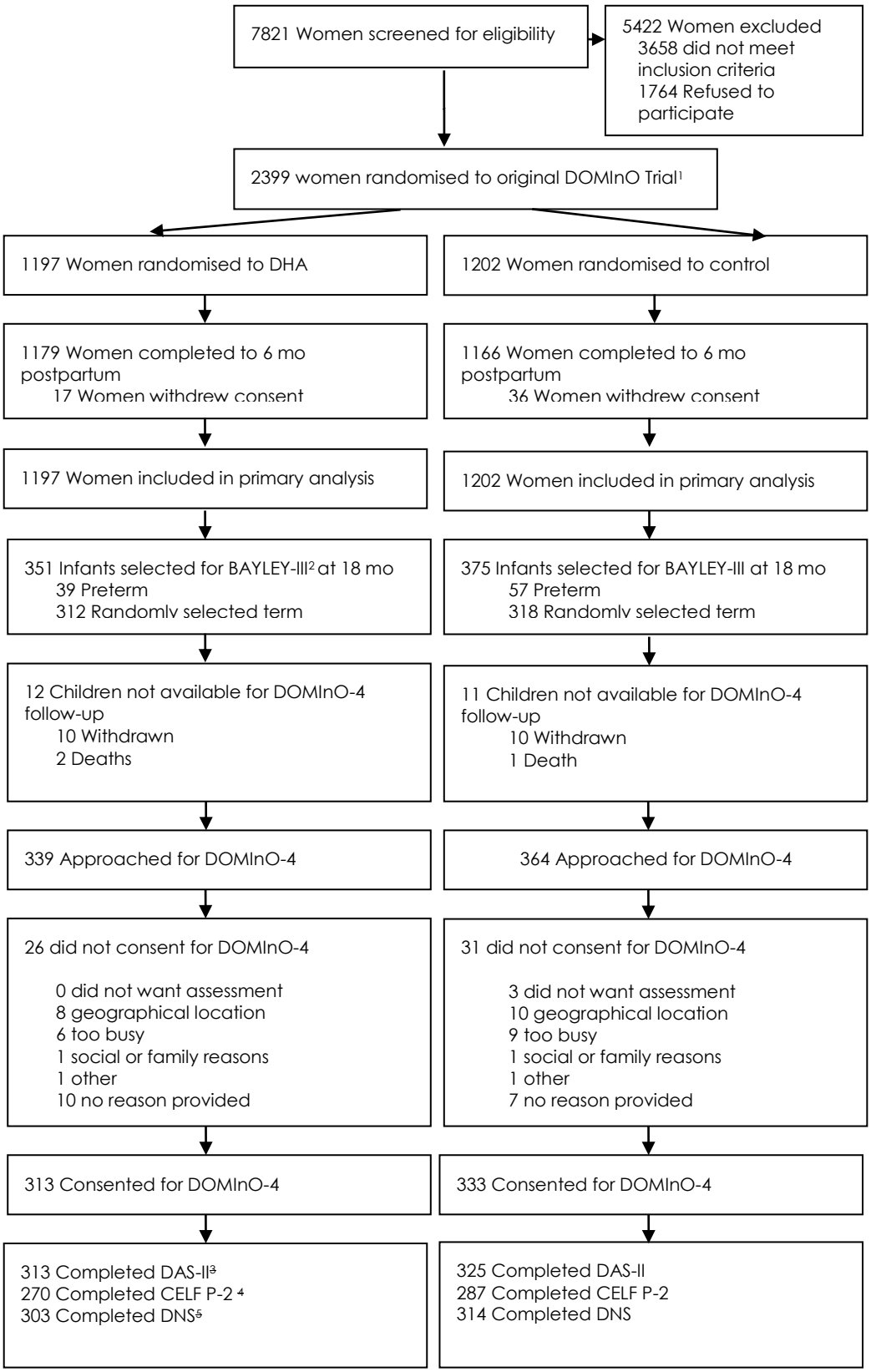


Figure 15. Participant flow in DOMInO trial. 1, DHA to Optimise Mother Infant Outcomes; 2, Bayley Scales of Infant Development, Third Edition; 3, Differential Abilities Scales, Second Edition; 4, Clinical Evaluation of language Fundamentals Preschool, Second Edition; 5, DNS, Day-Night Stroop.Outcome

2.2 THE ORIGINAL DOMINO TRIAL

The DOMInO trial was a double-blind, multicenter, randomised controlled trial in five Australian maternity hospitals (Women's and Children's Hospital, Flinders Medical Centre, Sunshine Hospital, Campbelltown Hospital, Royal Brisbane Women's Hospital). A total of 2,399 women were recruited (out of 7,821 approached) between October 31 2005 and January 11 2008. The trial protocol for the original study was approved by all participating centers. The original DOMInO trial methods have been published previously (210). Trial registrationanzctr.org.au Identifier: ACTRN12605000569606.

2.2.1 AIM

The aim of the original DOMInO study was to determine whether increasing DHA intake during the last half of pregnancy would result in fewer women with high levels of depressive symptoms and enhance the neurodevelopmental outcome of their children.

2.2.2 INCLUSION/EXCLUSION CRITERIA

Women who were less than 21 weeks' gestation with singleton pregnancies were invited to participate. Women were excluded if: they were already taking a prenatal supplement with DHA, their fetus had a known major abnormality, they had a bleeding disorder in which tuna oil was contraindicated, they were taking anticoagulant therapy, they had a documented history of drug or alcohol abuse, they were participating in another fatty acid trial,

they were unable to give written informed consent, or if English was not the main language spoken at home.

2.2.3 INTERVENTION

In the DOMInO trial, mothers allocated to the DHA group were asked to consume three 500 mg/day capsules of DHA-rich fish oil concentrate (providing 800 mg/day of DHA), while women in the control group were asked to take three 500 mg/day vegetable oil capsules without DHA. Women were asked to take their capsules daily, from study entry until the birth of their child.

2.2.4 RANDOMISATION

The randomisation schedule was produced by a statistician (other than the trial statistician) using *ralloc.ado* version 3.3 in Stata Release 9. Centre and parity were used to define the strata and blocks of size 2, 4, 6 and 8 were used in the ratio 1:3:3:1. Under this constraint, the size of the block was chosen at random and treatment allocations were randomly permuted and balanced within blocks. A telephone randomisation service was used to allocate consenting women to receive DHA or placebo.

2.2.5 BLINDING

DHA and placebo capsules were similar in size, shape, and colour and were donated by Efamol, Surrey, England. The blinding was

broken by the statistician during the analysis of the DOMInO Trial, however, the families, clinic staff and outcome assessors were not aware of the treatment allocations. Mothers were able to request details of their own treatment allocation after the 18-month analysis and the number of mothers requesting this information was reported.

2.3 THE 18-MONTH FOLLOW-UP

The aim of the 18-month follow-up was to determine whether increasing maternal DHA during the last half of pregnancy enhanced children's neurodevelopmental outcome of at 18 months of age. Neurodevelopment was measured by the Bayley-III (214). Follow-up of children was completed on December 16, 2009.

Participation was open to 726 children (96 preterm and 630 randomly selected term infants) from two of the original five Australian maternity hospitals, both of which are located in South Australia (Women's and Children's Hospital (WCH) and Flinders Medical Centre (FMC)). This sample size decision was made to enable statistical detection of any difference of five points between groups (a difference previously shown to be significant for families and clinicians) in Bayley-III scores with 80% power for boys and girls separately and allowing for a 10% loss to follow up. All preterm infants were also included to model the effect of DHA supplementation in pregnancy on all children.

The selection process of children involved in the 18-month follow-up specifically involved: identifying all previously participating infants born at the Women's and Children's Hospital and Flinders Medical Centre in an approximate six month period, excluding known withdrawals from the study and any deaths; selecting all preterm infants (<37 weeks gestational age (GA), calculated based on estimated due date obtained at enrolment and date of birth) for follow-up, and; randomly selecting a sample of term infants, half that were male and half that were female, for follow-up. This process was performed at four time points during the trial, approximately after every six months. Sampling by time period was necessary to meet the requirement for the selection to be performed in the period between birth (so the infant could be defined as term or preterm and the sex was known) and 12 months (so a letter could be sent with a birthday card indicating whether the infant had been selected for 18-month assessment or not). The probability of selection for the term infants depended on the time period, hospital and sex, and was defined based on the expected total sample size from these hospitals and the target sample size for the developmental assessment.

2.4 THE FOUR-YEAR FOLLOW-UP

The aim of the four-year follow-up study was to determine whether increasing maternal DHA during the last half of pregnancy enhanced children's neurodevelopmental outcome at four years of age. Follow-up of children was completed in September 2012. This involved a separate protocol and was registered separately to the

original DOMInO study and 18-month follow-up. Trial registrationanzctr.org.au Identifier: ACTRN12611001125910.

Participation was open to the same 726 children invited to participate in the 18-month follow-up. Of the 726 originally selected, 703 children were eligible for the four-year assessment (had not withdrawn from the study or died).

Mothers were able to request details of their own treatment allocation after the four-year analysis and the number of mothers requesting this information is reported in **Chapter 3**. All outcome assessors were not aware of treatment allocations. Eight outcome assessors administered the assessments (91.5% of which were completed by four personnel) and 25% of assessments were audited for consistency between assessors.

The four-year follow-up was designed with cognitive ability, as assessed by the second edition of the Differential Ability Scales (DAS-II) (215), as the primary outcome. However, as they became available, results from the 18-month follow-up prompted the need to investigate language development in greater depth and in turn the later addition of the Clinical Evaluation of Language Fundamentals Preschool (CELF P-2) (125). Other outcomes included executive functioning as measured by subtest scores from the second edition of the Differential Ability Scales (DAS-II) (215), impulse control and mental flexibility as measured by the Day-night Stroop (DNS) (216) and behaviour as measured by the parent-completed Behaviour Rating Inventory of Executive Function-Preschool (BRIEF-P) (217). Parents or caregivers were also asked to

complete the Strengths and Difficulties Questionnaire (SDQ) (218), the Home Screening Questionnaire (HSQ) (219), the Recent Life Events (RLE) questionnaire (220) and the General Functioning scale of the Family Assessment Device (FAD GF) (221).

A number of biological, demographic, and nutritional parameters were additionally recorded. These included the child's height, weight, and head circumference. Also whether they had in the past 12 months developed any health conditions (i.e. Type 1 or 2 diabetes, asthma, eczema, attention deficit hyperactivity disorder, autism), or learning or behavioural disorder, and whether they had had a hospital admission greater than 24 hours' duration. Their intake of fish (i.e. 60-80 grams, equivalent to one small can of tuna or four fish fingers), foods that included DHA (using a comprehensive list that included pictures of the product and definitions of what portion constituted a serving), and vitamin/mineral supplements was also recorded. Parental factors included the division of care for the child (i.e. full time, part time, sole parent, separated, or other), age, level of education, employment status, presence of cigarette smoking, and primary language spoken at home.

2.5 THE CURRENT STUDY

The objective of the current study is to ascertain the effect, if any, of increased prenatal DHA on the language abilities of children at four years of age, by which time any subtle-to-moderate benefits should have emerged and can be reliably assessed. The primary outcome

was the assessment of a broad range of language abilities measured by the CELF P-2 **(Chapter 3 for a full explanation)**.

2.5.1 PARTICIPANTS

2.5.1.1 RECRUITMENT

Participants were recruited using an identical process from WCH and FMC. Participant packs were mailed out six months prior to the child's fourth birthday. Each pack contained: one information sheet noting the caregiver's name which was an invitation to participate in the four-year follow-up study **(Appendices 2** and **3)**; a consent form specifying the study identification code and date of birth **(Appendices 4** and **5)**; a reply paid envelope, and; a DOMInO-4 updated contact details form **(Appendices 6** and **7)**. Returned consent was recorded on a computer system used for documenting participant information and also recording the details of each point of contact.

The clinic staff and assessors made courtesy calls to all participants approximately two weeks after participant packs were mailed out. At this call, caregivers were provided with a further explanation of the study and then given the opportunity to ask any questions as well as book an appointment for their child. Appointments could specifically be booked for those participants who would be aged between four years and four years + three months at the appointment. Upon request it was acceptable to book participants several months in advance. Appointments could only be booked once a hard copy consent form was returned or once people

agreed to attend during the courtesy call. The trial coordinator monitored follow-up, and directed any further attempts to reach families who were unable to be contacted after two months.

2.5.2 PROCEDURE

2.5.2.1 TRAINING

Assessors were familiar with and had relevant graduate training or professional experiences in developmental assessment. In order to confirm that assessors had an appropriate qualification level they undertook formal certified training in administering the CELF P-2. Essentially this involved: starting at an appropriate age entry point, performing all necessary teaching items, repeating items only once if asked, reading directions verbatim but naturally, making sure the child was paying attention, and making the correct discontinuation decision. Overall, I performed 225 assessments.

2.5.2.2 LOCATIONS FOR DATA COLLECTION

The data collection took place at the Child Nutrition Research Centre at Flinders Medical Centre and the Women's and Children's Hospital from 2010 to 2012. In some cases, participants needed to be assessed as a home visit or other local convenient location, such as a local child care centre, including those who had moved interstate, and this was organised accordingly. I performed all 72 out of clinic assessments. Some of the earlier 18 month assessments

were conducted as home visits and naturally some participants requested the same at four years.

2.5.2.3 ADMINISTRATION

All administered tests were carried out in a one-to-one manner between assessors and the child in a quiet, well-lit room that was free from interruptions and distractions. The physical arrangement for presentation of each test involved the child sitting across from the assessor. The age range for which the measures were designed required that the assessor have the ability to establish and maintain rapport with children and caregivers.

During administration of the tests demographic data were recorded from the caregiver **(Appendix 8)** who completed the 'questionnaire pack' in privacy in a room by themselves **(Appendix 9)**. Once assessment finished, the caregiver and clinic staff returned to the clinic room to: collect and check completion of the questionnaires, take anthropometric measurements (head circumference, height and weight) from the child as outlined by the standard operating procedures **(Appendices 10, 11** and **12)**, reimburse caregivers with $20 and the certificate of appreciation. On occasions when a home visit was required assessors completed these tasks that would otherwise have been completed by clinic staff.

2.5.2.4 POST APPOINTMENTS

Assessors marked and recorded attendance in the relevant trial files. Whoever assessed a participating child then produced a written report using a standard template summarizing the outcomes of the assessment for parents or caregivers. The report cover letter was then signed by the assessor and the head psychologist and then posted to the parents. Data were cross checked by the trial coordinator and then prepared for analysis. For each assessor the first 20 CELF P-2 tests and their corresponding reports were audited to ensure correct administration and scoring.

2.5.2.5 CONFIDENTIALITY AND DATA INTEGRITY

Attempts were made to protect data from physical damage as well as from tampering, loss, or theft. This was done by keeping data together in a locked cupboard in a safe secure location away from public access. Privacy and anonymity were assured by replacing names with unique randomisation study codes. Also, all members of the research team were fully educated about data protection procedures. Access to electronic data was protected by using usernames and passwords that could not be easily guessed, providing access to the data through a centralized system. Data files were regularly backed up (both on and offsite).

2.5.3 MEASURES

In order to answer the research questions about language development, data recorded during the original DOMInO trial, the 18-month follow-up and also the four-year follow-up were used **(Table 7)**. Notably, some data used in the current study, including

Bayley-III Language Composite scores (from the 18-month follow-up) and SDQ, BRIEF-P, FAD GF and HSQ scores (from the four-year follow-up) are plausibly outcomes of the intervention as well as predictors of language development.

TABLE 7. Variables used in the current study, time point at which they were collected, and chapter in which they are discussed.

Time data collected	Variable name	Chapter			
		3	4	5	6
Original DOMInO trial	Treatment group	✓	✓	✓	✓
	Cord blood DHA			✓	
	Maternal age		✓		
	Maternal education		✓		
	Maternal smoking		✓		
	Maternal alcohol consumption		✓		
	Maternal depression		✓		
	Child sex		✓		
18-month follow-up	Bayley-III			✓	
Four-year follow-up	SDQ				✓
	BRIEF-P				✓
	FAD GF				✓
	HSQ				✓
	Fish meals consumed			✓	
	DHA foods consumed			✓	
	CELF P-2	✓	✓	✓	✓

Bayley-III, The Bayley Scales of Infant Development, Third Edition; BRIEF-P, Behaviour Rating Inventory of Executive Function-Preschool; CELF P-2, Clinical Evaluation of Language Fundamentals Preschool, Second Edition; DHA, docosahexaenoic acid; DOMInO, DHA to Optimise Mother Infant Outcomes; FAD GF, General Functioning scale of the Family Assessment Device; HSQ, Home Screening Questionnaire, SDQ, Strengths and Difficulties Questionnaire

2.5.4 OVERALL ANALYTIC APPROACH

RCTs are commonly understood to provide the most reliable indication of cause and effect relationships in human clinical research, that is, they establish whether a treatment can work (efficacy). However, it is not really desirable to rely solely on such evidence from RCTs to inform or provide the most accurate picture to policy makers and healthcare professionals. Even when evidence strongly favors a treatment, its applicability in routine clinical practice and translation to the real world may be unclear. Thus it is also important to understand the effectiveness of that treatment or, in other words how it might work in different environments, or for different populations. This type of information moves beyond the biological efficacy of treatments and provides valuable data for policy makers and healthcare professionals on how to best use them in routine clinical practice. Improving our ability to understand these differences between efficacy and effectiveness of a treatment such as DHA may help more fully inform the dissemination of nutritional information to pregnant women. In particular, the current study sought to explore the potential variation in language development as a function not only of DHA but also of interacting biological and social variables.

The current study addressed such issues within its experimental framework by first and foremost determining whether DHA supplementation during the prenatal period had an effect on language development at four years of age **(Chapter 3)**, then by examining interactions between DHA and other individually contributing factors posed by the bio-ecological model **(Chapter 4)** and relationships between the biochemical availability of DHA and language development **(Chapter 5)** and, finally, by testing models that might provide a broader or more comprehensive conceptualization of the role of DHA within the larger system of influences on language development **(Chapter 6)**.

CHAPTER 3

3 THE EFFECT OF PRENATAL MATERNAL DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON CHILDREN'S LANGUAGE DEVELOPMENT AT FOUR YEARS OF AGE: A FOLLOW-UP OF A DOUBLE-BLINDED RANDOMISED CONTROLLED TRIAL

3.1 INTRODUCTION

This chapter seeks to investigate the effect of DHA on children's language development.

There is no more important period in human development than conception through early childhood in maximizing developmental potential. It is during the last trimester of pregnancy when the brain develops most rapidly (1, 2) and where the accumulation of DHA in neural tissues is at the greatest velocity (1, 3). Since maternal diet and stores of DHA during pregnancy and lactation are known to have important implications for the developing brain, the low DHA intake of women in Western countries has resulted in some worry for the developmental outcome of their children (4). Although this suggests a logical and theoretical biological basis for early intervention, via maternal prenatal DHA supplementation, which could make substantial difference to children's neurocognitive development and subsequent performance, evidence for efficacy

remains in doubt. However, as most research has looked at broad cognitive outcomes rather than specific underlying abilities it may have been lacking the focus needed in order to shed light on such specific effects. A more sensitive approach is to identify and measure specific aspects of cognitive ability such as language. Importantly, a child's language skills contribute to their subsequent success in life (168, 169). Existing RCTs of DHA supplementation in pregnant women (N=3), lactating women (N=3) or formula fed infants (N=3) have assessed children's language development in order to see whether supplementation had any benefit for enhancing language abilities. Results have been mixed and inconclusive largely due to methodological limitations **(Chapter 1)**. Thus it is important to evaluate the potential of DHA supplementation to optimize early language development so that informative health recommendations can be made. The DOMInO trial provides an ideal opportunity to examine whether high-dose DHA supplementation during pregnancy influences language development in children.

3.2 RESEARCH HYPOTHESES

The hypothesis is that children from the DHA group will have significantly better language development compared with those in the control group (H1). It is also hypothesised that more children from the DHA group will have language scores in the better functioning ranges (i.e. above average) than those in the control group (H2).

3.3 METHODS

3.3.1 PARTICIPANTS

The current study was a follow-up of the DOMInO Trial; trial details are described in **Chapter 2**. To summarize, women were randomised at enrolment (~18 weeks' gestation) and assigned to consume capsules containing 800 mg of DHA per day (treatment group) or a vegetable oil placebo (control group). A follow-up of the pre-specified sample which consisted of all 96 preterm children and 630 randomly selected term children from two centers in Adelaide, Australia (n=726) was conducted. The baseline characteristics of those selected for follow up were comparable to those not selected for follow up (published previously (183)).

3.3.2 PROCEDURE

All procedures were carried out in line with the trial protocol and approval by the local institutional ethics review boards of each centre **(Appendices 13** and **14)**. Written informed consent was obtained from the guardian of each participant. Psychological assessments were conducted between 18 June 2010 and 25 September 2012. Assessments were administered by eight trained personnel including myself (91.5% of which were completed by four personnel) blinded to group allocation and earlier follow-up assessment results. For children born preterm, their corrected age was used to standardize test scores.

3.3.3 MEASURES

3.3.3.1 CELF P-2: CLINICAL EVALUATION OF LANGUAGE FUNDAMENTALS PRESCHOOL, SECOND EDITION

The primary outcome of this thesis is language development, measured by the CELF P-2 as part of the four-year follow-up of the DOMInO trial. The CELF P-2 is a comprehensive measure of a range of language skills in children three to six years of age. Three subtests were administered to provide a Core Language Score which is an indicator of the general language ability. The Sentence Structure subtest uses 22 items to evaluate the child's ability to interpret spoken sentences of increasing difficulty. The abilities evaluated relate to creating meaning and context in response to pictures. The Word Structure subtest uses 24 items to evaluate the child's ability to a) apply rules related to morphology, and to mark inflections, derivations, and comparison; and b) select and use appropriate pronouns to refer to people, objects, and possessive relationships. The Expressive Vocabulary subtest uses 20 items to evaluate a child's ability to label pictures of people, objects, and actions. Here, abilities assessed relate to the expression of meaning in home and academic settings.

3.3.3.1.1 SCORING AND INTERPRETATION

The Core Language Score is calculated by adding the scaled scores from the Sentence Structure, Word Structure, and Expressive Vocabulary subtests. As indicated in **Figure 16**, this involves following a number of steps. Firstly, it was necessary to determine the raw

score for each subtest by summing the item scores. For each of the subtests, a standard score of 10 is considered to be within the average range, with three points above or below that score equal to one standard deviation, with a standard score seven to 13 within the average range. Next, using the age-appropriate table in the manual, subtest raw scores were converted to a norm referenced index score and percentile rank. The norm referenced scores of the CELF P-2 are standardized to a mean of 100 with a standard deviation of 15.

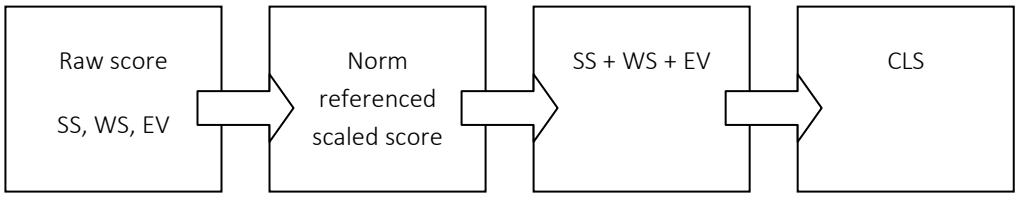


Figure 16. The process for scoring the CELF P-2 subtests of Sentence Structure (SS), Word Structure (WS) and Expressive Vocabulary (EV), and determining the Core Language Score (CLS)

A standard score of 85 – 115 is considered within the average range. Core Language Scores of 85 or below suggest that further testing is warranted to establish whether there is a language disorder and determine the child's eligibility for special services. See **Table 8** for a description of the range of language outcomes based on CELF P-2 Australian results.

TABLE 8. Guidelines for describing the quality of language proficiency according to the CELF P-2 manual

Core Language Score	Classification	Relationship to mean
115 and above	Above average	+1 SD and above
86 to 114	Average	Within + or – 1 SD
78 to 85	Marginal/Borderline	Within – 1 to – 1.5 SD
71 to 77	Low range	Within – 1.5 to – 2 SD
70 and below	Very low range	-2 SD and below

SD, Standard Deviation.

3.3.3.1.2 PSYCHOMETRIC PROPERTIES OF THE CELF P-2

The psychometric properties of the aforementioned CELF P-2 subtests are robust. The average corrected stability coefficients for those aged four years - four years, 11 months are 0.80 for Sentence Structure, 0.77 for Word Structure, 0.90 for Expressive Vocabulary and 0.89 for Core Language Score. The average internal consistency reliability coefficients are 0.74 and 0.73 for Sentence Structure, 0.90 and 0.91 for Word Structure, 0.78 and 0.80 for Expressive Vocabulary, and 0.91 and 0.92 for Core Language Score for those aged four years – four years, five months and for those aged four years, six months - four years, 11 months respectively (125).

In regards to validity, intercorrelations of the CELF P-2 subtests and composites provide evidence of convergent and discriminant

validity **(Appendix 15)**. Specifically, it is important to note that higher correlations are observed between subtests and composites of which they are a part than between subtests that are independent of the composites. Moreover, when factor analyzed, the CELF P-2 items have been shown to load meaningfully on four separate factors (Receptive Language, Expressive Language, Language Content, Language Structure), thus showing divergent validity (125).

3.3.4 DATA ANALYSIS

Previous research revealing nutritional or environmental interventions with a four to five-point change or greater have been a catalyst for changes in health policy (176, 177) and thus it was decided that only differences that reached this threshold would be considered indicative of a phenomenon likely to warrant health consideration for changes to health policy or recommendations to the public. A follow-up sample of 536 children was needed to reliably detect any difference of at least four points in the CELF P-2 Core Language Scores between the treatment groups (mean [SD], 100 [15]), with 80% power (alpha=0.05), after inflating by 10% to allow adjustment for potential confounders including sex and a further 10% to allow for loss to follow up.

Using SPSS version 20, analyses were performed for those who completed the language assessment. Sampling design and probability weights were not taken into account and no adjustments were made. Results based on imputed data have been published elsewhere (222).

Differences in language development as measured by CELF P-2 Core Language Scores and the subtests that comprise these scores (i.e. Sentence Structure, Word Structure, and Expressive Vocabulary scores) between treatment groups were analyzed using an independent-samples t-test. A Chi-square test (with Pearson Chi-Square) was also performed to determine whether there were significant differences between treatment groups in terms of the numbers of participants whose language development as measured by CELF P-2 Core Language Scores fell into the below average, average and above average range. Statistical significance was assessed at the two-sided P<.05 level.

3.4 RESULTS

3.4.1 SAMPLE AND PARTICIPANT FLOW

Of the 726 children originally selected for the 18-month follow-up study, 703 children were eligible for the four-year assessment (had not withdrawn from the study or died). A total of 646 families consented to the assessment at four years (89.0% of the 726 originally selected for neurodevelopment follow-up and 91.9% of the 703 invited) and 557 had CELF P-2 data (76.7% of consenters). See **Figure 15 (Chapter 2)** for an overview of the participant flow.

3.4.2 CHARACTERISTICS OF STUDY PARTICIPANTS

The characteristics of the randomised groups in the subset consenting to the four-year follow-up were comparable at baseline

(Table 9). Overall, there were more participants from the Women's and Children's Hospital (62.5%) and proportions of male and female children were approximately equal within and between treatment groups. The average age at which mothers consented to participate in the initial DOMInO trial was 28.8 years which is representative of the national mean age at first birth in Australia (i.e. 28.9 years) with half of participating mothers falling within the "middle" age group (i.e. 28-37 years). 67.1% had pursued an education subsequent to their high school studies. 31.2% smoked and 67.2% consumed alcohol (either in the two to three months leading up to their pregnancy and/or during their pregnancy). 19.5% of the mothers had a history of depression at study entry. Compared with the DHA group, almost 3% more families from the control group requested the information about which group they had originally been assigned to.

TABLE 9. Comparison of baseline characteristics between treatment groups among consenters to DOMInO-4 who participated in the language assessment at four years of age.

Characteristic	Categorisation	DHA N (%)	Control N (%)	Total N (%)
		270 (48.5)	287 (51.5)	557 (100)
Centre	FMC[1]	105 (38.9)	104 (36.2)	209 (37.5)
	WCH[2]	165 (61.1)	183 (63.8)	348 (62.5)
Child sex	Female	141 (52.2)	153 (53.3)	294 (52.7)
	Male	129 (47.8)	134 (46.7)	263 (47.3)
Maternal age[3]	Younger *(<28 y[4])*	110 (40.7)	117 (40.8)	227 (40.8)
	Middle *(28-37 y)*	145 (53.7)	155 (54.0)	300 (53.8)
	Older *(≥38 y)*	15 (5.6)	15 (5.2)	30 (5.4)
Maternal education[3]	Certificate/Diploma	119 (44.0)	134 (46.7)	253 (45.4)
	Degree/Higher Degree	55 (20.4)	66 (23.0)	121 (21.7)
	None	96 (35.6)	87 (30.3)	183 (32.9)
Maternal smoking[3]	Yes	70 (25.9)	104 (36.2)	174 (31.2)
	No	200 (74.1)	182 (63.4)	382 (68.6)
	Missing	0 (0)	1 (0.4)	1 (0.2)
Maternal alcohol intake	Yes	161 (59.6)	188 (65.5)	349 (62.7)
	No	109 (40.4)	99 (34.5)	208 (37.3)
History of depression[1]	Yes	59 (21.8)	50 (17.4)	109 (19.5)
	No	133 (49.3)	132 (46.0)	265 (47.6)
	Missing	78 (28.9)	105 (36.6)	183 (32.9)

1, Flinders Medical Centre; 2, Women's and Children's Hospital; 3, at study entry; 4, years

3.4.3 MAIN FINDINGS

H1: There was no significant difference in CELF P-2 Core Language Scores for the DHA group (M = 92.95, SD = 14.51) and the Control Group (M = 95.35, SD = 14.24; t (555) = 1.95, p = 0.052, two tailed) **(Table 10)**. The magnitude of the differences between means (mean difference = 2.39, 95% CI: -0.02 to 4.80) was very small (eta squared = 0.006).

TABLE 10. Treatment group differences in Core Language Scores and subtests.

	DHA N=270	**Control N=287**	**Mean difference (95% CI)**	**p-value**
Core Language Score	92.95 (14.51)	95.35 (14.24)	2.39 (-0.02, 4.80)	0.052
Sentence Structure	8.67 (2.84)	9.02 (2.80)	0.36 (-0.11, 0.83)	0.14
Word Structure	8.49 (3.17)	8.89 (2.99)	0.40 (-0.11, 0.92)	0.12
Expressive Vocabulary	9.24 (2.82)	9.70 (2.87)	0.46 (-0.01, 0.94)	0.12

Values are mean (SD), and treatment effects are differences in means (95% CI)

There was no significant difference in CELF P-2 Sentence Structure scores for the DHA group (M = 8.67, SD = 2.84) and the Control Group (M = 9.02, SD = 2.80; t (555) = 1.50, p = 0.14, two tailed). The magnitude of the differences between means (mean difference = 0.36, 95% CI: -0.11 to 0.83) was very small (eta squared = 0.004).

There was no significant difference in CELF P-2 Word Structure scores for the DHA group (M = 8.49, SD = 3.17) and the Control Group (M = 8.89, SD = 2.99; t (555) = 1.55, p = 0.12, two tailed). The magnitude of the differences between means (mean difference = 0.40, 95% CI: -0.11 to 0.92) was very small (eta squared = 0.004).

There was no significant difference in CELF P-2 Expressive Vocabulary scores for the DHA group (M = 9.24, SD = 2.82) and the Control Group (M = 9.70, SD = 2.87; t (555) = 1.92, p = 0.56, two tailed). The magnitude of the differences between means (mean difference = 0.46, 95% CI: -0.01 to 0.94) was very small (eta squared = 0.007).

H2: A Chi-square test for independence indicated no significant association between treatment group and those who had Core Language Scores in the above average, average, borderline, low and very low ranges, χ^2 (4, n = 557) = 3.37, p = 0.50, Cramer's V = 0.08 **(Table 11)**

TABLE 11. Treatment group N differences in Core Language Score classification

Core Language Score	Classification[1]	DHA N=270 (48.47)	Control N=287 (51.53)	Total N (%) 557 (100)
115 and above	Above average	13 (4.81)	20 (6.97)	33 (5.92)
86 to 114	Average	182 (67.41)	202 (70.38)	384 (68.94)
78 to 85	Borderline/Mild	33 (12.22)	33 (11.50)	66 (11.85)
71 to 77	Low range/Moderate	24 (8.89)	18 (6.27)	42 (7.54)
70 and below	Very low range/Severe	18 (6.67)	14 (4.88)	32 (5.75)

1, Severity of language disorder according to CELF P-2 manual, Values are N (%)

3.5 DISCUSSION

DHA supplementation during pregnancy was expected to enhance the language development of children at four years of age. The current study found no significant effect of DHA supplementation during pregnancy on children's language development at four years of age as measured by the primary outcome of the current study: mean Core Language Scores as assessed using the CELF P-2. Results also revealed no differences between the DHA and Control groups in terms of the numbers of children with Core Language Scores clinically classified as being in the above average, average, borderline, low and very low ranges for children of comparable age.

Both treatment groups' language development was considered to be normal as mean Core Language Scores fell within the average ranges (85-115). 22% and 27% of children in the control and DHA groups respectively as well as 25% of the whole sample had Core Language Scores in the below average ranges. Despite being selected from one state only (South Australia) it is likely that the sample is representative of the wider population of children in Australia as results are comparable to findings from the AEDC which suggested that 23% of children demonstrate a lower than average ability in language development prior to starting school (23, 24).

The null findings of the current study are in agreement with most of the developmental findings of previous RCTs with language outcomes (161, 163-166, 171-173, 175, 176, 182, 184-186, 188, 194, 210, 223, 224) including the larger DOMInO trial (210). One of the biggest criticisms around the lack of effect of DHA supplementation in pregnancy on child development is that nearly all tests have used global tests of child development. Global tests lack the specificity to detect functioning of specific cognitive abilities including language. The current study used a comprehensive measure of a broad range of children's language abilities and also did not find an effect of DHA supplementation. This suggests that prenatal DHA supplementation confers no specific benefit to language development in early childhood.

It may be that other Person characteristics moderate the relationship between DHA and children's language development. For instance, the majority of studies to date investigating

neurocognitive development have not examined the effect of maternal genetic variation.

In line with the bio-ecological theory the current study proposed that provision of a higher amount of DHA during the prenatal period could be considered to be a proximal process important for language development. Related to the power of proximal processes is the notion that language development might depend on a higher dose of DHA, or both groups may have been in receipt of sufficient DHA to support their language development, regardless of supplementation. To date only one study has investigated the effect of multiple DHA concentrations (i.e. 0%, 0.32%, 0.64% and 0.96%) on language development with no evidence for a consistent effect at different time points (161, 162, 176). Particularly with regard to those supplemented with 0.32% DHA this suggests that DHA might have bidirectional effects on language outcome. Alternatively, it may be that no additional DHA is required during pregnancy as the placenta will act as an in-built mechanism and concentrate sufficient amounts for the fetus.

It is important to note that there is just as much ambiguity surrounding what, if any, the implications of too much DHA may be. Although not statistically significant, the lower language scores in the DHA group might even be suggestive of DHA being a particularly strong stimulus that may have resulted in overstimulation as this has been shown to impact neuronal functioning. Here it is worthwhile considering the potential effect of DHA on the neurodevelopmental events that take place after birth that surround language learning, namely synaptogenesis – the process

through which neurons receive their connections. Notably, more in development does not necessarily mean better, more complex, or more mature.

It is also possible that the effect of varying DHA levels in early life may have consequences that will become apparent in the longer term, or are too subtle to detect at this point in time (21) and so noticeable effects on language may emerge in older individuals. DHA may enhance later learning or in other words may be the best stimulus for which children have been tuned to learn more complex language abilities later on in life, such as those relating to grammar that tend to develop after four years of age. This idea is also consistent with findings of other RCTs of DHA supplementation with language outcomes that have found an effect later on in the child's life, particularly in the area of reading and spelling, but not during earlier periods of infancy.

Brain development occurs earlier than when supplementation started. The DOMInO trial might have missed a critical point/important period for DHA to have maximal effect. Alternatively, as brain development continues beyond when supplementation ceased and considering infant red blood cell and plasma DHA concentrations rapidly decrease by 50% within four months after birth without an exogenous source of DHA (225) prenatal supplementation studies may have been more likely to identify a positive effect on language development had the supplementation period been extended beyond pregnancy (although this was not demonstrated in the one trial in which this occurred (27)).

The current study had satisfactory internal validity as there was a low dropout rate, equal involvement in each group, and blinding was continued from the original trial, although some participants did request and receive their group allocation. Thus, there was a low risk of attrition or participant bias (Results based on missing data that were imputed data are published elsewhere (222)). The trial also had satisfactory power (>80%) to distinguish a difference of at least four points between the groups in Core Language Scores, minimizing the risk of a Type II error.

Within this study a critical assumption was that all individuals should react similarly to the same level of DHA exposure and that a single dose response relationship should link DHA exposure to language development scores. However, the validity of this assumption of equivalent reactivity could be questioned given evidence on the inter-individual variation of DHA on language outcome **(see Chapter 1, Section 1.5)**. Such questioning is consistent with the conceptual framework of this thesis; namely that the impact of bio-ecological influences can only be understood when such influences are viewed as operating within a complex system of linked multiple influences upon development. This requires a more thorough investigation of what other influences may interact with DHA exposure. This means looking at how the nature of the prenatal DHA supplementation proximal process varies according to aspects of the Person and Context.

3.6 CONCLUSION

In this population of children from largely normal pregnancies, increasing fetal exposure to DHA during late pregnancy did not influence a comprehensive measure of language development at four years of age.

CHAPTER 4

4 INTERACTIONS BETWEEN PRENATAL MATERNAL DOCOSAHEXAENOIC ACID SUPPLEMENTATION AND OTHER ENVIRONMENTAL VARIABLES INFLUENCING CHILDREN'S LANGUAGE DEVELOPMENT AT FOUR YEARS OF AGE

4.1 INTRODUCTION

This chapter seeks to investigate the interactions between DHA and other biological and social factors influencing children's language development.

It is important to explore the potential variation in language development as a function not only of DHA but also of interacting biological and social variables. The point of efforts to understand individual differences in the influence of DHA on children's language development is to inform as well as know how to respond to these inequalities with public health interventions.

Evidence has pointed to the potential for inter-individual, biologically based, differences to moderate DHA synthesis. As children grow up, the social environment increasingly plays a part while biological factors play a decreasing part in influencing language development. For example, reports from Australia's Early

Language in Victoria Study (ELVS) study suggests that environmental variables account for 10% and 20% of the variance in expressive and receptive language skills at two and four years of age respectively (226, 227). An environment that provides enriched activities that stimulates language development will enhance synaptogenesis and bring about a change in a child's brain (228). Human interaction with children has been shown to have a particularly strong influence on language acquisition abilities. This has been dramatically demonstrated by cases in which children have been reared in socially isolating environments, revealing that social deprivation has negative repercussions for language development, such that 'typical' language skills do not develop (229).

As DHA could drive the creation of neural pathways, or links between neurons for acquiring and storing language, it may enhance the language abilities of children likely to be raised in an impoverished language environment or an environment unlikely to provide them with appropriate social interaction for them to develop the neural pathways for acquiring language optimally.

The following sections will present biological and social factors influencing language development and discuss how DHA may be related.

4.1.1 CHILD SEX

Females commonly have better linguistic abilities than males, even from an early age (230, 231). They begin talking sooner (3) and attain vocabulary more rapidly (232). These advantages have been shown to continue through the school years (233) and into adulthood (234).

Research suggests that females have a greater ability to convert ALA into DHA than males (74), which results in them having more DHA circulating in their plasma (235). The impact of estrogen and other hormones on the activity and expression of the delta-5 desaturase and delta-6 enzymes in the liver have been proposed to be attributable to this process (236, 237). While some research also suggests that females manifest a greater capacity to incorporate dietary DHA into blood plasma, cells and tissues (238), other findings suggest that this may have negative implications for girls' language development (210). Although applicability of such findings in children is limited, some research in a Western Australia pregnancy cohort reported sex-specific long-term neurocognitive benefits from lactation, particularly for males (239).

This suggests that males may have higher requirements for DHA in the diet and their language development may be more likely to benefit.

4.1.2 MATERNAL AGE

In Australia, around 1 in 25, or about 11,700 babies are live born to teenage mothers (240). Research suggests that children raised by older mothers have better language development compared to those raised by younger mothers, although exactly what age range constitutes older versus younger varies between the studies. Language development at 3 and 5 years of age is significantly better in children of mothers aged 40 compared to those of mothers aged 20 (241). There are also findings that preschool children of teenage mothers have lower IQ scores than children of older mothers (matched for socioeconomic status) (229, 242). Considering many IQ tests comprise an assessment of language abilities it is plausible to suggest that having "younger" parents may negatively affect children's language development. Reasons for differences in language development related to maternal age may be due to children having different language experiences. Younger mothers have been found to talk less frequently to their children than mothers who were a little older and matched for education (243). Furthermore, their speech was less complex in general. For instance, they articulated fewer object labels and asked fewer questions.

Although the relationship between age and omega 3 LCPUFA levels, including DHA, has been reported several times before (244-246) little information is available concerning age related differences in DHA metabolism. Results from one trial which looked at whether incorporation of dietary omega-3 FAs into plasma phospholipids changes depending on age showed that older adults

incorporated significantly more DHA into plasma lipids compared to younger adults (247). Another trial reported no differences between older and younger subjects' overall oxidation of DHA (248). Notably, these trials included men, had few participants and differently defined the 'older' age group (i.e. to be 74±4 years old) which could soften any potential effects.

This suggests a potential role for DHA to influence the language development of children with young mothers, who may not be able to provide appropriate linguistic stimulation, and older mothers, who might have age related difficulties in their ability to properly transfer DHA to the developing fetus.

Considering the age related differences in the communicative environment that mothers can provide for their children, this suggests that DHA may improve the language development of children of younger mothers.

4.1.3 MATERNAL EDUCATION

There is considerable evidence that maternal education influences children's language performance. Language development is better in children of mothers who were either college graduates (compared to those who were either high school graduates or did not complete high school) or high school graduates (compared to those whose mothers had not graduated from high school) (249). As with age, variation in education is associated with variation in the language experience provided to children. This has been linked to

academic competence and attitudes towards education (250, 251) and knowledge and beliefs about child development (252, 253). It is reasonable to suggest that a mother's educational achievement might affect the characteristics of the language she uses towards her child (254, 255). A brain imaging study of young children revealed maternal education impacted brain regions related to attention skills (256) which are related to language development (257).

Considering the education related differences in the communicative environment that mothers can provide for their children, this suggests that DHA may improve the language development of children of mothers with a lower educational level.

4.1.4 MATERNAL SMOKING

Cigarettes are a common "non-medicinal drug" used during pregnancy, particularly in Western cultures (258). Alarmingly, although rates of smoking are declining in the general population, rates for pregnant women are declining the most slowly (259). In Australia, approximately 1 in 7, or 42,600, women who gave birth in 2009 smoked during pregnancy (240). Maternal smoking while pregnant is known to be detrimental to child development. Specifically, it has been shown to have a negative influence on brain development. In particular, previous studies have posited a role for the association with poorer language development in their infants and children compared with those whose mothers did not smoke during pregnancy (41, 258-263).

Maternal smoking during pregnancy has a negative impact on the central nervous system (CNS) in the fetus as it restricts utero–placental blood flow and the availability of oxygen (264). Of note, breastfeeding has been shown to buffer such an impact during pregnancy on children's cognitive development (265). It is plausible to suggest that something in breast milk itself, like high concentrations of LCPUFAs, may have positive influence on early brain development and function by counteracting the detrimental consequences that mothers smoking during pregnancy has on the fetus.

This suggests that DHA supplementation may mitigate the negative effects that prenatal exposure to maternal smoking has on children's language development. While the aforementioned findings focus on more severe cases of smoking, the current study has the opportunity to explore such relationships in a group whose alcohol use may be considered to be more moderate and applicable to real life situations.

4.1.5 MATERNAL ALCOHOL CONSUMPTION

Alcohol is one of the most commonly used substances that impacts the developing brain and is a key avoidable cause of neurodevelopmental disorders (266). The 2010 National Drug Strategy Household Survey revealed that, of the estimated 395,000 women in Australia who were pregnant in the year before the survey, half (51%) reported drinking alcohol during pregnancy (240). The impact of prenatal alcohol exposure depends on the frequency and intensity of alcohol consumed and the developmental period

that the fetus is at. Those children with Fetal Alcohol Syndrome (FAS) have mothers who have previously used alcohol heavily, either chronically or intermittently. However, it is important to note that even prenatal alcohol exposure at lower doses can result in a range of milder outcomes that are still of practical importance (267). Fetal Alcohol Spectrum Disorders (FASD) is the broad name used to encompass the variety of such alcohol related harms (268, 269). A large number of experimental studies have reported the susceptibility of the developing central nervous system (CNS) to the effects of alcohol. Alcohol seems to have the biggest impact on the developing brain during the last trimester of pregnancy when, as previously noted, the brain growth spurt occurs (270). Impairment to language abilities has been found to be a prominent characteristic of brain damage in children with FAS and FASD (271-273).

Prenatal alcohol exposure may compromise essential fatty acid status in two ways. First, the fetus is fully dependent on maternal placental transport of essential fatty acids, and alcohol can disturb this process (274). Second, alcohol increases fatty acid catabolism which may lead to decreased concentrations of LCPUFAs (275). The loss of DHA in particular from the CNS may lead to suboptimal CNS development and function. Furthermore, these findings are supported by a study showing that newborn guinea pigs have clear reductions of DHA concentrations in brain phospholipids after they were exposed to alcohol on a daily basis, and that the deficit could be reversed, at least partially, if they were supplemented with tuna oil (276).

This suggests that prenatal alcohol exposure can leave the fetus vulnerable to lower DHA concentrations in the brain and that there is potential for DHA supplementation to atone for this and in turn optimize the language development of children whose mothers drink during pregnancy.

4.1.6 MATERNAL DEPRESSION

National research in Australia shows that 15.5% of postnatal mothers are affected by depression (277). The impact of maternal depression on fetal and infant development is multifaceted as there are direct biological, genetic, and indirect environmental, influences that extend from early pregnancy well into early childhood. There is a growing body of evidence for maternal psychological functioning altering fetal and neonatal physiological outcomes. The mother's capacity to provide a communicative environment that their child needs to develop language is influenced by her mental well-being. On average, depressed mothers spend less time providing the experiences that their infants need for optimal language development such as touching and talking to their children (278). Research also reveals a significant relation between maternal depression and neonatal brain activity related to well-being (specifically stress reactivity and vulnerability for mood and anxiety disorders (279)), which is related to language development. Higher and consistently elevated levels of cortisol, a hormone linked to stress, have been found in infants of mothers with depression compared to those without depression (280). Other studies suggest that cortisol levels that are raised for prolonged periods of time are related to deteriorated hippocampus function,

which is associated with learning and memory (281) that is particularly sensitive to dietary DHA.

This suggests that maternal depression may possibly have a long lasting effect on a child's ability to retain memories and therefore learn language and that DHA supplementation which may target brain regions central to these processes may mitigate such effects.

4.2 RESEARCH HYPOTHESES

It was hypothesized that DHA would statistically significantly benefit the language development of boys more than girls (H1), children of the youngest group of mothers, more than those of older mothers (H2), children of mothers of a lower educational status (H3), children exposed to maternal smoking relative to those whose mothers did not smoke (H4) and those exposed to maternal alcohol consumption relative to those who were not (H5) prenatally and, finally, children whose mothers had a diagnosis of depression (prior to the intervention) relative to those whose mothers did not (H6).

4.3 METHODS

4.3.1 PARTICIPANTS, PROCEDURE AND MEASURES

The methods for participants and procedures are described in **Chapter 2**. Language development was measured by the CELF P-2 as part of the four-year follow-up of the DOMInO trial. A detailed

description is provided in **Chapter 3**. A description of additional measures/grouping variables uses are described below.

Maternal age

In the absence of any agreed upon age cut-offs the current study based the age groups on reproductive maturity. The 'younger' age group was defined as <28 years of age, as 28 years is the national mean age at first birth in Australia (282). In defining the 'older' motherhood group the period during which children can be conceived and carried in the reproductive life cycle which is usually between 15 and 44 years of age (283) was taken into account, although it is noted that fertility is considerably lowered from 38 years onward (284). Furthermore, women 39 - 44 years of age have a higher risk of not being able to bear children compared to those less than 30 years (285). As the age of 38 therefore represents a biological indicator for which the expectancy of fertility is diminished (284) the 'older' age group was accordingly defined as being ≥38 years of age. Thus women who had their child at ages between those considered to be 'younger' or 'older' (i.e. those aged from 28-37 years) comprised the 'middle' age group.

Maternal education

Mothers were asked whether they completed further study after school and were able to answer that they had not (i.e. "None") or that they had, either in the form of university studies (i.e. a "Degree", or a "Higher Degree") or an alternative vocational course (i.e. a

"Certificate/Diploma"). For the purpose of data analysis in the current study women were classified either as having completed a 'Certificate/Diploma' or 'Degree/Higher degree' or 'None' (i.e. as not having completed further study).

Maternal smoking

At entry into the DOMInO trial mothers were asked if they currently smoked cigarettes and if they smoked cigarettes in the two to three months leading up to their current pregnancy. For both questions they were able to respond either with "No" or "Yes". If mothers answered "Yes" on either occasion then they were asked to specify how many cigarettes (i.e. "1 – 20 per day", "21 – 40 per day" or "more than 41 per day"). For the purpose of data analysis in the current study mothers smoking status was classified as either 'DID NOT smoke' (i.e. did not currently smoke and did not smoke in the 2 to 3 months leading up to their current pregnancy) or 'DID smoke' (i.e. did currently smoke and/or did smoke in the two to three months leading up to their current pregnancy).

Maternal alcohol consumption

At entry into the DOMInO trial mothers were asked if they currently drank alcohol and if they drank alcohol in the two to three months leading up to their current pregnancy. For both questions they were able to respond either with "No" or "Yes". If mothers answered "Yes" on either occasion then they were asked to specify the average number of standard alcoholic drinks (i.e. "up to 1 to 2 per week",

"up to 7 to 10 per week" or "more than 10 per week"). For the purpose of data analysis in the current study mothers drinking patterns were classified as either 'DID NOT consume alcohol' (i.e. did not currently drink and did not drink in the two to three months leading up to their current pregnancy) or 'DID consume alcohol' (i.e. did currently drink and/or did drink in the 2 to 3 months leading up to their current pregnancy).

Maternal depression

Before commencing the DOMInO trial mothers were asked if they had a previous diagnosis of depression, a previous diagnosis of postnatal depression and a current diagnosis of depression. For these questions they were able to respond either with "No" or "Yes" or, in relation to having a previous diagnosis of postnatal depression "Not applicable". For the purpose of data analysis in the current study maternal depression status was classified as either 'NO Depression diagnosis' (i.e. did not have a diagnosis of depression at any of the aforementioned time points) or 'Depression diagnosis' (i.e. did have a diagnosis of depression during at least one of the aforementioned time points).

4.3.2 DATA ANALYSIS

Using SPSS version 20, analyses were performed for those who completed the language assessment. Sampling design and probability weights were not taken into account and no adjustments were made.

A two-way between-groups analysis of variance was conducted to explore the impact of treatment group (DHA, Control) and: child sex (female; male) (H1), maternal age (Younger: ≤27 years; Middle: 28-37 years; Older: ≥38 years) (H2), maternal education (Certificate/Diploma, Degree/Higher Degree, None) (H3), maternal smoking in utero (Yes, No) (H4), maternal alcohol consumption in utero (Yes, No) (H5), and maternal depression (Diagnosis of depression, NO diagnosis of depression) (H6) on language development as measured by CELF P-2 Core Language Scores and for Sentence Structure, Word Structure and Expressive Vocabulary subtest scores individually. Statistical significance was assessed at the 0.05 level.

4.4 RESULTS

4.4.1 SAMPLE AND PARTICIPANT FLOW

See **Figure 15 (Chapter 2)** for an overview of the participant flow.

4.4.2 CHARACTERISTICS OF STUDY PARTICIPANTS

For demographic information refer to **Chapter 3**.

4.4.3 MAIN FINDINGS

4.4.3.1 CHILD SEX

H1: The interaction between treatment group and sex of child was not statistically significant, F (1, 553) = 1.38, p = 0.24 **(Figure 17).** There was a statistically significant main effect for sex, where girls (M = 95.91, SD = 14.05) had higher Core Language Scores than boys (M = 92.27, SD = 14.78; F (1, 553) = 8.63, p < 0.01); however, the effect size was small (partial eta squared = 0.02).

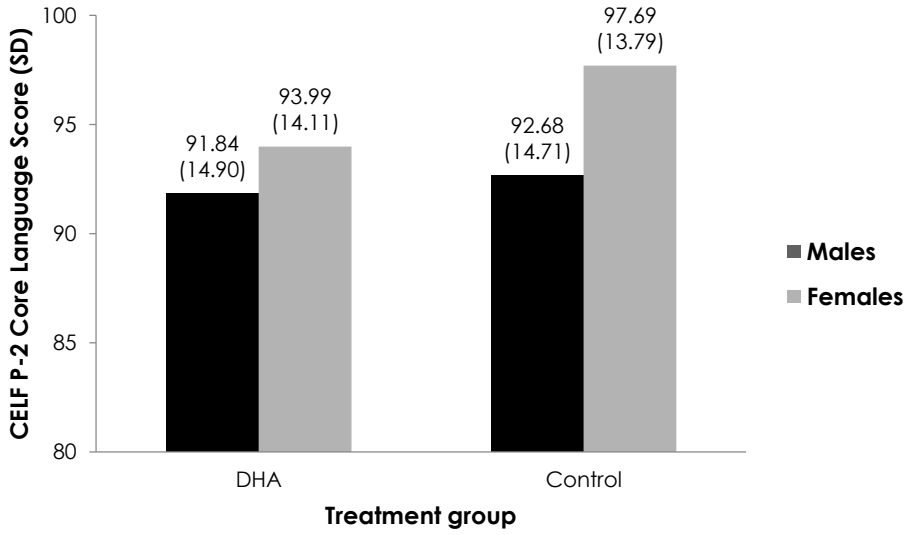


Figure 17. Treatment group differences in Core Language Scores by sex of child. Results presented are Mean (Standard Deviation).

Sentence Structure

The interaction effect for the Sentence Structure subtest was not statistically significant, F (1, 553) = 0.00, p = 0.98. There was a

statistically significant main effect for sex, where girls (M = 9.19, SD = 2.71) had higher Sentence Structure scores than boys (M = 8.47, SD = 2.90; F (1, 553) = 9.22, p <0.01); however, the effect size was small (partial eta squared = 0.02).

Word Structure

The interaction effect for the Word Structure subtest was not statistically significant, F (1, 553) = 3.00, p = 0.08. There was a statistically significant main effect for sex, where girls had higher Word Structure scores than boys (M = 8.25, SD = 3.13; F (1, 553) = 9.89, p <0.01); however, the effect size was small (partial eta squared = 0.02).

Expressive Vocabulary

The interaction effect for the Expressive Vocabulary subtest was not statistically significant, F (1, 553) = 1.29, p = 0.26. The main effect for sex, F (1, 553) = 1.37, p = 0.24, also did not reach statistical significance.

4.4.3.2 MATERNAL AGE

H2: The interaction between treatment group and maternal age was not statistically significant, F (2, 551) = 0.95, p = 0.39 **(Figure 18)**. There was a statistically significant main effect for maternal age, F (2, 551) = 8.81, p <0.01, however, the effect size was small (partial eta squared = 0.03). Post hoc comparisons using the Tukey HSD test indicated that the children of mothers in the 'Middle' age group had significantly better Core Language Scores (M = 96.20, SD = 0.82) than those children of mothers in the 'Younger' age group (M = 91.09, SD = 0.95).

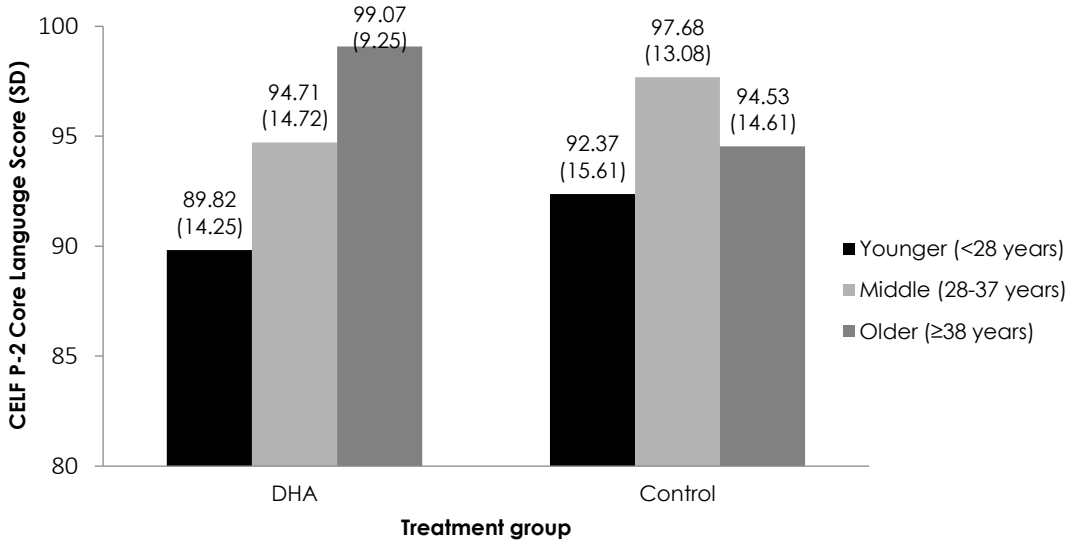


Figure 18. Treatment group differences in Core Language Scores by maternal age group. Results presented are Mean (Standard Deviation).

Sentence Structure

The interaction effect for the Sentence Structure subtest was not statistically significant, F (2, 551) = 2.45, p = 0.09. There was a statistically significant main effect for age, F (2, 551) = 0.01; however, the effect size was small (partial eta squared = 0.02). Post-hoc comparisons using the Tukey HSD test indicated that the children of mothers in the 'Younger' age group (M = 8.42, SD = 2.96) had significantly poorer Sentence Structure scores than those children of mothers in the 'Middle' age group (M = 9.12, SD = 2.69).

Word Structure

The interaction effect for the Word Structure subtest was not statistically significant, F (2, 551) = 0.63, p = 0.53. There was a statistically significant effect for age, F (2, 551) = 4.95, p = 0.07; however, the effect size was small (partial eta squared = 0.02). Post-hoc comparisons using the Tukey HSD test indicated that the children of mothers in the 'Younger' age group (M = 8.21, SD = 3.16) had significantly poorer Word Structure scores than those children of mothers in the 'Middle' age group (M = 9.05, SD = 3.01).

Expressive Vocabulary

The interaction effect for the Expressive Vocabulary subtest was not statistically significant, F (2, 551) = 0.24, p = 0.79. There was a

statistically significant main effect for age, F (2, 551) = 10.17, p <0.01; however, the effect size was small (partial eta squared = 0.04). Post-hoc comparisons using the Tukey HSD test indicated that the children of mothers in the 'Younger' age group (M = 8.84, SD = 2.84) had significantly poorer Expressive Vocabulary scores than those children of mothers in the 'Middle' (M = 9.89, SD = 2.79) and 'Older' age groups (M = 10.20, SD = 2.70). The main effect for treatment group, F (1, 551) = 0.80, p = 0.37, did not reach statistical significance.

4.4.3.3 MATERNAL EDUCATION

H3: The interaction effect between treatment group and maternal education was statistically significant, F (2, 551) = 3.05, p = 0.05 **(Figure 19)**. There was a statistically significant main effect for maternal education, F (2, 551) = 8.00, p <0.01; however, the effect size was small (partial eta squared = 0.03). Post hoc comparisons using the Tukey HSD test indicated that the children of mothers who had a Degree/Higher degree (M = 98.16, SD = 1.30) had higher Core Language Scores than those whose mothers had a Certificate/Diploma (M = 94.02, SD = 0.90) or No degree (M = 91.47, SD = 1.05).

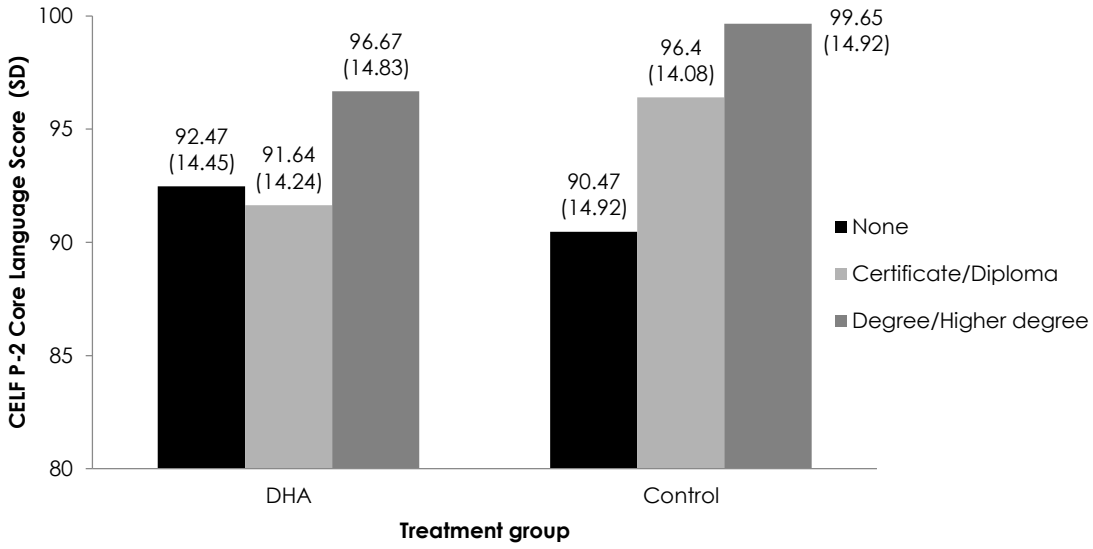


Figure 19. Treatment group differences in mean Core Language Scores by maternal education. Results presented are Mean (Standard Deviation).

Sentence Structure

There was a statistically significant interaction for the Sentence Structure subtest, F (2, 551) = 5.36, p = 0.01; however, the effect size was small (partial eta squared = 0.02). Specifically, for those children whose mothers had a Certificate/Diploma, mean Sentence Structure scores for the Control group (M = 9.16, SD = 2.85) were higher than those in the DHA group (M = 8.31, SD = 2.84; mean difference = 0.85, 95% CI = 0.16 to 1.54), F (1, 551) = 5.90, p = 0.02; however, the effect size was small (partial eta squared = 0.01).

Word Structure

The interaction effect for the Word Structure subtest was not statistically significant, F (2, 551) = 0.96, p = 0.38. There was a statistically significant main effect for maternal education, F (2, 551) = 5.22, p = 0.01; however, the effect size was small (partial eta squared = 0.02). Post-hoc comparisons using the Tukey HSD test indicated that children whose mothers had a Degree/Higher degree (M = 9.36, SD = 3.04) had higher Word Structure scores than the children of mothers with no educational qualifications (None) (M = 8.20, SD = 3.05).

Expressive Vocabulary

The interaction effect for the Expressive Vocabulary subtest was not statistically significant, F (2, 551) = 1.99, p = 0.14. There was a statistically significant main effect for maternal education, F (2,551) = 9.52, p <0.01; however, the effect size was small (partial eta squared = 0.03). Post-hoc comparisons using the Tukey HSD test indicated that the children of mothers in the Certificate/Diploma group (M = 9.51, SD = 2.76) had lower Expressive Vocabulary scores than the children of mothers in the Degree/Higher degree group (M = 10.32, SD = 2.69) as well as the group with no educational qualifications (None) (M = 8.87, SD = 2.96). The latter group also had significantly lower Expressive Vocabulary scores than the Degree/Higher degree group.

4.4.3.4 MATERNAL SMOKING

H4: The interaction effect between treatment group and maternal smoking was not statistically significant, F (1, 552) = 0.23, p = 0.63 **(Figure 20)**. The main effect for smoking, F (2,552) = 1.41, p = 0.03, did not reach statistical significance.

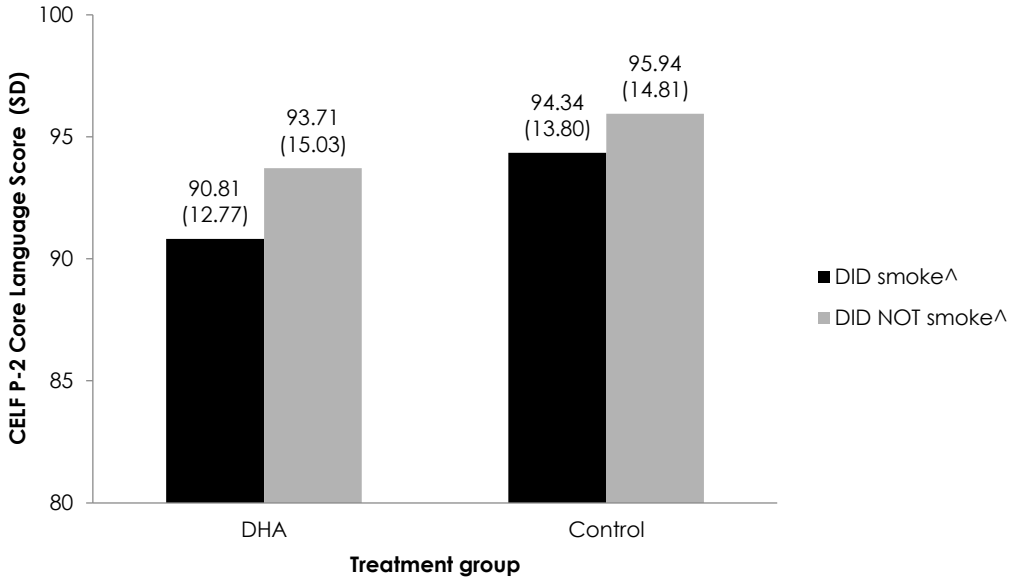


Figure 20. Treatment group differences in Core Language Scores by maternal smoking. ^ Maternal smoking defined as "in the 2-3 months before pregnancy and/or during pregnancy". Results presented are Mean (Standard Deviation).

Sentence Structure

The interaction for the Sentence Structure subtest was not statistically significant, F (1, 552) = 0.11, p = 0.74. The main effect for

smoking, F (1, 552) = 0.80, p = 0.45, did not reach statistical significance.

Word Structure

The interaction for the Word Structure subtest was not statistically significant, F (1, 552) = 0.74, p = 0.39. The main effect for smoking, F (2, 552) = 1.52, p = 0.22, did not reach statistical significance. The main effect for treatment group, F (1, 552) = 3.63, p = 0.06, also did not reach statistical significance.

Expressive Vocabulary

The interaction for the Expressive Vocabulary subtest was not statistically significant, F (1, 552) = 0.42, p = 0.52. The main effect for smoking, F (2, 552) = 0.76, p = 0.47, did not reach statistical significance.

4.4.3.5 MATERNAL ALCOHOL CONSUMPTION

H5: The interaction effect between treatment group and maternal alcohol consumption was not statistically significant, F (1, 553) = 0.63, p = 0.43 **(Figure 21)**. The main effect for maternal alcohol consumption, F (1, 553) = 2.68, p = 0.10 did not reach statistical significance.

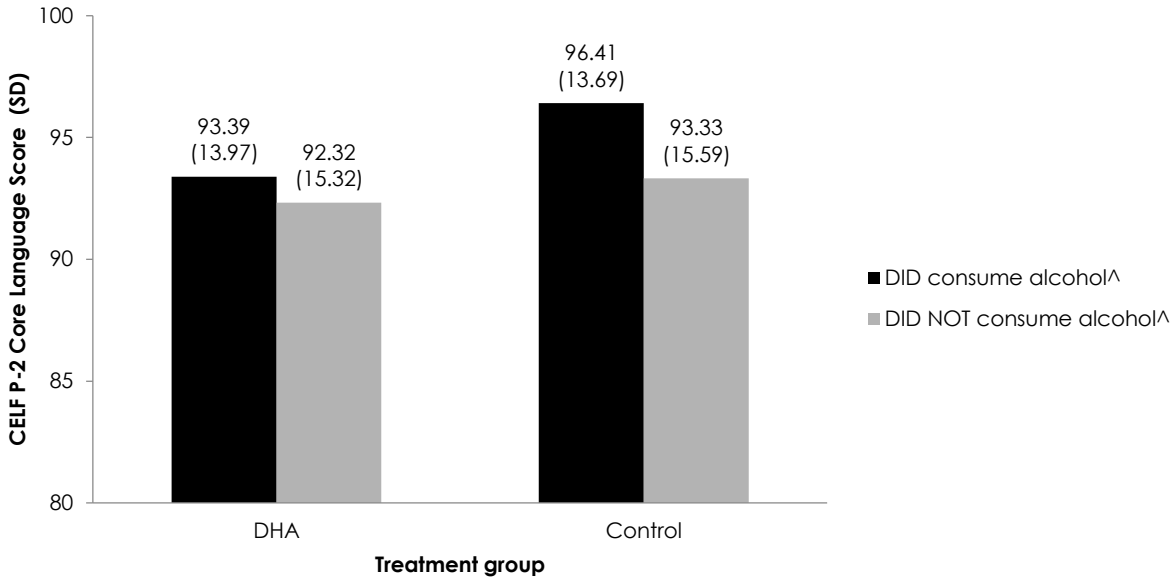


Figure 21. Treatment group differences in Core Language Scores by maternal alcohol consumption. ^ Maternal alcohol consumption defined as "in the 2-3 months before pregnancy and/or during pregnancy". Results presented are Mean (Standard Deviation).

Sentence Structure

The interaction for the Sentence Structure subtest was not statistically significant, F (1, 553) = 0.97, p = 0.32. The main effect for maternal alcohol consumption, F (1, 553) = 0.03, p = 0.87, did not reach statistical significance.

Word Structure

The interaction for the Word Structure subtest was not statistically significant, F (1, 553) = 0.00, p = 0.99. The main effect for maternal

alcohol consumption, F (1, 553) = 2.54, p = 0.11, did not reach statistical significance.

Expressive Vocabulary

The interaction for the Expressive Vocabulary subtest was not statistically significant, F (1, 553) = 1.08, p = 0.29. There was a statistically significant main effect for maternal alcohol consumption, with the children of mothers who consumed alcohol in the 2 to 3 months leading up to their current pregnancy having higher Expressive Vocabulary scores than those who did not, F (1, 553) = 6.01, p = 0.02; however, the effect size was small (partial eta squared = 0.10).

4.4.3.6 MATERNAL DEPRESSION

H6: The interaction effect between treatment group and maternal depression was not statistically significant, F (1,553) = 0.43, p = 0.51 **(Figure 22)**. The main effect for maternal depression, F (1, 553) = 2.64, p = 0.11, did not reach statistical significance.

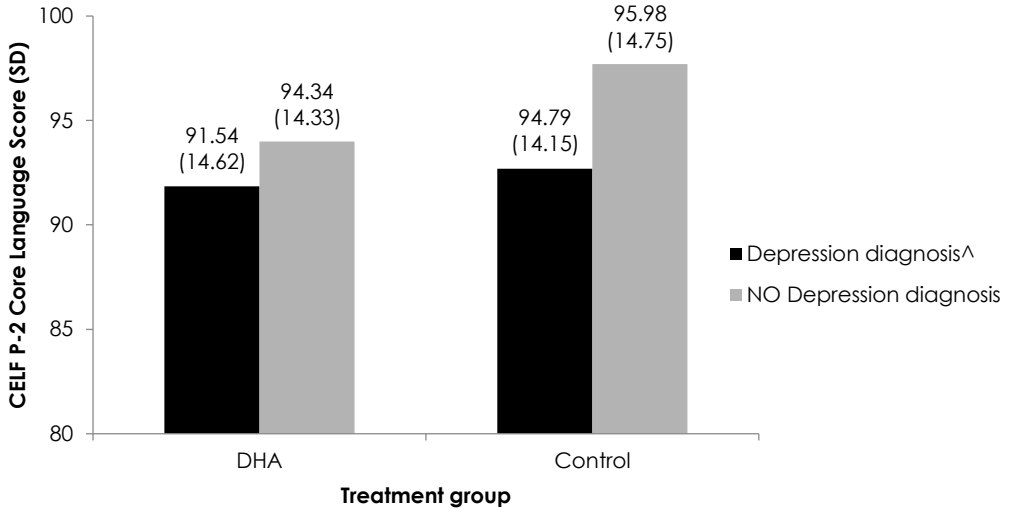


Figure 22. Treatment group differences in Core Language Scores by maternal depression. ^ Maternal depression defined as "a diagnosis of depression at study entry". Results presented are Mean (Standard Deviation).

Sentence Structure

The interaction for the Sentence Structure subtest was not statistically significant, F (1, 553) = 0.14, p = 0.71. The main effect for depression, F (1, 553) = 2.35, p = 0.13, did not reach statistical significance.

Word Structure

The interaction for the Word Structure subtest was not statistically significant, F (1, 553) = 1.25, p = 0.26. The main effect for depression, F (1, 553) = 1.27, p = 0.26, did not reach statistical significance.

Expressive Vocabulary

The interaction for the Expressive Vocabulary subtest was not statistically significant, F (1, 553) = 0.70, p = 0.40. The main effect for depression, F (1, 553) = 1.97, p = 0.16, did not reach statistical significance.

4.5 DISCUSSION

DHA supplementation during pregnancy was expected to benefit the language development of children from vulnerable subgroups. The current study found no significant interactions between treatment group and child sex, maternal age, in utero exposure to maternal cigarette smoking or alcohol consumption, or maternal depression for overall language development as indicated by mean Core Language Scores. However, results did reveal a significant interaction for maternal education.

4.5.1 CHILD SEX

Few trials have described sex subgroup analyses (160, 161, 183, 188) and consequently it is not known whether DHA might be beneficial for, or detrimental to, the language development of one sex more than the other. The current study found no interaction between treatment group participants and sex for overall language development as indicated by mean Core Language Scores or for any subtest scores. This is consistent with the majority of trials investigating sex differences which have found no significant

treatment group differences in the language development of females (160, 161, 188) or males (161, 183, 188). This is also consistent with the 18-month data wherein females in the control group had significantly better language development than those in the DHA group (183). This may be a chance finding or alternatively it may be indicative of an adverse effect, ultimately suggesting that the DHA dosage may have been too high and impeded the language performance for girls at four years of age. Other trials have conversely reported positive results for females (188) and a negative effect for males (160). In many cases the sample size of other trials was a limiting factor.

Consistent with literature, females' language development tended to be better than that of males, irrespective of treatment group. For females, 23% in the DHA group and 16% in the control group had Core Language Scores that were below average **(Table 12)**. These findings are again comparable with the national average and also consistent with the 18-month data which suggested that girls exposed to a higher amount of DHA were more likely to have delayed language development than the control group (183). For males, 32% in the DHA group and 25% in the control group had Core Language Scores that were below average **(Table 13)**. This is surprising considering that DHA supplementation should benefit males the most. Compared to females they are physiologically likely to have lower circulating DHA concentrations and, from a social perspective, less linguistic stimulation.

TABLE 12. Treatment group N differences in Core Language Score classification (females). N, number.

Core Language Score	Classification	Control N=153	DHA N=141
115 and above	Above average	13	5
86 to 114	Average	114	103
78 to 85	Borderline	9	8
71 to 77	Low range	13	15
70 and below	Very low range	4	10

TABLE 13. Treatment group N differences in Core Language Score classification (males). N, number.

Core Language Score	Classification	Control N=134	DHA N=129
115 and above	Above average	7	8
86 to 114	Average	88	79
78 to 85	Borderline	9	16
71 to 77	Low range	20	18
70 and below	Very low range	10	8

4.5.2 MATERNAL AGE

The current study found that there were no significant differences in Core Language Scores or any of the subtests between the DHA and control groups when comparing children with younger, middle aged or older mothers. Although not statistically significant, the children of younger and middle aged mothers in the control group appeared to have better Core Language Scores than the children in the DHA group with mothers in these age groups, reflecting a pattern consistent with overall findings for the whole group. This suggests that DHA does not buffer against the relationship that younger maternal age can have on impeding language development. In contrast to overall findings, children of older mothers in the DHA group had better language development than those children of older mothers in the control group. Again although this did not reach statistical significance, the difference between groups was interestingly of greater than four points. This is preliminarily suggestive of a beneficial effect of DHA for the language development of children of older mothers, but the possible relevance of age related changes in maternal DHA metabolism to children's cognitive development, particularly language, remains to be established. More generally, finding an overall effect of maternal age on language performance, specifically the significant differences between the Younger and Middle age groups but not between the Older group and either of the latter two, partially confirms the observations made in previous literature.

4.5.3 MATERNAL EDUCATION

Children of mothers with a higher level of education in the DHA group had significantly poorer language development overall and also significantly poorer Sentence Structure scores than children of mothers with a higher level of education in the control group. Furthermore, the difference in Core Language Scores between groups was of more than four points, suggesting this might manifest as a difference in clinical functioning. Despite there currently being no consensus regarding the benefits of DHA for cognitive development, DHA has been added to a number of prenatal supplements which have been marketed with this benefit. Considering women with a higher educational achievement are more likely to be aware of the importance of nutrition during pregnancy it may be that they do not need DHA supplementation to benefit their child's language development.

Admittedly, it is not clear whether for these women there was a relationship between maternal education and the level of nutritional knowledge and the use of DHA in pregnancy per se. A recent study involving pregnant women in Australia (n=190) revealed that their knowledge about n-3 LCPUFA during pregnancy was limited (mostly derived from magazines) and that health-care services do not provide adequate information (286). As this lack of knowledge could prompt information-seeking behaviours, the information that is provided by such sources is important. The accuracy of the information accessed and, furthermore, how this translated to decisions and behaviour regarding supplementation

was not assessed as part of the study and should be a priority for future research.

4.5.4 MATERNAL SMOKING

In my study prevalence of in utero smoking was considerably higher compared to previous reports of these behaviours of women in Australia. On the one hand, this higher rate is alarming as it may potentially be an under reporting of the true situation. On the other hand, data from the population in the current study may be limited as it might not have been representative of the average population in the community. My study confirmed earlier studies which show that in utero exposure to cigarette smoking resulted in poorer language development **(see Section 4.4.3)**. Specifically, irrespective of treatment group mean Core Language Scores were lower for those children whose mothers smoked during pregnancy and/or in the lead up to pregnancy compared to those whose mothers did not. Surprisingly, comparisons of smoking subgroups showed that the DHA group appeared to have poorer language development than the control group, although this effect did not reach statistical significance and could therefore have occurred by chance.

Few other trials of DHA supplementation with language outcomes have reported maternal smoking status (172, 174, 186, 194, 210, 224) and participant numbers for these trials were low. Furthermore, some trials excluded women who were smokers from participation (163, 167). To date there has been only one other trial of DHA supplementation with language outcomes that has reported subgroup analyses by smoking status during pregnancy (189). In

contrast to findings of the current study this trial reported significantly better language development in the children in the treatment group exposed to smoking prenatally. Comparatively, in the current study there were considerably more smokers and overall prevalence rates were higher. While from this perspective results from the current study may be more reliable, this is purely speculative. It is also important to note differences between the current and aforementioned trials in methods for recording smoking behaviour. While the current study could more precisely quantify the number of cigarettes smoked per day the other trial only recorded whether women smoked greater than 5 cigarettes per day. These women may have smoked considerably more during pregnancy than those in the current study. It may be only those children whose mothers smoke frequently during pregnancy that have compromised DHA synthesis and in turn whose language development will benefit. Alternatively, the timing during which maternal smoking occurred may explain current findings. Maternal smoking behaviour was recorded early on in the study and it may have markedly diminished by the conclusion when the brain is developing and when drinking may have had the most pronounced effect on language development. This suggests that more work is needed in order to elucidate the effects of maternal smoking during pregnancy.

4.5.5 MATERNAL ALCOHOL CONSUMPTION

In the current study prevalence of in utero alcohol consumption was considerably higher compared to previous reports of this behaviour of women in Australia (62% in the DOMInO trial compared to 51%

nationwide) (240). This is alarming, and may reflect an under reporting of the true situation. In contrast to literature, irrespective of treatment group, children with prenatal alcohol exposure had better language development than those who were not exposed. Comparisons of drinking subgroups showed that the DHA group had poorer language development than the control group as indicated by lower Core Language Scores, although again this did not reach statistical significance, and nor were there any significant differences in subtest scores.

The frequency of the mother's alcohol consumption may well be critical in determining fatty acid concentration and in turn functional output. On the one hand, it may be that women in the current study did not consume enough alcohol to decrease DHA concentration which may have impacted our ability to determine whether children's language ability would benefit. Consistent with this notion, no alcohol-related neurodevelopmental disorders were identified in the group and so the likelihood of maternal alcohol consumption altering DHA concentrations (and in turn having functional consequences) is unclear. Conversely, it has been suggested that alcohol in low doses may actually lead to stimulation of fatty acid metabolism thereby increasing DHA concentrations (287). In addition to frequency, the intensity of alcohol consumption during pregnancy is another factor to consider. It has been suggested that for those who consume large amounts of alcohol, fatty acid catabolism may overwhelm the rate of fatty elongation, desaturation and transport which may therefore decrease tissue concentrations of DHA (287). The extent to which this may explain findings of the current study is limited considering alcohol consumption was low. Alternatively, the timing during which

maternal alcohol consumption occurred may explain current findings. Maternal alcohol consumption behaviour was recorded early on in the study and it may have markedly diminished by the conclusion when the brain is developing and when drinking may have had the most pronounced effect.

To date only one other trial of DHA supplementation with language outcomes has reported maternal alcohol consumption (224) and none have reported subgroup analyses by alcohol consumption during pregnancy. Furthermore, women with high alcohol use were reported as excluded from some trials (161, 174, 176, 210, 224). This suggests that more work is needed in order to elucidate the effects of maternal alcohol consumption during pregnancy perhaps with particular attention paid to whether and how effects might differ by frequency and intensity of the behaviour.

4.5.6 MATERNAL DEPRESSION

Maternal depression in the current study was more prevalent than the national average (i.e. 50% in the DOMInO trial compared to 15% nationwide) (277) although this may be because The current study took into account a diagnosis at a number of different time points. Children of mothers with depression who were in the DHA group had poorer language development than children of mothers with depression who were the control group as indicated by lower Core Language Scores, although this did not reach statistical significance, and again and nor were there any significant differences in subtest scores. To date no other RCTs of DHA supplementation with child language outcomes have considered a

role for DHA in protecting children from the suboptimal linguistic outcomes associated with maternal depression, that is, either due to the physiological effects or reduced stimulation. Notably, the current study did not take into account the type, nature or duration of depressive disorder that women were originally diagnosed with, all of which could account for not finding a significant interaction between maternal depression and DHA in influencing children's language development. Children of a mother whose depression includes periods of positive affect or have less severe symptoms are arguably going to be less susceptible to poor language outcomes. Furthermore, it may be that mothers' depression did not remain for long enough to influence children's language development.

One other important factor to consider in understanding the interaction between depression and DHA intake during pregnancy in influencing children's language development is the role for medication used to treat depression. Selective Serotonin Reuptake Inhibitors (SSRIs), for instance, increase central synaptic 5HT (serotonin) concentrations and easily cross the placenta ultimately possibly constraining 5HT reuptake in the developing fetus. This is of concern because 5HT plays a key role in cognitive capacities. Higher levels of DHA have been found to be associated with higher levels of 5HT. The language development of children whose mothers who took SSRIs may have been protected from the negative consequences that a depressive disorder may have. Although medication use and type was recorded as part of the original DOMInO trial, using a sample who did not receive any such treatment for their depression in the analysis would have resulted in a sample that was too small to analyze.

4.6 CONCLUSION

This section presented a range of exploratory analyses in an attempt to understand potential important interactions between DHA and other social and biological variables. These included child sex, maternal age, maternal education, maternal smoking, maternal alcohol consumption and maternal depression. Importantly, although these analyses may be subject to random error, this was a large enough study to make such an exploration useful. Overall, DHA could not overcome the majority of effects that many of the variables under investigation have in this population (i.e. excluding maternal education).

CHAPTER 5

5 RELATIONSHIPS BETWEEN MARKERS OF DOCOSAHEXAENOIC ACID STATUS AND LANGUAGE DEVELOPMENT OVER TIME

5.1 INTRODUCTION

This chapter seeks to determine the relationship between the markers of DHA and language development.

Many studies have highlighted the value of examining different levels of DHA in order to uncover possible dose-response relationships and potential immediate and long-term benefits. Although it is important that such information is derived from an adequate and well-controlled study (aka a RCT) this does not preclude the examination of associations within the entire sample. Considering several studies have demonstrated positive relationships between infant blood concentrations of DHA and neurocognitive outcomes (16, 99) such correlational analyses may provide additional insights to the weight of evidence of an acceptable risk/benefit relationship between DHA and language development.

This suggests that it is important to consider the predictive value of markers of DHA status and the impact for language development.

5.2 RESEARCH HYPOTHESES

It was hypothesized that there would be a significant positive correlation between cord blood DHA recorded at birth and language development at both four years (H1) and 18 months (H2) of age. It was further hypothesized that children who had cord blood DHA in the 75th percentile would have significantly better language development at four years of age than those who had cord blood DHA in the 25th percentile (H3). Finally, it was hypothesized that there would be a significant positive correlation between the number of fish meals (H4) and DHA foods (H5) consumed and language development at four years of age.

5.3 METHODS

5.3.1 PARTICIPANTS, PROCEDURE AND MEASURES

The methods for participants and procedures are described in **Chapter 2.** Language development was measured by the CELF P-2 as part of the four-year follow-up of the DOMInO trial. A detailed description is provided in **Chapter 3**. A description of additional measures/grouping variables used is provided below.

Language development

Language development at 18 months was assessed using the Language Composite Scale of the Bayley-III. The language scale is

a composite of receptive (i.e. verbal comprehension, vocabulary) and expressive (babbling, gesturing, and utterances) language abilities. The raw scores for each of the scales are standardized to a mean of 100 with a standard deviation of 15 (range, 50-150). The standardized scores were also classified into the categories of advanced (>115), within normal (85-115), and delayed performance (<85).

Cord blood DHA

The amount of DHA in cord blood was assessed as part of the original DOMInO trial using capillary gas chromatography (288) to offer an independent biomarker of compliance. Cord blood samples were collected at birth for the measurement of plasma phospholipid DHA. Plasma samples were stored frozen until transported to the laboratory for analysis. The method used has been previously established (14).

Number of fish meals and DHA enriched foods consumed

The number of fish meals and DHA enriched foods consumed was recorded based on parents or caregivers selecting from a predetermined list, including serving sizes, of fish meals and DHA enriched foods that the child had consumed in the past month (from the time of the four-year assessment).

5.3.2 DATA ANALYSIS

Analyses were performed using SPSS version 20. Analyses were performed for those who completed the language assessment and, for the purpose of this section, were not undertaken 'by group', although sex differences were explored. Sampling design and probability weights were not taken into account and no adjustments were made.

Pearson product-moment correlation coefficient was used to investigate the relationship between cord blood DHA and language development at four years of age as measured by CELF P-2 Core Language Scores (H1) and at 18 months of age as measured by the Bayley-III Language Composite scores (H2). An independent-samples t-test was conducted to determine differences in language development, as measured by CELF P-2 Core Language Scores at four years of age and Bayley-III Language Composite scores at 18 months of age, between those with cord blood DHA in the 25th percentile and with those with cord blood DHA in the 75th percentile (H3). Considering the similar levels of both the treatment and control groups, this analysis was undertaken in order to understand what implications, if any, particularly low and high (for the sample) levels of DHA may have on language development. Pearson product-moment correlation coefficient was used to investigate the relationship between the number of fish meals (H4) and DHA foods (H5) consumed in the month prior to language assessment at four years of age and language development at four years of age as measured by CELF-P2 Core Language Scores. An independent t-test was performed to

compare the post-randomisation variables; number of fish meals and DHA foods consumed) for consenters to DOMInO-4 who participated in the language assessment. Statistical significance was assessed at the 0.05 level.

5.4 RESULTS

5.4.1 SAMPLE AND PARTICIPANT FLOW

See **Figure 15 (Chapter 2)** for an overview of the participant flow. Notably, the current study had fewer cord bloods available (N=196 in the DHA group and N=202 in the Control group)

5.4.2 CHARACTERISTICS OF STUDY PARTICIPANTS

For demographic information refer to **Chapter 3**. **Table 14** shows indicators of DHA status and language outcomes.

TABLE 14. Indicators of DHA status and language outcomes

	Whole group	Females	Males
DHA % in plasma total phospholipid fatty acids in cord blood mean (±SD)	7.01 (1.88) N = 398	6.85 (1.86) N = 222	7.21 (1.89) N = 176
Number of fish meals consumed	4.14 (3.98) N = 555	4.11 (4.07) N = 294	4.17 (3.89) N = 261
Number of DHA foods consumed	15.99 (25.52) N = 551	16.87 (25.23) N = 292	14.99 (25.85) N = 259
CELF P-2 Core Language Score	94.19 (14.50) N = 557	95.91 (14.05) N = 294	92.27 (14.78) N = 263
Bayley-III Language Composite Score	98.39 (13.88) N = 550	101.31 (13.16) N = 289	95.16 (13.96) N = 261

5.4.3 MAIN FINDINGS

H1: There was no significant correlation between cord blood DHA and Core Language Scores, r = 0.05, p = 0.37 with cord blood DHA explaining 0.25% of the variance in Core Language Scores for the group as a whole **(Figure 23)**. For females, there was no significant correlation between cord blood DHA and Core Language Scores, r = 0.09, p = 0.20 with cord blood DHA explaining 0.81% of the variance in Core Language Scores for the group of females as a whole **(Figure 24)**. For males, there was no significant correlation between cord blood DHA and Core Language Scores, r = 0.02, p = 0.82 with cord blood DHA explaining none of the variance in Core Language Scores for the group of males as a whole **(Figure 25)**.

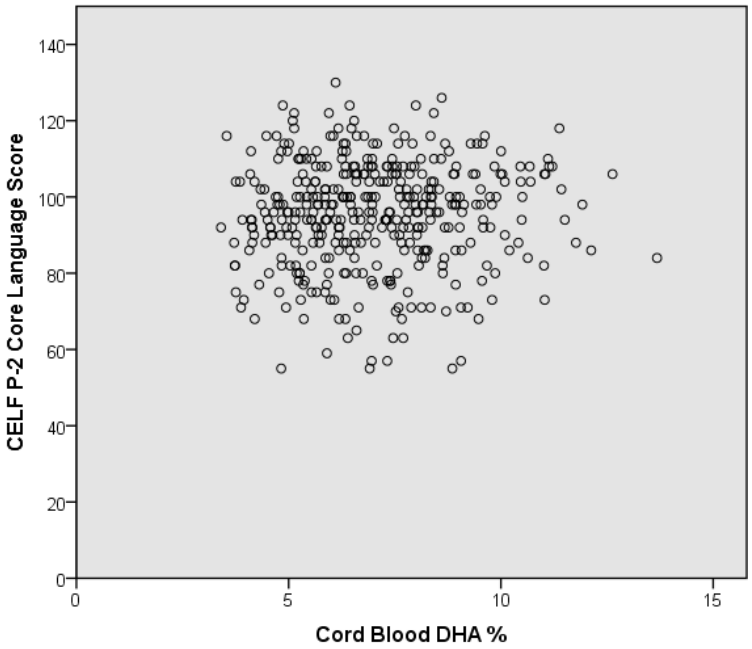


Figure 23. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Core Language Scores, r = 0.05, p = 0.37.

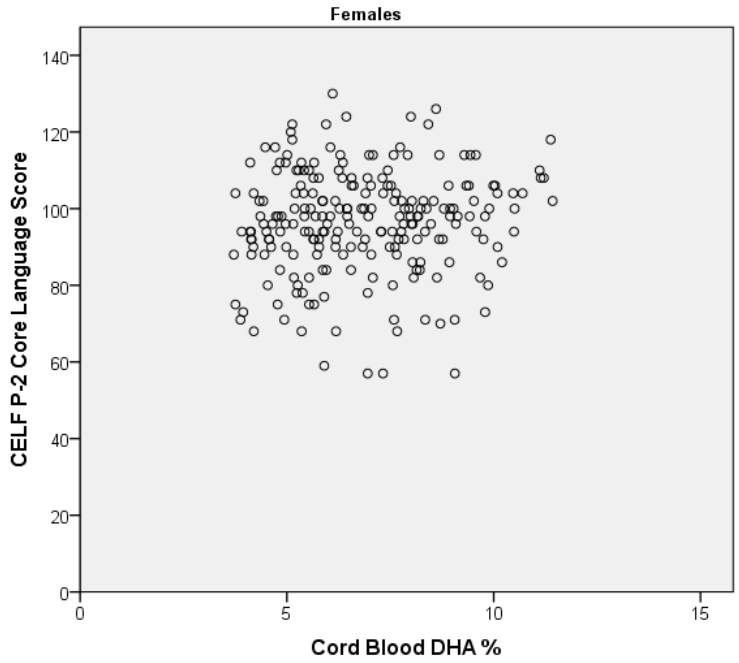


Figure 24. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Core Language Scores for females only, r = 0.09, p = 0.20.

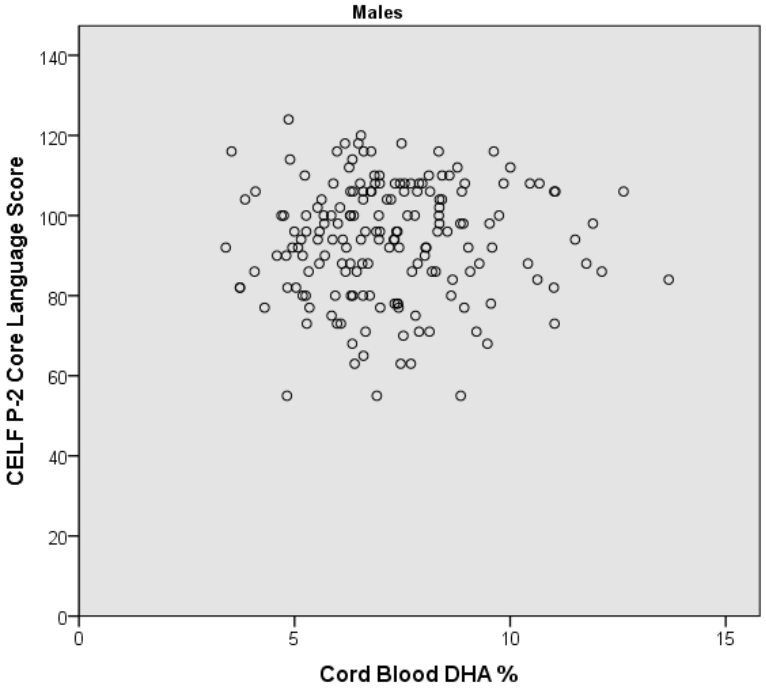


Figure 25. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Core Language Scores for males only, r = 0.02, p = 0.82.

H2: There was no significant correlation between cord blood DHA and Bayley-III language composite scores, r = -0.03, p = 0.51 with cord blood DHA explaining 0.09% of the variance in Bayley-III language composite scores for the group as a whole **(Figure 26)**. For females there was no significant correlation between cord blood DHA and Bayley-III language composite scores, r = 0.03, p = 0.64 with cord blood DHA explaining 0.09% of the variance in BAYLEY-III language composite scores for the group of females as a whole **(Figure 27)**. For males there was no significant correlation between cord blood DHA and Bayley-III language composite scores, r = -0.07, p = 0.39 with cord blood DHA explaining 0.49% of the variance in BAYLEY-III language composite scores for the group of males as a whole **(Figure 28)**.

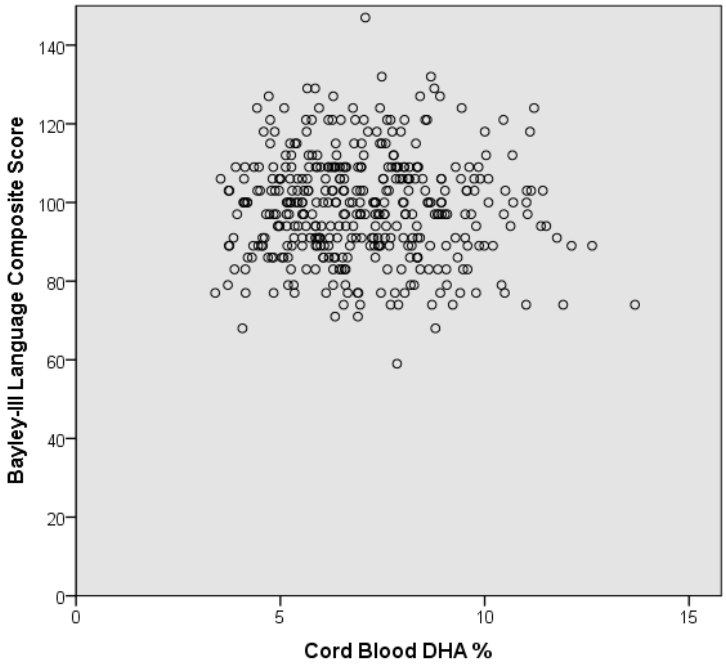


Figure 26. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Bayley-III Language Composite Scores, r = -0.03, p = 0.51.

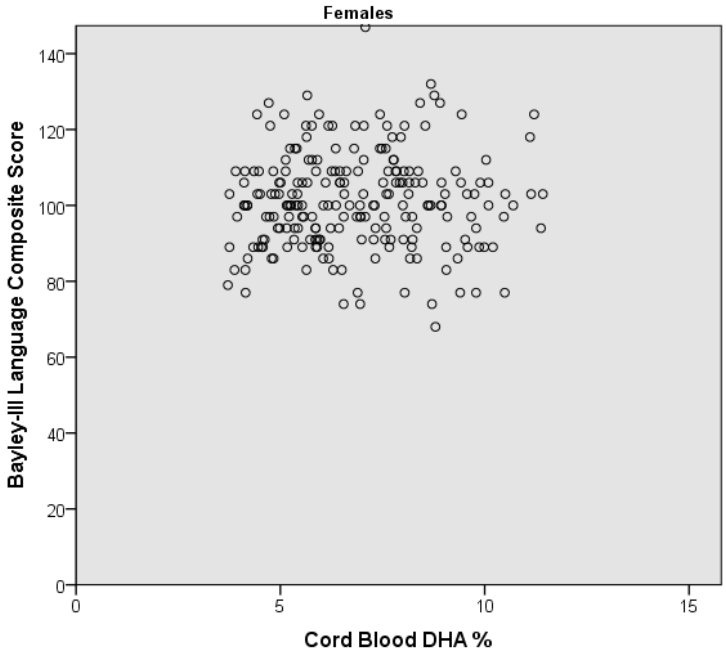


Figure 27. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Bayley-III Language Composite Scores for females only, r = 0.03, p = 0.64.

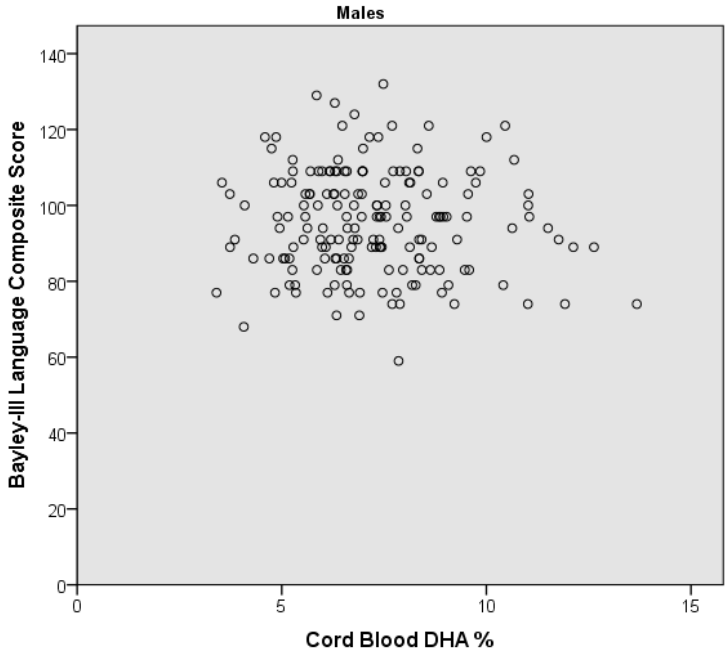


Figure 28. Scatter plot of the relationship between cord blood plasma DHA (percentage of total phospholipid fatty acids) plotted against Bayley-III Language Composite Scores for males only, r = -0.07, p = 0.39.

H3: There was no significant difference in Core Language Scores for those with cord blood DHA in the 25th percentile (M = 94.24, SD = 13.78) and 75th percentile (M = 96.30, SD = 13.71; t (195) = -1.05, p = 0.30, two-tailed). The magnitude of differences in the means (mean difference = -2.06, 95% CI: -5.92 to 1.80) was very small (eta squared = 0.01). There was no significant difference in Core Language Scores specifically for males with cord blood DHA in the 25th percentile (M = 92.00, SD = 13.95) and 75th percentile (M = 94.78, SD = 13.78; t (76) = -0.87, p = 0.39, two-tailed). The magnitude of differences in the means (mean difference = -2.78, 95% CI: -9.13 to 3.57) was very small (eta squared = -0.023). There was also no significant difference in Core Language Scores specifically for females with cord blood DHA in the 25th percentile (M = 95.33, SD = 13.66) and 75th percentile (M = 97.62, SD = 13.65; t (117) = -0.91, p = 0.37, two-tailed). The

magnitude of differences in the means (mean difference = 2.78, 95% CI: -9.13 to 3.57) was very small (eta squared = -0.016)

There was no significant difference in Bayley-III language composite scores for those with cord blood DHA in the 25th percentile (M=98.00, SD = 11.82) and 75th percentile (M = 97.45, SD = 14.10); t (193) = 0.30, p = 0.77, two tailed). The magnitude of differences in the means (mean difference = 0.55, 95% CI: -3.13 to 4.23) was very small (eta squared = 0.0005). There was no significant difference in Bayley-III Language Composite scores specifically for males with cord blood DHA in the 25th percentile (M = 94.10, SD = 13.41) and 75th percentile (M = 94.33, SD = 13.11; t (75) = -0.08, p = 0.94, two-tailed). The magnitude of differences in the means (mean difference = 0.23, 95% CI: -6.35 to 5.89) was very small (eta squared = -0.002). There was no significant difference in Bayley-III Language Composite scores specifically for females with cord blood DHA in the 25th percentile (M = 99.83, SD =10.62) and 75th percentile (M = 100.21, SD = 14.48; t (116) = -0.16, p = 0.87, two-tailed). The magnitude of differences in the means (mean difference = 0.38, 95% CI: -4.96 to 4.20) was very small (eta squared = -0.002).

H4: There was a significant, small positive correlation between the number of fish meals children consumed and CELF P-2 Core Language Scores, r = 0.11, p = 0.01, with a higher number of fish meals consumed associated with higher CELF P-2 Core Language Scores **(Figure 29)**. Here, the number of fish meals consumed explained 1.21% of the variance in CELF P-2 Core Language Scores for the group as a whole. For females there was no significant correlation between the number of fish meals consumed and CELF

P-2 Core Language Scores, r = 0.09, p = 0.15 **(Figure 30)**. Here, the number of fish meals consumed explaining 0.81% of the variance in CELF P-2 Core Language Scores for the group of females as a whole. For males, there was a significant, small positive correlation between the number of fish meals consumed and CELF P-2 Core Language Scores, r = 0.12, p = 0.03 **(Figure 31)**. Here, the number of fish meals consumed explaining 1.44% of the variance in CELF P-2 Core Language Scores for the group of males as a whole.

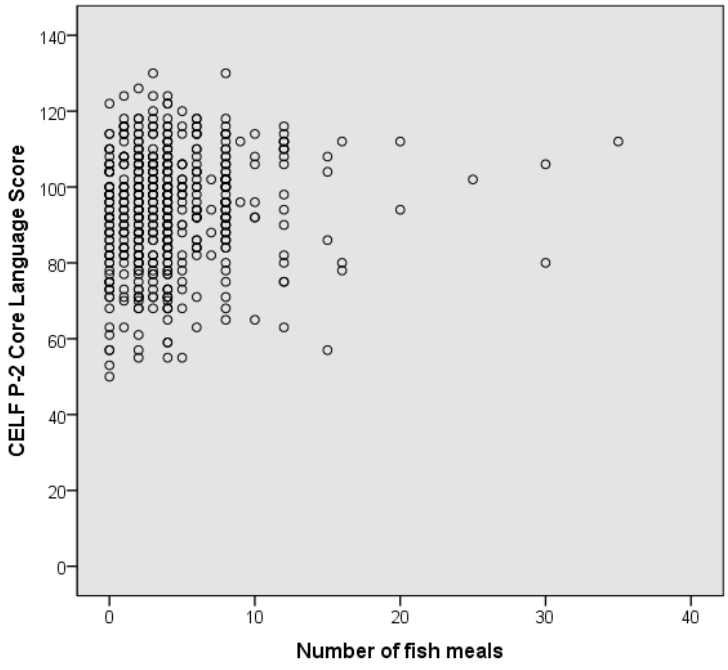


Figure 29. Number of fish meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores, r = 0.11, p = 0.01.

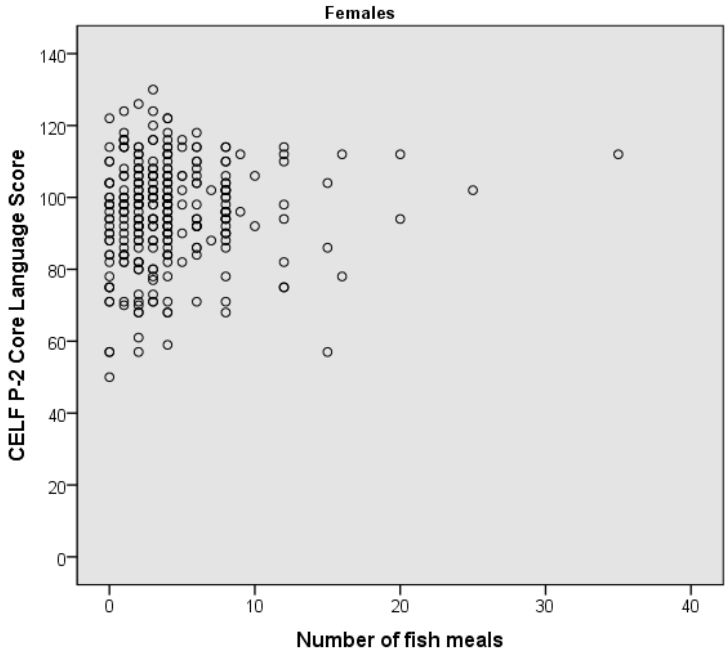


Figure 30. Number of fish meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores for females only, r = 0.09, p = 0.15.

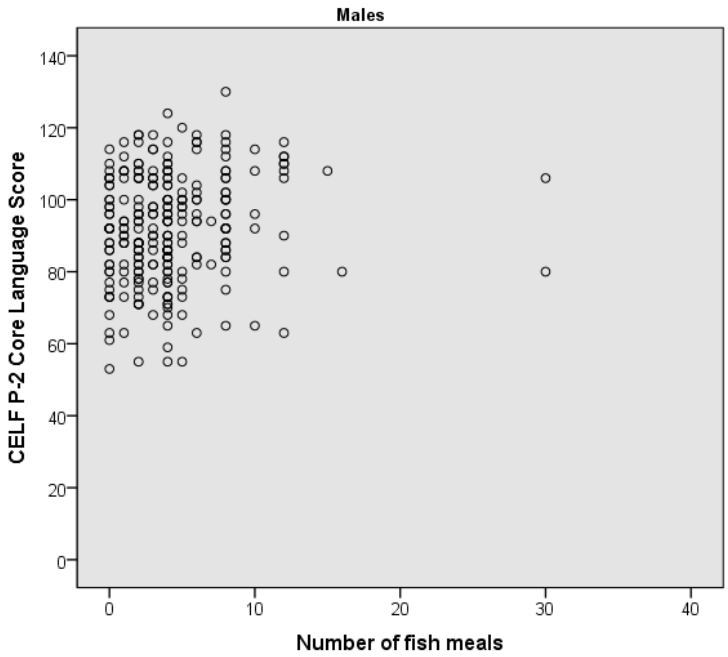


Figure 31. Number of fish meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores for males only, r = 0.12, p = 0.03.

H5: There was a significant, small, negative correlation between the number of DHA foods children consumed and CELF P-2 Core Language Scores, r = -0.10, p = 0.02, with a higher number of DHA foods consumed associated with lower CELF P-2 Core Language Scores **(Figure 32)**. Here, the number of DHA foods consumed explained 1.00% of the variance in CELF P-2 Core Language Scores for the group as a whole. For females, there was no significant correlation between the number of DHA foods consumed and CELF P-2 Core Language Scores, r = -0.06, p = 0.28 **(Figure 33)**. Here, the number of DHA foods consumed explaining 0.36% of the variance in CELF P-2 Core Language Scores for the group of females as a whole. For males, there was a significant small negative correlation between the number of DHA foods consumed and CELF P-2 Core Language Scores, r = -0.16, p = 0.01 **(Figure 34)**. Here, the number of DHA foods consumed explaining 2.56% of the variance in CELF P-2 Core Language Scores for the group of males as a whole.

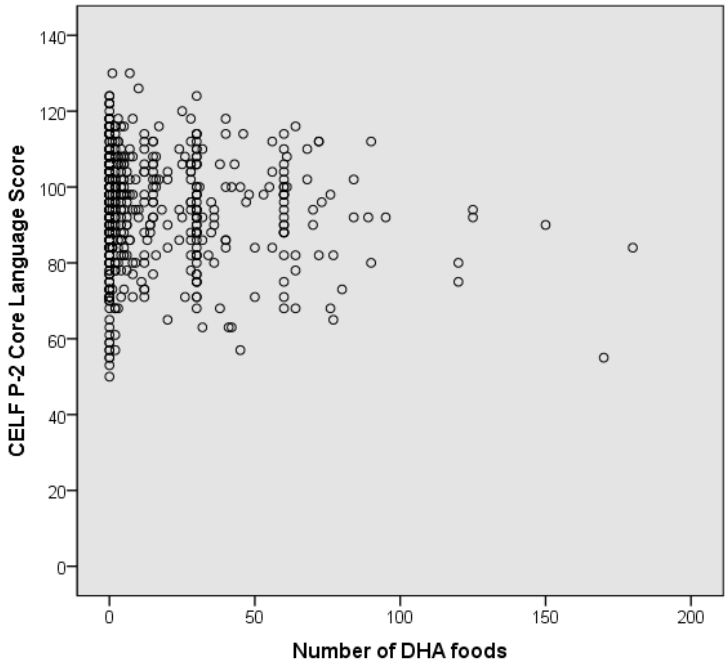


Figure 32. Number of DHA meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores, r = -0.10, p = 0.02.

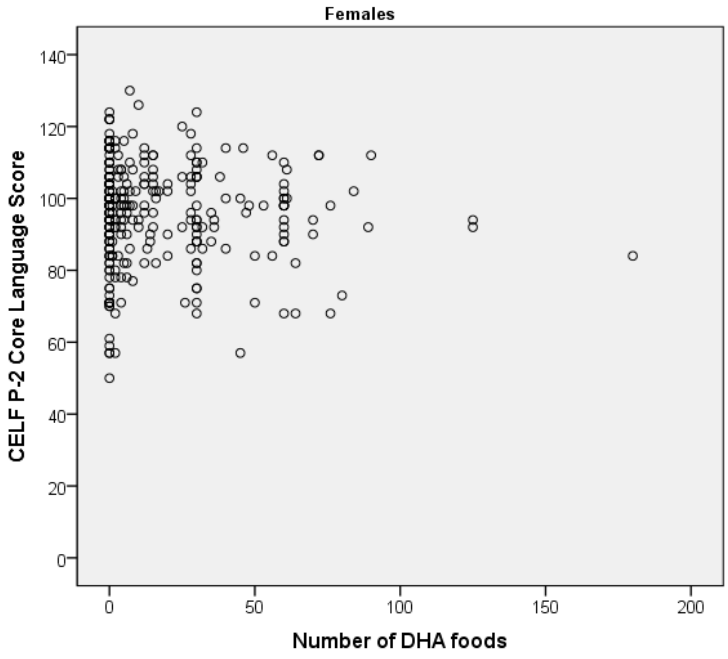


Figure 33. Number of DHA meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores for females only, r = -0.06, p = 0.28.

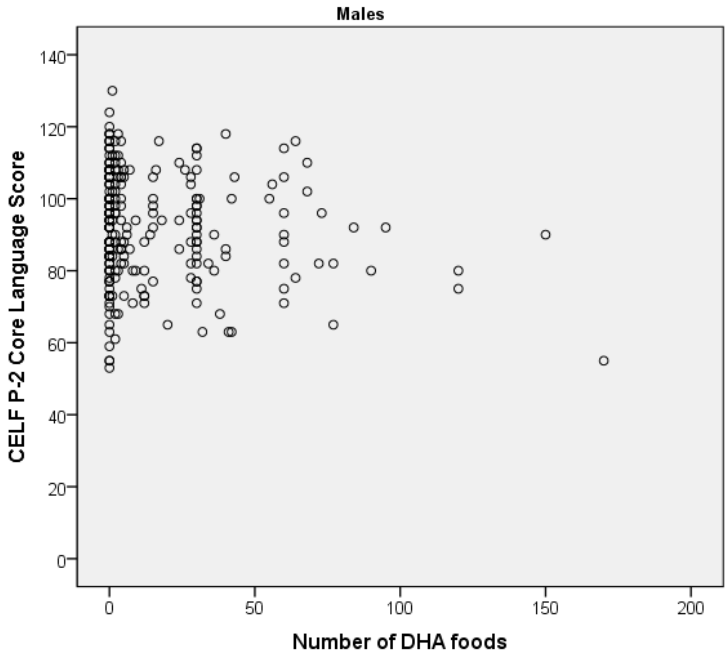


Figure 34. Number of DHA meals consumed by the child in the past month (from the time of assessment) plotted against Core Language Scores for males only, r = -0.16, p = 0.01.

5.5 DISCUSSION

Markers of DHA were expected to be significantly positively associated with language development.

The current study found no significant relationship between cord plasma phospholipid DHA and language development for the whole group and when analyzing females and males separately (at either 18 months as measured by Bayley-III Language Composite scores or four years of age as measured by CELF P-2 Core language Scores). Furthermore, results revealed no significant difference in Core Language Scores or Language Composite scores between those with cord blood DHA in the 25th and 75th percentile (again, for

the whole group and when analyzing females and males separately). However, find a small but significant positive relationship was found between the number of fish meals the child consumed in the month prior to the four-year assessment and language development at 4 years of age. This relationship remained significant for males only. Additionally, a small but significant negative relationship between the number of DHA foods the child consumed in the month prior to the four-year assessment and language development at four years of age, a relationship which, again, remained significant for males only.

Finding no association between cord blood DHA and language outcomes is consistent with previous RCTs (160, 163-165, 167). However, this was surprising considering the DHA dosage was considerably higher than that used by these trials albeit with the exception of Dunstan (167) who supplemented pregnant women with 2200mg of DHA. Notably, negative (160, 161, 176) as well as positive (161, 163) correlations between cord blood DHA and language outcomes have also been reported by RCTs. Unlike the current study which had a large sample size those that did find associations had comparatively smaller sample sizes. Thus their significant findings may be because there were fewer observations and therefore respectively fewer potential groupings of the values of the variables and, thus, the likelihood of finding by chance a grouping of those values suggestive of a significant relationship between cord blood DHA and language development is relatively higher. Notably, the current study also investigated whether there were any sex differences in these relationships with further null findings contributing to the robustness of data in the current study suggesting no effect **(Chapter 3)**.

It has been emphasized that dichotomization is suitable only when an upper limit/level actually exists. That is, if some division of the continuous covariate that creates two reasonably distinct but similar groups in relation to a specific end point can be assumed. Although this was arguably already done when comparing the language development of children in the DHA and control groups **(Chapter 3)** it is important to note that there was substantial overlap between cord blood DHA in these groups. Thus it made more sense to collapse the groups and compare the language development of children with cord blood DHA % in the upper and lower quartiles.

As cord blood DHA indicates DHA intake it stands to reason that the reverse would be true – that is, that intake would be a sufficient indicator of status. Interestingly, the relationship between these measures and language development were found to be inconsistent. While the number of fish meals consumed was positively associated with language development, lending support to observational studies demonstrating a positive relationship between mothers' fish intake and their child's language outcomes (31, 33), the number of DHA foods consumed was negatively associated. This suggests that it may be something else in fish that is beneficial for neurodevelopment. Furthermore, these relationships remained significant for males only. These findings are not consistent with evidence suggesting males language development may benefit most (239) or with data from the current study suggesting a negative effect for females but not males **(Chapter 4)**. It may be that these indicators of intake are not suitable, or accurate, proxies as they reflect other properties than physiologic measures of DHA. Furthermore, cross sectional data of this nature is less reliable than longitudinal data.

5.6 CONCLUSION

Correlational findings provide a preliminary indication that a higher DHA status does not necessarily enhance language abilities. It should be cautioned, however, that associations between DHA status and child language development do not necessarily demonstrate causality. Considering the correlations found were consistently small and in opposite directions, it is likely that these are chance associations that are not meaningful and, moreover, have negligible clinical significance. These results ultimately lend increasing support to the idea that in a relatively well nourished population, prenatal DHA supplementation does not have any significant influence on language development. Future studies pursuing this line of investigation should rely on physiologic measures of DHA status rather than proxy variables.

CHAPTER 6

6 USING STRUCTURAL EQUATION MODELLING TO TEST A MODEL OF LANGUAGE DEVELOPMENT PROPOSED BY THE BIO-ECOLOGICAL THEORY

6.1 INTRODUCTION

This chapter seeks to measure the microsystem, individual and language development constructs with multiple indicators and test the bio-ecological model regarding how the DHA intervention might affect children's language development.

This final part of the current study acknowledges that the microsystem and individual levels of the bio-ecological model are hypothetical constructs that are not directly observable and can only be inferred or measured indirectly through observed variables. While **Chapters 3, 4** and **5** of the current study have focused on observed variables in order to understand how these elements of the bio-ecological model influence language development, **Chapter 6** proposes that the complexity of underlying processes that might be responsible for language development might not have been realistically captured by these observed variables, henceforth referred to as indicators, as 1) they are generally not free from the effects of random error, which implies that their scores are not perfectly reliable, and 2) not all of the variance may reflect

the construct, which implies that the scores are not perfectly valid. This final chapter seeks to address these problems by using structural equation modelling in order to measure the microsystem, individual and language development constructs with multiple indicators and test the bio-ecological model regarding how the DHA intervention might affect children's language development.

Structural equation modelling has been used in only a few studies investigating the effects of early childhood intervention and non-intervention variables on child outcomes (e.g. (289-291)). The model that constitutes the focus of this chapter is not only plausible but more importantly may contribute to our understanding of the interrelationships among variables of practical significance that inform plans for action.

Importantly, structural equation modelling needs to be based on a priori model with a sound theoretical basis. According to the bio-ecological model, development, including that of language, resides in the relation between the individual child and his or her environment: that is, in a two-way interaction. Generally, nutritional deficiencies are hypothesized to lead to altered brain structure which in turn is associated with difficulties in specific aspects of development. Also, nutritional deficiencies are hypothesized to be associated with reduced environmental involvement by the child as well as with developmentally inappropriate caregiver-child transactions such as treating children as if they were younger than they are. As previously noted **(Chapter 2)**, some data used in the current study are plausibly outcomes of the intervention as well as predictors of language development, and it is the integrative study

of these that will permit a more holistic understanding of children's language outcomes.

Children exposed to an insufficient amount of DHA in utero might have more developmentally vulnerable characteristics (psychological well-being and behaviour) which could lead to poorer language development than if they had a sufficient, or higher, amount of DHA in utero. The child's immediate environment, in terms of their family functioning and materials provided in their home, also affects their ability to develop language. Parents or caregivers might treat children exposed to an insufficient amount of DHA in utero more negatively or as developmentally younger than they actually are - which could be linked to them providing less appropriate linguistic stimulation - which in turn would lead to the child's sub optimal linguistic function. Alternatively, DHA exposed children might be more precocious and elicit more input from their immediate environment. Additionally, having developmentally vulnerable characteristics may limit the child's exploration of the home environment and initiation of parent/caregiver interactions which in turn could also impede language acquisition.

The following provides a more in depth discussion of the relationship between indicators of the individual and microsystem constructs and children's language development.

6.1.1 PSYCHOLOGICAL WELL-BEING

A child's psychological well-being has been associated with their language development. An association between early difficulties in language development and conduct problems is well documented (292). Children who have emotional problems often have difficulty with language and those who have language difficulties often have ensuing emotional problems (93). From 6 to 18 months of age infants' engagement with adults, particularly in joint attention, is important for their language development wherein they coordinate their visual attention with that of another person regarding objects and events (293). From the preschool years children with language difficulties are commonly identified as having problems with attention (256, 257). Preschoolers' language difficulties have been shown to interfere with their peer relationships in terms of entering into peer group conversations and forming and maintaining friendships (294). Linguistic abilities and psychological well-being are intertwined. While it is difficult to separate dimensions of psychological problems with difficulties with language and understanding language development overall it is still imperative to contemplate the implications psychological well-being has on language development.

6.1.2 BEHAVIOUR

The relationship between children's language abilities and their behaviour is also well documented, with difficulties in one area contributing to susceptibility to developing difficulties in the other (295). Children with linguistic difficulties have been reported to

exhibit noticeably disruptive behaviour (296) and commonly meet the diagnostic criteria for psychological disorders (257).

6.1.3 HOME ENVIRONMENT

Children's physical home environment plays an important role in shaping their development (297). The home in which children learn is made up of a range of factors, comprising not just the provision of learning materials but also the educational interactions between parents and their children (277). Although particular elements of home may be more likely to influence development of specific skills it is the collective qualities of the overall home environment that is related to children's future language abilities (298-302). There is evidence that problematic home environments can interfere with healthy brain development for children living in those environments. Those lacking contact with age-appropriate learning resources commonly display difficulties with language.

6.1.4 FAMILY FUNCTIONING

Poor family functioning and stress on child development has demonstrated a negative impact on child cognitive outcomes, including language (303). However, it may be that children whose mothers were supplemented with DHA are buffered from the negative association that poor family functioning has on their language development.

6.2 RESEARCH HYPOTHESES

It was hypothesized that treatment group moderates measurement of the individual, microsystem, and language development variables (the measurement model) (H1) and also moderates the structural paths between the individual, microsystem and language development latent variables (the structural model) (H2). Furthermore, it was hypothesized that there are differences between treatment groups in the ability of the individual to mediate the relationship between their microsystem and their language development (H3).

6.3 METHODS

6.3.1 PARTICIPANTS, PROCEDURE AND MEASURES

The methods for participants and procedures are described in **Chapter 2**. Language development was measured by the CELF P-2 as part of the four-year follow-up of the DOMInO trial. A detailed description is provided in **Chapter 3**. A description of additional measures/grouping variables uses are described below.

SDQ: Strengths and Difficulties Questionnaire

The psychological adjustment of children was measured by the Total Difficulties index from the SDQ. The SDQ is a short survey suitable for screening and both clinical and outcome assessment

(304-306). Completed by parents, the SDQ inquires about 5 domains, including emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems and prosocial behavior. There are 25 'favourable' and 'unfavourable' characteristics (5 per each domain) and parents/caregivers use a 3-point Likert scale to show how far each characteristic is true of the child (0 = not true, 1 = somewhat true and 2 = certainly true). Scores for each of these domains can range from 0 to 10. Items 7, 11, 14, 21 and 25 are reverse scored and A Total Difficulties score is calculated through adding the first four subscales. A Total Difficulties score of 0-13 is considered to be average, or unlikely to be clinically significant. A score of 14-16 may indicate clinically significant problems. A score of 17-40 is suggestive of a considerable risk. Interpretation of other scores derived from the SDQ is illustrated in **Appendix 16**. The SDQ is also seen to have good psychometric properties. It has shown good correlation with the Achenbach Child Behaviour Checklist (0.87, $p<0.001$) (307). It also has good internal consistency and Cronbach's alphas (hyperactivity 0.74, emotional symptoms 0.70, conduct problems 0.63, peer problems 0.60, pro-social 0.74) (308). When employed with upwards of 10,000 adolescents and children in the UK the SDQ has also proven to be stable. For the purpose of the current study, the SDQ, variable scores were recoded (reversed) such that higher scores signified better functioning. This was done for consistency of scoring directions in the creation of latent variables.

BRIEF-P: Behaviour Rating Inventory of Executive Function–Preschool

Behaviour was assessed as part of the four-year follow-up of the DOMInO trial with the BRIEF-P (217). The BRIEF-P is a questionnaire designed to offer insight into everyday behaviours related to particular areas of executive functioning in children aged 2 to 5 years. The BRIEF-P is made up of 63 items that form a Global Executive Composite and three corresponding indexes. The Inhibitory Self-Control Index characterizes a child's capacity to regulate his or her actions, responses, emotions, and behaviour in the presence of other distractions. The Flexibility Index represents a child's capacity to alternate between any of these depending on the situation. The Emergent Metacognition Index reflects their capacity to hold on to thoughts and events in their working memory and to strategize problem-solving approaches. Raters' responses are reproduced as circled item scores on the scoring sheet, with 1, 2 and 3 reflecting Never (N), Sometimes (S) and Often (O) respectively. The recommended cut-off for possible clinical significance is a T score of 65, which indicates a score 1.5 standard deviations above the normative mean, or a percentile of 93 or higher (217). The BRIEF-P has been found to have good reliability, with high test-retest reliability (0.82) and internal consistency (-0.98) (106). Convergent and divergent validity has also been proven with other emotional and behavioural assessments. The BRIEF-P has also has the ability to differentiate children with and without clinical functioning and also adolescents with Attention Deficit Hyperactivity Disorder (106). For the purpose of the current study, the BRIEF-P variable scores were recoded (reversed) such that higher scores signified more optimal functioning. This was done for consistency of scoring directions in the creation of latent variables.

FAD GF: Family Assessment Device – General Functioning subscale

Family functioning was assessed as part of the four-year follow-up of the DOMInO trial using the FAD General Functioning scale. The General Functioning scale is a validated measure of overall family health and also members' views of overall family operations with respect to important responsibilities. The General Functioning scale consists of 12 items. Half refer to healthy family functioning (e.g. "In times of crisis we turn to each other for support") and the other half refer to unhealthy family functioning (e.g. "There are lots of bad feelings in our family"). Parents rate how accurately each reflects their family by choosing from four replies on a unidimensional scale from 1 to 4, (i.e. "strongly agree" = 1, "agree" = 2, "disagree" = 3, and "strongly disagree" = 4). The item scores are totaled and then divided by 12 resulting in a total score from 1 to 4, with higher scores suggesting more family dysfunction. A score of 2 or greater suggests problematic family functioning (221). The higher the score the more problematic the family member identifies the family's overall functioning to be. Poor family function is associated with difficulty discussing concerns with, being supported by or being able to confide in family members and not accepting family members as they are (221, 309). The internal consistency estimates (Cronbach's alpha) for the General Functioning scale is 0.92 and test-retest reliability is 0.71 (309). There is also substantiation of the scale's construct validity as an assessment of family functioning demonstrating its association with a range of family factors, including marriage difficulties and violence (309). For the purpose of the current study, the FAD General Functioning variable score was recoded (reversed) such that higher scores signified better

functioning. This was done for consistency of scoring directions in the creation of latent variables.

HSQ: Home Screening Questionnaire

Features in children's home environment that are related to children's growth and development were assessed as part of the four-year follow-up of the DOMInO trial using the HSQ. The HSQ has 34 items and a checklist of toys that questions their presence in the home. The scale includes features such as language-stimulating activities, organization such as presence of a schedule, use of punishment and family activities. Parents/caregivers answer multiple-choice, fill-in-the-blank, and yes/no questions. Following the scoring guidelines, a question's subtotal score and a toy checklist's subtotal are obtained. The total score equals the sum of the two subtotals. A total score of 32 or below is a "suspect" screening result. A score of 33 or higher is a "non-suspect" screening result. A home environment in the suspect range is likely to have few books or toys or adults available to offer activities that promote development. According to its manual, the alpha coefficient is 0.80 and the test-retest coefficient is 0.86. Evidence for concurrent validity was proven using the HOME (Home Measurement of the Environment Inventory (310)) as the criteria.

6.3.2 THE BASIC BUILDING BLOCKS OF STRUCTURAL EQUATION MODELLING

The basic building blocks of structural equation modelling analyses, which follow a progression of five steps: model specification, model identification, model estimation, model testing, and model modification are considered essential to conducting structural equation models (311). These will be addressed in this final part of the methods with the exception of model modification as it is beyond the scope of this thesis.

6.3.2.1 SPECIFICATION OF THE HYPOTHESISED MODEL

Model specification includes using the appropriate theory and findings from previous studies to create a theoretical model, as delineated in the introduction of this chapter. This includes deciding which variables to include and how these variables are related (311). As illustrated in **Figure 35,** latent variables (represented by the ovals) were created for the hypothetical microsystem, individual, and language development constructs with measured variables, or indicators, (represented by the rectangles) from the DOMInO study. The "microsystem" latent variable was created from HSQ Home Environment and FAD General Functioning scores. The "individual" latent variable was created from SDQ Total Difficulties and BRIEF-P Inhibitory Self Control, Flexibility and Emergent Metacognition scores. The "language development" latent variable was created from CELF P-2 Sentence Structure, Word Structure and Expressive Vocabulary scores.

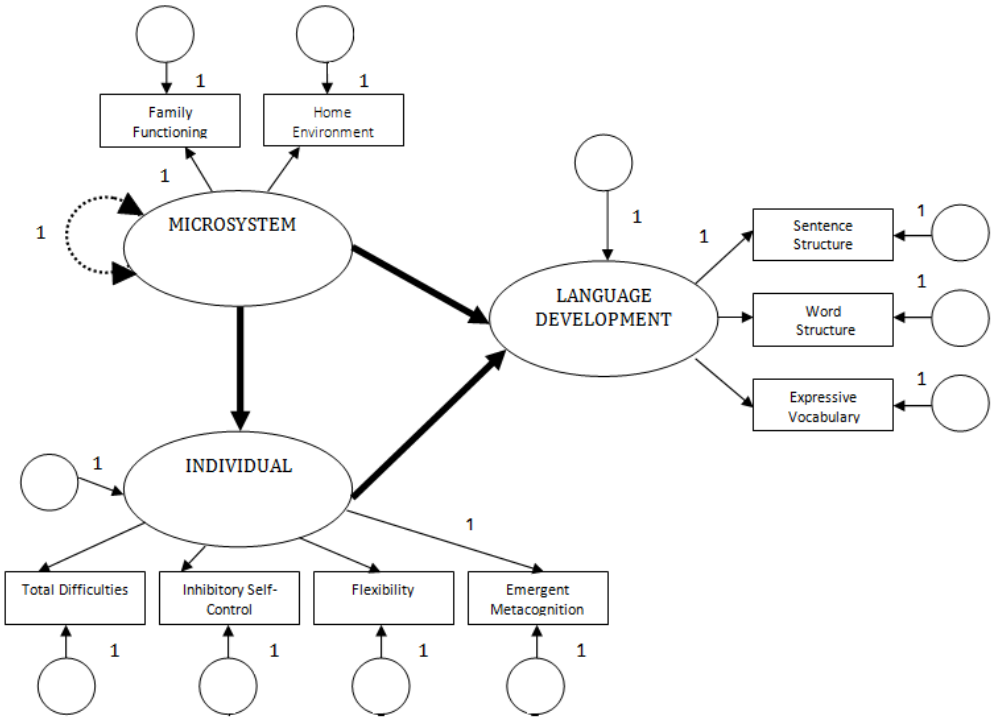


Figure 35. Proposed structural regression model of language development

Lines with a single arrowhead (→) signify a theorized *direct effect* that one variable has on another. The arrowhead points to the effect and the line comes from the cause. Direct effects are similarly called *paths*. Additionally, *indirect effects* involve intervening, or mediator, variables thought to "transmit" some of the causal effects. The individual is a mediating variable (e.g. microsystem →individual → language development). Error terms associated with variables (represented by the circles) are explained in the following paragraphs.

The measurement part of the model (aka the measurement model) concerns the hypothesized direct effect of the latent variables on their respective indicators. Conceptually, these represent the extent

to which the microsystem, individual and language development latent variables are reflected in the scores of their respective indicators (e.g. microsystem → HSQ Home Environment). Statistical estimates of direct effects for the measurement model are also known as factor loadings. In order to give each latent variable an interpretable scale one factor loading was fixed to 1. Error associated with indicators, also known as measurement error, is a proxy variable for all sources of residual variation in indicator scores that isn't explained by the latent variable. Accordingly, the arrows that point from the measurement error to the indicators represent the direct effect of all unmeasured sources of variance on the indicators.

The structural part of the model (aka the structural model) concerns the hypothesised direct effects among the latent variables (e.g. individual → language development). Error associated with latent variables reflects omitted causes of latent variables. The double-headed arrow that exits and re-enters the microsystem variable represents its variance as its cause is not represented in the model.

6.3.2.2 MODEL IDENTIFICATION

In structural equation modelling identification is an important consideration because the unknown model parameters are equated to the known variances of, and covariances amongst, the measured variables. Therefore, if the number of parameters to be estimated in a model exceeds the number of variances of, and covariances amongst, the measured variables then the model is said to be 'unidentified'. The t-rule procedure (312) was used to

assess whether the model in the current study was identified. According to the t-rule for identification, the number of unknown parameters to be estimated in the model must be less than or equal to the number of non-redundant elements in the sample variance-covariance matrix of the observed variables. That is: $t \leq p(p+1)/2$ where t is the number of free parameters to be estimated and p is the number of observed variables. In the model posed by the current study there are 9 observed variables, this means that there are $9(9+1)/2=45$ data points. Thus, with 45 data points and 23 parameters to be estimated there is an overidentified model with 22 degrees of freedom and is thus identified.

6.3.2.3 MODEL ESTIMATION

In fitting the structural equation model, missing data was taken into account, or imputed, using the Maximum Likelihood Estimation method provided by Stata which "aims to find the parameter values that make the observed data most likely (or conversely maximize the likelihood of the parameters given the data)" (p. 63 (313)). It is similar to the ordinary least squares criterion used in multiple regression (314).

6.3.2.4 MODEL TESTING

(See Section 6.3.3, Data analysis)

6.3.3 DATA ANALYSIS

Structural equation modelling using Stata 13.1 was used to address the three research questions.

As with other statistical techniques, there needs to be a suitable sample size in order to get reliable estimates (315). While some studies advise at least 5 participants for each construct and a minimum of 100 individuals for each analysis of the data (316) others propose a sample size of at least 200 (314, 317). A descriptive analysis and independent t-test was performed to compare the post-randomisation variables (SDQ Total Difficulties scores, BRIEF-P Inhibitory Self Control Index scores, BRIEF-P Flexibility Index scores, BRIEF-P Emergent Metacognition Index scores, FAD the General Functioning subscale scores, HSQ Home Environment) for consenters to DOMInO-4 who participated in the language assessment. Frequencies and percentages for these variables as they are clinically classified/categorized are reported.

The current study used multiple-groups structural equation modelling to assess the equivalence of the loadings in the measurement model and the equivalence of the path coefficients in the structural model across treatment groups. This method affords the means by which to ascertain if differences exist between the DHA and control groups and, if they do, where they are. This involved testing a series of hierarchically nested models. Measurement invariance is no change in the factor loadings (the scale units relating each item to its latent underlying construct) across samples). In the current study each lot of models was said to

be nested as a series of parameters were forced to be the same across groups in the model that was more restricted. The series of tests that constitutes invariance testing are summarized below.

An important first step in analyzing invariance of the bio-ecological model is to evaluate the overall fit of the measurement model (Model 0). If the first unconstrained models (permitting for treatment group differences) does not fit the data well, additional constraints will worsen this. So, it was first necessary to verify a model with a good fit that was unconstrained. Then, to answer H1 regarding treatment group interactions in measuring elements of the bio-ecological model parameters of the measurement model were first constrained to be equal between the DHA and control groups (Model 1) and compared the fit of this constrained and unconstrained models (Model 0). If the model fit is not worse when the factor loadings are constrained to be equal, then the measurement models are the same (318). Following Bollen (1989) (312) results of adding equality constraints for the error variances (Model 2) and latent (microsystem) variable variance across groups (Model 3) are shown. If the fit of the model becomes significantly worse when each set of parameters are constrained to be equal, then that component of the model does not fit similarly for the DHA and control groups. Once treatment group invariance or difference in measurement of elements of the bio-ecological theory is assessed in the measurement model, in order to answer H2 treatment group invariance of the structural paths were tested. Doing this involved beginning with a model with equal factor loadings (in accordance with the results from the measurement model), and then constraining the three structural paths to be equal (Model 4). Constraints were also added on the coefficients among elements of

the bio-ecological theory (Model 5), although this is a more rigorous test than the theory suggests is needed.

Assessing model fit

As no model will fit the data flawlessly and each measure has limitations, using several indices of fit has been recommended (314, 319-321). The following fit statistics were used: a) the X^2 (with df and p value for significance wherein if the X^2 difference test is significant it indicates that the more constrained model may be too stringent) and, b) the Comparative Fit Index (CFI; should be >0.95) and c) the Tucker and Lewis Index (TLI; should be <0.95).

6.4 RESULTS

6.4.1 SAMPLE AND PARTICIPANT FLOW

See **Figure 15 (Chapter 2)** for a fuller overview of the participant flow.

6.4.2 CHARACTERISTICS OF STUDY PARTICIPANTS

For demographic information refer to **Chapter 3**. Of those who participated in the language assessment at four years of age, 85% had SDQ Total Difficulties scores in the close to average range, suggesting that the majority of these children had good psychological well-being. Greater than 90% had BRIEF-P Inhibitory

Self Control, Flexibility and Emergent Metacognition scores in the normal range, suggesting that their everyday behaviours were not clinically significant. 77% had FAD General Functioning scale scores in the range considered not to be problematic, suggesting that the majority of respondents did not perceive the child's family's overall functioning to be difficult. 97% had HSQ Home Environment scores in the non-suspect range, suggesting that the majority of children had materials in their home environment that are supportive of their growth and development. **(Table 15)**

TABLE 15. Comparison of post randomisation variables between treatment groups

Characteristic	Classification	Control	DHA	Total
		287	270	557
SDQ Total Difficulties	High risk	14	21	35
	Maybe risk	20	20	40
	Unlikely risk	250	227	477
	Missing	3	2	5
BRIEF-P Inhibitory Self Control	Abnormal	20	30	50
	Normal	265	237	502
	Missing	2	3	5
BRIEF-P Flexibility	Abnormal	14	21	35
	Normal	271	246	517
	Missing	2	3	5

BRIEF-P Emergent metacognition	Abnormal	34	41	75
	Normal	249	226	475
	Missing	4	3	7
FAD GF General Functioning	Problematic	51	40	91
	Not problematic	225	208	433
	Missing	11	22	33
HSQ Home Environment	Suspect	5	5	10
	Not suspect	280	263	543
	Missing	2	2	4

Results are for consenters to DOMInO-4 who participated in the language assessment at 4 years of age. SDQ, Strengths and Difficulties Questionnaire; BRIEF-P, Behaviour Rating Inventory of Executive Function–Preschool; FAD GF, Family Assessment Device General Functioning scale; HSQ, Home Screening Questionnaire.

On examination, t-tests indicated there were some statistically significant differences between the DHA and Control groups on a number of sub measures that are of interest in the research questions to be discussed **(Table 16)**.

TABLE 16. Treatment group differences in post randomisation variables

	DHA	Control	Mean difference (95% CI)	p-value
SDQ Total Difficulties	8.86 (5.04)	7.98 (4.73)	-0.89 (-1.70, -0.07)	0.03
BRIEF-P Inhibitory Self Control	52.42 (10.34)	50.73 (9.90)	-1.69 (-3.39, -0.001)	0.05
BRIEF-P Flexibility	50.75 (10.58)	49.02 (9.31)	-1.73 (-3.40, -0.07)	0.04
BRIEF-P Emergent Metacognition	54.13 (11.19)	52.12 (10.41)	-2.01 (-3.82, 0.20)	0.03
FAD GF General Functioning	1.52 (0.41)	1.48 (0.43)	-0.04 (-0.11, 0.03)	0.28
HSQ Home Environment	43.80 (4.28)	44.04 (4.38)	0.23 (-0.49 to 0.96)	0.53

Results are for consenters to DOMInO-4 who participated in the language assessment at four years of age. Values are mean (SD), and treatment effects are differences in means (95% CI) Results presented are unadjusted. SDQ, Strengths and Difficulties Questionnaire; BRIEF-P, Behaviour Rating Inventory of Executive Function–Preschool; FAD GF, Family Assessment Device General Functioning scale; HSQ, Home Screening Questionnaire.

There was a significant difference in SDQ Total Difficulties scores for the DHA group (M = 8.86, SD = 5.04) and the Control Group (M = 7.98, SD = 4.73; t (551) = -2.13, p = 0.03, two tailed), suggesting that the DHA group had better psychological functioning than the Control group. The magnitude of the differences between means (mean difference = -0.89, 95% CI: -1.70 to -0.07) was very small (eta squared = -0.008).

There was a significant difference in BRIEF-P Inhibitory Self Control scores for the DHA group (M = 52.42, SD = 10.34) and the Control Group (M = 50.73, SD = 9.90; t (550) = -1.97, p = 0.05, two tailed), suggesting that children in the DHA group were better able to regulate their actions, responses, emotions, and behaviour in the presence of other distractions. The magnitude of the differences between means (mean difference = -1.69, 95% CI: -3.39 to -0.001) was very small (eta squared = -0.007).

There was a significant difference in BRIEF-P Flexibility scores for the DHA group (M = 50.75, SD = 10.58) and the Control Group (M = 49.02, SD = 9.31; t (550) = -2.04, p = 0.04, two tailed), suggesting that children in the DHA group were better able to alternate between any of these actions, responses, emotions, and behaviours depending on the context. The magnitude of the differences between means (mean difference = -1.73, 95% CI: -3.40 to -0.07) was very small (eta squared = -0.007).

There was a significant difference in BRIEF-P Emergent Metacognition scores for the DHA group (M = 54.13, SD = 11.19) and the Control Group (M = 52.12, SD = 10.41; t (548) = -2.18, p = 0.03, two tailed), suggesting that children in the DHA group were better able to remember ideas and activities and prepare problem-solving activities. The magnitude of the differences between means (mean difference = -2.01, 95% CI: -3.82 to -0.20) was very small (eta squared = -0.008).

There was no significant difference in FAD General Functioning scores for the DHA group (M = 1.52, SD = 0.41) and the Control Group (M = 1.48, SD = 0.43; t (550) = -1.08, p = 0.28, two tailed), suggesting that there was no difference in the DHA or Control groups' perception of overall family health and pathology and functioning with regard to essential tasks. The magnitude of the differences between means (mean difference = -0.04, 95% CI: -0.11 to 0.03) was very small (eta squared = -0.004).

There was no significant difference in HSQ Home Environment scores for the DHA group (M = 43.80, SD = 4.28) and the Control Group (M = 44.04, SD = 4.38; t (551) = 0.63, p = 0.53, two tailed), suggesting that there was no difference in the provision of developmentally appropriate features, including toys and activities, of the homes of children in the DHA and Control groups. The magnitude of the differences between means (mean difference = 0.23, 95% CI: -0.49 to 0.96) was very small (eta squared = 0.001).

6.4.3 MAIN FINDINGS

Tables 17 and **18** display correlations between scores on measured variables including the HSQ, FAD GF, SDQ Total Difficulties, BRIEF-P Inhibitory Self Control, Flexibility and Emergent Metacognition indices and CELF P-2 Sentence Structure, Word Structure and Expressive Vocabulary subtests. Relationships between measured variables related to latent variables including the individual (i.e. SDQ and BRIEF-P), the microsystem (i.e. HSQ and FAD) and language development (i.e. CELF P-2) were stronger than they were to measured variables related to other latent variables.

TABLE 17. Bivariate correlations between microsystem, individual and language development variables (control group).

		1.	2.	3.	4.	5.	6.	7.	8.	9.
1.	FAD General Functioning	1								
2.	HSQ Home Environment	-0.33*	1							
3.	SDQ Total Difficulties	0.30*	-0.34*	1						
4.	BRIEF-P Inhibitory Self Control	0.35*	-0.30*	0.70*	1					
5.	BRIEF-P Flexibility	0.34*	-0.33*	0.57*	0.78*	1				
6.	BRIEF-P Emergent Metacognition	0.32*	-0.30*	0.63*	0.74*	0.60*	1			
7.	CELF P-2 Sentence Structure	-0.11	0.22*	-0.20*	-0.14*	-0.14*	-0.22*	1		
8.	CELF P-2 Word Structure	-0.10	0.25*	-0.19*	-0.13*	-0.19*	-0.24*	0.58*	1	
9.	CELF P-2 Expressive Vocabulary	-0.17*	0.32*	-0.14*	-0.14*	-0.18*	-0.20*	0.54*	0.61*	1

* indicates $p < 0.05$.

TABLE 18. Bivariate correlations between microsystem, individual and language development variables (DHA group).

		1.	2.	3.	4.	5.	6.	7.	8.	9.
1.	FAD General Functioning	1								
2.	HSQ Home Environment	-0.30*	1							
3.	SDQ Total Difficulties	0.27*	-0.18*	1						
4.	BRIEF-P Inhibitory Self Control	0.27*	-0.15*	0.74*	1					
5.	BRIEF-P Flexibility	0.24*	-0.15*	0.66*	0.81*	1				
6.	BRIEF-P Emergent Metacognition	0.24*	-0.15*	0.65*	0.75*	0.56*	1			
7.	CELF P-2 Sentence Structure	-0.11	0.13*	-0.20*	-0.19*	-0.17*	-0.24*	1		
8.	CELF P-2 Word Structure	-0.70	0.29*	-0.25*	-0.18*	-0.16*	-0.23*	0.60*	1	
9.	CELF P-2 Expressive Vocabulary	-0.5	0.09	-0.17*	-0.06	-0.06	-0.15*	0.49*	0.57*	1

* indicates $p < 0.05$.

H1: As illustrated in **Table 19**, the measurement models for the DHA and control group that were unconstrained had fitted the data sufficiently (Model 0). Notably, although there were dissimilarities in DHA and control group means on some of the observed variables that made up the individual, the relationships between these elements and the latent constructs were comparable for the DHA and control groups.

TABLE 19. Baseline measurement model (Model 0) comparing DHA and control groups regression loadings[1].

		DHA		Control	
		Factor	**Standardized**	**Factor**	**Standardized**
Individual	SDQ Total Difficulties	1.00	0.78	1.00	0.74
	BRIEF-P Inhibitory Self Control	2.56	0.97	2.67	0.95
	BRIEF-P Flexibility	2.24	0.83	2.17	0.81
	BRIEF-P Emergent Metacognition	2.21	0.77	2.33	0.78
Microsystem	HSQ Home Environment	9.40	0.52	11.75	0.61
	FAD General Functioning	1.00	0.58	1.00	0.53
Language development	CELF P-2 Sentence Structure	1.00	0.70	1.00	0.71
	CELF P-2 Word Structure	1.34	0.85	1.20	0.80
	CELF P-2 Expressive Vocabulary	0.94	0.67	1.09	0.76

[1]The variables that are fixed for scaling have a "1" for their factor loading.

As illustrated in **Table 20** when the loadings were constrained to be equal between the DHA and control groups (Model 1), the fit of the models did not get significantly worse. As demonstrated by the sequence of additional comparisons, the fit did not get significantly worse when equality constraints for the error variances (Model 2) and latent (microsystem) variable variance across groups (Model 3) were imposed. The equality of measurement loadings is a suitable condition for deducing that two measurement models (i.e. DHA and control groups measurement models) are not significantly different (322). By this criterion the analysis showed that the factor loadings for the latent variables did not differ between the DHA and control groups, or that treatment group did not moderate measurement of elements of the bio-ecological theory that were examined.

TABLE 20. Fit statistics for the model

Model	X^2 [1]	Df[2]	Comparison model	X^2 diff	Df diff	p-value	CFI[3]	TLI[4]
Model 0 No constraints across groups	94.62	48					0.98	0.97
Model 1 Overall measurement invariance across groups	100.11	54	0	5.49	6	0.52	0.98	0.97
Model 2 Measurement error invariance across groups	107.57	63	1	7.46	9	0.41	0.98	0.98
Model 3 Variance of exogenous variable (Micro) across groups	108.00	64	2	0.43	1	0.49	0.98	0.98
Model 4 Structural variances across groups	116.03	66	3	8.04	2	0.98	0.98	0.98
Model 5 Structural coefficients across groups	116.61	69	4	0.57	3	0.10	0.98	0.98

1, chi square; 2, degrees of freedom; 3, Comparative Fit Index; 4, Tucker-Lewis Index

H2: To evaluate treatment group invariance of the structural paths between the individual and microsystem and language development a model in which the factor loadings and structural paths were constrained to be equal (Model 5) and compared to a model in which only the factor loadings were equal (results from the measurement model analysis) (Model 4). The p-value for the change in X^2 was greater than 0.05, indicating that treatment group does not moderate the relationships between elements of the bio-ecological model. Model 4 was the best fitting, most parsimonious model of language development and is presented in **Table 21**.

TABLE 21. Model 4: treatment group invariance of the structural paths between the individual and microsystem and language

	Unstandardised coefficient	Standard error	Standardized coefficient	z	p-value	95% confidence interval
INDIVIDUAL > LANGUAGE DEVELOPMENT						
DHA	0.04	0.05	0.08	0.84	0.40	-0.05, 0.14
Control	-0.01	0.06	-0.02	-0.20	0.84	-0.12, 0.10
MICROSYSTEM > INDIVIDUAL						
DHA	8.24	1.79	0.52	4.60	<0.005	4.73, 11.76
Control	8.47	1.48	0.53	5.73	<0.005	5.57, 11.37
MICROSYSTEM > LANGUAGE DEVELOPMENT						
DHA	2.82	1.20	0.33	2.35	0.02	0.46, 5.18
Control	3.64	1.19	0.42	3.05	0.002	1.29, 5.98

H3: With a view to investigating the mechanisms linking children's microsystem to their language development the current study tested a mediational model proposing that the child's individual characteristics facilitate this relationship. In the first stage of the analysis, as expected, the prerequisites were met. That is, for the whole group, indicators of the microsystem latent variable (FAD General Functioning and HSQ Home Environment) were significantly correlated with indicators of the individual latent variable (SDQ Total Difficulties, BRIEF-P Inhibitory Self-Control, Flexibility and Emergent Metacognition), and indicators of the individual latent variable were in turn significantly correlated with indicators of the language development latent variable (CELF P-2 Sentence Structure, Word Structure and Expressive Vocabulary). For the direction and significance of each correlation see **Table 17** and **18**. It should be noted that when looking at DHA and control groups there was not always a significant relationship between all indicators of the microsystem latent variable and individual latent variable and between individual latent variable and language development latent variable. However, the lack of consistency/pattern to the non-significant relationships was not reason enough not to test for language development as a mediated outcome.

For Model 4, the microsystem did not indirectly effect children's language development through their individual characteristics for the DHA group (Standardised Indirect effect = 0.35, p = 0.40, 95% CI = -0.43 to1.13) or the control group (Standardised Indirect effect =-0.09 p = 0.47, 95% CI = -1.01 to 0.83).

6.5 DISCUSSION

Although structural equation modelling has been used extensively in recent studies, most have used the method in non-experimental contexts. The application of structural equation modelling in experimental studies such as the current study represented a significant but relatively untapped potential area of application. This contemporary effort to introduce a level of analysis that conjoins the child and environmental variation in understanding language development differs from traditional studies of the interaction between two discrete variables. In this analysis the variables were not considered independent of each other but instead the relevance of each for language development involved that of others.

Results from the structural equation modelling analyses revealed that the fit of the model (that is, of the bio-ecological model of development) for the DHA and control groups was good, suggesting that the correlation matrix and reproduced model implied correlation matrix were alike. In line with previous research measurement variables related to the individual (that is, their psychological well-being and behaviour) and microsystem (in this case family functioning and home environment) were significantly positively related to language development. This was the case for the DHA and control groups separately. That is, children who were more psychologically stable and had good behaviour, good family functioning and access to developmentally appropriate materials at home tended to have better language development.

For the group as a whole, the path from microsystem to language development was significant, although there were no significant differences between the DHA and control groups in the strength of this relationship. There was no significant path between the individual and language development for the group as a whole or between the DHA and control groups. Furthermore, there were no significant differences between groups in the ability of the individual child to mediate the relationship between their microsystem and their language development.

Only examining the impact of the microsystem on the individual does not provide a holistic picture, as the parent child relationship is dynamic, meaning that children's behaviour also influences parenting actions. Although addressing reciprocal relationships is clearly required when exploring changes in the home, this was beyond the scope of this study.

6.6 CONCLUSION

Taken together, these findings provide only partial support for understanding children's language development through focusing on the relationships between the individual and his or her microsystem as proposed by the bio-ecological model but not necessarily any support for understanding how DHA is related. It may be that alternative individual and microsystem measurement variables not used in the current study might reveal significant differences between DHA and control groups. Alternatively, consistent with the majority of results in this study, not finding any significant differences between groups (i.e. for the microsystem-language development and individual-language development paths) might suggest that prenatal DHA supplementation is not an important element in language development at least at four years of age.

CHAPTER 7

7 GENERAL DISCUSSION

7.1 OVERVIEW OF STUDY RATIONALE AND OBJECTIVES

The role of DHA in early life, particularly from the last trimester of pregnancy up to 2 years of age when the brain growth spurt occurs, is of considerable importance. The relationship between DHA and early neurodevelopment has captured attention because DHA is related to specific physiological and neurological processes. The Western diet is low in DHA, raising doubt as to whether children's cognitive development is optimal. Basic and epidemiological studies provide strong evidence for the role of DHA in brain function.

However, it is necessary to properly establish whether a higher DHA intake by those with low consumption enhances cognitive performance. Just as findings can be used to accelerate application of discoveries it can also be just as useful in avoiding the hasty implementation of ideas that have no scientific evidence supporting them. The easiest way to deal with the outstanding complexity of establishing causality in epidemiological studies is to use the design of a RCT. RCTs have been advocated as the "gold standard" for establishing efficacy of treatments. Recent systematic reviews and meta-analyses have proposed that a reason for inconsistencies in previous findings for an effect of DHA supplementation on brain function is the use of insensitive measures

for developmental assessment. Many trials used global assessments, which are not designed to detect subtle changes in brain function, or the umbrella concept of cognition. Notably, outcomes related to language were particularly controversial.

The human capacity for language is one of the most important neurodevelopmental achievements of childhood. The acquisition of language, however, is not a straightforward process for all children, with early disparities proving to persist overtime and influence later achievements. In Australia 23% of children struggle to develop language optimally, suggesting that this is a problem that needs to be addressed.

In light of recommendations to avoid global tests for assessing efficacy of interventions in favor of using more specialized measures of function, the literature was systematically reviewed according to the Cochrane handbook and identified RCTs of DHA supplementation in pregnant women (N=3), lactating women (N=2) or formula fed infants (N=13) that have assessed children's language development in order to see whether supplementation had any benefit for enhancing language acquisition. Results have been mixed and inconclusive, largely due to methodological limitations including post-randomisation exclusion, high attrition, lack of power and absence of suitable measures for assessing language in particular. Investigations of effectiveness have also been limited. Moreover, RCTs can show what can cause development but not necessarily what shapes it in natural settings.

With this in mind the current study sought to contribute to an emerging trend of interdisciplinary research that seeks to understand brain development and linguistic functioning as adaptations not only to DHA but also to broader experiential contexts. Research investigating whether, to what extent, and how the biochemical availability of DHA and elements of the bio-ecological theory, that is, Process, Person, Context and Time related variables, interacted to influence children's language development was undertaken. The current study approached the overarching research question from multiple angles with the aim of permitting a more holistic understanding of language outcomes. That is, by maximizing the potential to add to current knowledge about the relationships among intervention and non-intervention variables and how these both independently and together play a role in differences in children's language development.

The DOMInO trial is the largest published RCT of DHA supplementation during pregnancy with childhood neurodevelopmental outcomes and it has been identified as one of the few trials free from bias. It therefore presented a valuable opportunity to investigate the effect of DHA on children's language development. The study was well-designed, with strengths including carefully controlled, randomised design, blinded assessments of language development, large sample size and excellent follow-up at four years of age. Unlike other RCTs of DHA supplementation with language outcomes, the lack of treatment effect cannot be attributed to common study limitations, particularly lack of statistical power. This also presented as a particularly unique occasion to compare the effect of a DHA supplement with a non-DHA supplement due to the increasing use of prenatal supplements

containing DHA. Considering intervention during the early years is effective for improving child outcomes and often yields higher returns on investment than programs later in life, the current study was very well timed.

7.2 STUDY FINDINGS IN THE CONTEXT OF OTHER STUDIES

Findings of the DOMInO trial indicate that DHA supplementation in pregnancy had no effect on language development in children aged 18 months. However, treatment group girls had lower mean language scores as well as an increased risk of delayed language development compared with girls in the control group. As both of these outcomes were secondary they may have been due to chance and therefore needed further validation.

Follow up of this group at four years of age revealed that supplementing women with a higher amount of DHA during the second half of pregnancy did not impact children's language development as there was no effect on the primary outcome CELF P-2 Core Language Scores or any of the subtests that comprised them (Sentence Structure, Word Structure and Expressive Vocabulary), regardless of sex. This also suggests that any potential negative effect of DHA on language experienced by girls at 18 months is resolved by four years of age.

7.3 SUPPORT FOR THEORETICAL FOUNDATIONS AND ALTERNATIVE POSSIBILITIES

Despite the evidence for the relationship between children's language acquisition and elements of Process, Person, Context and Time and the plausible theoretical basis for how DHA may be implicated in such relationships, a role for DHA supplementation was not supported by findings of the current study. It is important to note that the bio-ecological model is not a model of language development per se but a model of sources of influence on development in general. This is not to say that if it were conceptualized differently that it would not be a model for language development. DHA may enhance language development but only through other Process, Person, Context or Time variables. Further studies would be required to investigate this proposition more deeply.

It might be that children in the current study received the appropriate stimuli (or environmental support or experience) for their language development which may have mitigated the true effect of the intervention. The plasticity of the human brain in early life may have lessened the effects of DHA insufficiency on the brain by adapting or compensating in response to environmental experiences. In particular, the current study could only infer from characteristics of the mother the sort of language experience likely to have been provided to the child, but did not specifically measure maternal language itself. Importantly, mothers provide support that enables language learning and development by providing children with the necessary stimulation for learning.

Mothers of children in the current study may have adapted their speech to assist their own child's language skills. That is, they may have provided their children with language at an appropriate level for language to develop optimally. Interestingly, research suggests that maternal language mediates the relationship between socioeconomic status and children's language skills (198, 323, 324). That is, associations diminish when controlling for the quantity and/or quality of maternal language. This suggests that using social variables such as the ones the current study examined as proxies for maternal language might not be as useful as measuring maternal language itself.

Subgroup analyses were undertaken in an effort to assess whose language abilities may benefit most from DHA. As no power calculation was done these analyses may have been limited by poor statistical power. An alternative is to conduct a risk stratified analysis to investigate the advantage of DHA supplementation based on the baseline risk of suffering a negative outcome such as delayed language development. These models can often conquer the usual limitations of usual subgroup analyses, including poor statistical power and multiple comparisons, and can be particularly useful when a treatment may have adverse consequences (325-327).

7.4 STRENGTHS AND LIMITATIONS

Language is a developmental domain that shows evidence of stage-like change and the potential for DHA to optimize acquisition of each stage has been the focus of this thesis. However, the

approach taken by studies of its effectiveness to date demands some rethinking. Notably, while environmental effects and individual differences abound, potential sources of difference cannot count as causes of developmental sequence. Arguably, any variation as a result of DHA supplementation can only affect the speed of development through sequence. Thus, rethinking the approach of current studies of effectiveness should involve a better understanding of what development means and, importantly, what it means to say that language development is effected by DHA supplementation.

7.5 FUTURE DIRECTIONS

Findings underscore the importance of longer-term follow-up studies of early DHA supplementation and language development. Additional studies are required to determine whether apparent disadvantages are real, whether they persist and whether delayed effects of early DHA availability emerge.

The baseline DHA status probably varied significantly between mothers. It is plausible to suggest that individuals with poor status benefit more from additional DHA. It might be worth screening pregnant or lactating mothers for a DHA deficiency or insufficiency, for example, by using the omega 3 index, in order to identify mothers and infants with low DHA status.

While the focus of the current thesis has been on term infants, valuable information can be gained from studying the preterm

population. Preterm infants are particularly susceptible to DHA deficiency as they have not been able to access their mother's lipid stores for the full length of gestation (328). Considering language impairments are higher in preterm infants compared to gender and age matched term born children (329, 330) it is possible that lower DHA status in preterm infants during important periods of brain development may compound such impairments (21).

In elucidating the DHA-language relationship it might be more informative for future research to move beyond focusing on the causal effect of DHA on language outcome (whether DHA causes variation in language outcome) towards a broader understanding of how DHA contributes to the developmental trajectory of children's language abilities. That is to say, consideration should be given to whether, as a function of DHA, children's linguistic performance is accelerated, delayed or remains within the normal limits and how this may change (or remain stable) as they grow up as measured at further time points of assessment. New discoveries about patterns and predictors of what stays the same, what changes, what improves and even what declines in language abilities in the first decade of life may provide the best guidance for how DHA intervention efforts might look in the future.

Lately, there has been growing curiosity in what effect n-3 PUFA supplementation has on brain physiology. While findings of the current study could not necessarily be impetus for further exploration of the effect of DHA supplementation on language function, the use of brain imaging in other studies has proven valuable in ascertaining the effects that n-3 PUFAs have on brain

function in general in the absence of explicit behavioural manifestation. It may be that the relevant neural wiring may be in place and ready to work from the beginning, but is not being captured by the assessments of language development. An area may become active at some point not because it has finally attained the necessary wiring but because the child has finally figured out how the area can and should be employed within a given task. Thus, to resolve the seemingly conflicting claims future studies should determine whether or not an area is working at all as versus whether the area is activated for a given task.

CONCLUSION

In progressing knowledge about the role that DHA supplementation plays in children's language development forward this thesis took a comprehensive approach. While a randomised controlled trial design was employed, its rigidity in fully shedding light on the topic at hand was taken into account. In turn, a more productive strategy was used by considering what multiple sources of evidence contributes and undertaking a variety of further analyses accordingly. Overall, findings add to the increasing evidence base suggesting that prenatal DHA supplementation confers no benefit to children's language development. Longer-term follow-up studies of early DHA supplementation are required to determine whether delayed effects emerge.

APPENDICES

APPENDIX 1: SYSTEMATIC REVIEW SEARCH STRATEGY

COCHRANE (CENTRAL)

(Docosahex*enoic acid OR docosahex*enoate OR omega 3 OR lcpufa OR long chain polyunsaturated fatty acid OR fish oil OR algal oil OR marine oil) AND (language OR linguistics OR verbal OR vocabulary OR literacy OR reading OR communication OR language test OR neurodevelopment OR cognitive development)

PUBMED

("DHA"[All] OR "Fatty Acids, Omega-3"[Mesh] OR "Docosahexaenoic Acids"[Mesh] OR "Fish Oils"[Mesh] OR "Fish Oils"[All Fields] OR "Marine Oil"[All Fields] OR "Algal Oil"[All fields] OR "long chain polyunsaturated fatty acids"[All Fields] OR "lcpufa"[All Fields]) AND (Language[Mesh] OR "Language tests"[Mesh] OR Linguistics[Mesh] OR Literacy[All] OR "Verbal"[All] OR Vocabulary[All] OR Reading[All] OR "Cognitive development"[All Fields] OR "Neurodevelopment"[All] OR Communication [All Fields]) AND ("RCT"[All Fields] OR "randomised"[All Fields] OR "randomised"[All] OR "Intervention"[All Fields] OR "Placebo"[All] OR "Control"[All] OR "Trial"[All])

EMBASE

('DHA' OR 'omega 3'/syn OR 'docosahexaenoic acid'/syn OR 'long chain polyunsaturated fatty acid' OR 'lcpufa' OR 'fish oil'/exp OR 'marine oil' OR 'algal oil') AND ('linguistics'/syn OR 'literacy'/exp OR 'language'/exp OR 'reading'/syn OR 'language test'/syn OR 'verbal' OR 'vocabulary'/exp OR 'cognitive development'/exp OR 'neurodevelopment' OR 'communication'/syn) AND ('randomised' OR 'randomised' OR 'RCT' OR 'intervention' OR 'placebo'/exp OR 'control' OR 'trial')

CINAHL

(DHA OR omega 3* OR docosahexaenoic acid* OR long chain polyunsaturated fatty acid* OR lcpufa OR fish oil* OR marine oil* OR algal oil*) AND (linguistic* OR literacy OR language OR reading OR verbal OR vocabulary OR "cognitive development" OR neurodevelopment OR communication) AND (randomi* OR placebo OR rct OR intervention OR control OR trial)

PSYCHINFO

(DHA OR omega 3* OR docosahexaenoic acid* OR long chain polyunsaturated fatty acid* OR lcpufa OR fish oil* OR marine oil* OR algal oil*) AND (linguistic* OR literacy OR language OR reading OR verbal OR vocabulary OR "cognitive development" OR neurodevelopment OR communication) AND (randomi* OR placebo OR rct OR intervention OR control OR trial)

WEB OF SCIENCE

(DHA OR "omega 3" OR docosahexaenoic acid* OR long chain polyunsaturated fatty acid* OR lcpufa OR fish oil* OR marine oil* OR algal oil*) AND (linguistic* OR literacy OR language OR reading OR verbal OR vocabulary OR reading OR "cognitive development" OR neurodevelopment OR communication) AND (randomi* OR placebo OR rct OR intervention OR control* OR trial)

CURRENT CONTENTS CONNECT

(DHA OR "omega 3" OR docosahexaenoic acid* OR long chain polyunsaturated fatty acid* OR lcpufa OR fish oil* OR marine oil* OR algal oil*) AND (linguistic* OR literacy OR language OR reading OR verbal OR vocabulary OR reading OR "cognitive development" OR neurodevelopment OR communication) AND (randomi* OR placebo OR rct OR intervention OR control* OR trial)

SUMMARY

"DHA OR Docosahex*enoic acid OR docosahex*enoate OR omega 3 OR LCPUFA OR long chain polyunsaturated fatty acid OR fish oil OR marine oil OR algal oil" AND "Language OR linguistics OR verbal OR vocabulary OR literacy OR reading OR communication OR language test OR neurodevelopment OR cognitive development" AND "RCT OR randomi*e* OR intervention OR placebo OR control

APPENDIX 2: PARTICIPANT INFORMATION SHEET (WOMEN'S AND CHILDREN'S HOSPITAL)

Government of South Australia
Children, Youth and Women's Health Service

Women's & Children's Health Research Institute Inc.

Follow-up of children whose mothers participated in the DOMINO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

(Scientific title: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood?)

Dear

We would like to invite you and your child to participate in a follow-up study of children involved in the DOMInO trial.

You may remember that the DOMInO trial aimed to determine whether supplementing pregnant women with a special omega 3 fat called DHA would improve the development of children at 18 months of age. We will let you know the findings from the 18-month old development appointment during 2010, soon after all the children have completed their appointments.

Invitation to participate in a new follow up study of 'DOMInO children'

This invitation is a new part of the DOMInO trial that will go on to determine whether supplementing pregnant women with DHA can lead to differences in children's development at 4 years of age. It is important that we measure the longer-term effects of omega 3 fats as children grow older and learn new skills. Other research suggests that taking omega 3 supplements during pregnancy and while breastfeeding may improve development of children at 4 years of age, but not at younger ages.

What does the follow up study involve?

You and your child will be asked to attend an appointment at the Women's and Children's Hospital when your child is 4 years of age. The appointment will take approximately 2 hours.

There is no need for any dietary supplements to be taken as a part of this follow-up study.

At the appointment, a research psychologist will measure your child's development. The psychologist will administer a variety of activities designed to look at how your child solves problems. Some activities will also consider how your child uses and understands language. The majority of children (90%) will take approximately 1¼ hour to complete the tasks but a small number of children may take 1½ hours. Children generally enjoy the appointment because the tasks are appealing to 4-year-old children. We can take a break during the appointment if your child is tired or hungry. These procedures usually do not cause any discomfort to your child. If you

wish, you will have the opportunity to discuss the outcome of the assessment with the psychologist. If the cognitive assessment suggests abnormalities the psychologist will discuss this with you and referral with an appropriate health professional will be made. We will then measure your child's weight, height, head circumference.

If your child has been in hospital in the last 12 months we may access your child's medical record to record any new and relevant diagnosis that may affect development.

Parental / Carer's involvement

In order to minimise potential distractions it is preferable that your child completes the activities with the psychologist and without other people in the room. If for any reason, you or your child are not comfortable with this arrangement, you may remain present during the assessment.

While your child is working with our researcher, we will ask you to complete four questionnaires that will take approximately 20 to 30 minutes to complete. The questionnaires ask about your child's behaviour, and how they cope with everyday situations, the impact of recent events on your child's development and the child's home environment. In addition we will ask a few questions about foods with omega-3 fats in your child's diet and whether your child takes an omega-3 supplement. If any of the questionnaires suggest a problem we will notify you and discuss a referral to an appropriate health professional with your permission.

You will be given $20 to offset car parking and travelling expenses associated with attending the appointment.

Your rights

If you decide to participate you are free to withdraw from the follow-up study at any time without any explanation of why you have chosen to do so and without prejudice to you or your child's future care.

All information gathered will be treated with confidence and no information that could identify you or your child will be released to any person who is not directly associated with the study except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility. Results from the study will be published in medical journals and at professional meetings, but neither you nor your child will be identified in any way. If you would like further information about the study please contact our research staff on (08) 8161 8045.

To better understand the long term influence of omega 3 supplementation during pregnancy on child development and growth, our research staff may contact you to discuss the possibility of your child being involved in future follow-up studies.

How to take part

One of our research staff will contact you shortly to answer any questions you may have about the *'DOMInO trial development follow-up study'. If you decide that you would be interested in participating with your child we will organise an appointment closer to their 4 year mark.*

Any Questions?

If at any time during the study you have any problems or questions, please ring our office on 8161 8045 and leave a message on our answering machine; one of our nurses will return your call as soon as possible. If you have a problem and would like to talk to us immediately please ring 8161 7000 and ask for pager 5864, and one of our research nurses will answer your call.

This study has been reviewed and approved by the Children, Youth Women's Health Service (CYWHS) Human Research Ethics Committee Approval number REC2242/12/12. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the executive secretary of the Human Research Ethics Committee, Ms Brenda Penny, CYWHS (8161 6521).

Yours Sincerely

The DOMInO team

Child Nutrition Research Centre
Women's and Children's Hospital
72 King William Road
North Adelaide. 5007
Tel: 8161 8045

APPENDIX 3: PARTICIPANT INFORMATION SHEET (FLINDERS MEDICAL CENTRE)

Child Nutrition Research Centre
a joint venture of the Women's & Children's Health Research Institute,
Women's and Children's Hospital of the Children, Youth and Women's Health Service
and Flinders Medical Centre of the Southern Adelaide Health Service

Follow-up of children whose mothers participated in the DOMINO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

Scientific Title: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood?

Dear

We would like to invite you and your child to participate in a follow-up study of children involved in the DOMInO trial.

You may remember that the DOMInO trial aimed to determine whether supplementing pregnant women with a special omega 3 fat called DHA would improve the development of children at 18 months of age. We will let you know the findings from the 18-month old development appointment during 2010, soon after all the children have completed their appointments.

Invitation to participate in a new follow up study of 'DOMInO children'

This invitation is a new part of the DOMInO trial that will go on to determine whether supplementing pregnant women with DHA can lead to differences in children's development at 4 years of age. It is important that we measure the longer-term effects of omega 3 fats as children grow older and learn new skills. Other research suggests that taking omega 3 supplements during pregnancy and while breastfeeding may improve development of children at 4 years of age, but not at younger ages.

What does the follow up study involve?

You and your child will be asked to attend an appointment at the Flinders Medical Centre when your child is 4 years of age. The appointment will take approximately 2 hours.

There is no need for any dietary supplements to be taken as a part of this follow-up study.

At the appointment, a research psychologist will measure your child's development. The psychologist will administer a variety of activities designed to look at how your child solves problems. Some activities will also consider how your child uses and understands language. The majority of children (90%) will take approximately 1¼ hour to complete the tasks but a small number of children may take 1½ hours. Children generally enjoy the appointment because the tasks are appealing to 4-year-old children. We will then measure your child's weight, height, head circumference.

If your child has been in hospital in the last 12 months we may access your child's medical record to record any new and relevant diagnosis that may affect development.

Parental / Carer's involvement

In order to minimise potential distractions it is preferable that your child completes the activities with the psychologist and without other people in the room. If for any reason, you or your child are not comfortable with this arrangement, you may remain present during the assessment.

While your child is working with our researcher, we will ask you to complete four questionnaires that, in total, will take approximately 20 to 30 minutes to complete. The questionnaires ask about your child's behaviour, and how they cope with everyday situations, the impact of recent events on your child's development and the child's home environment. We will also ask you a few questions about foods in your child's diet that contain omega-3 fats and whether your child takes an omega-3 supplement.

You will be given $20 to offset car parking and travelling expenses associated with attending the appointment

Benefits and Risks

The cognitive assessments do not pose any apparent physical risk to children. However, if any of the assessments or questionnaires suggest a problem we will contact you to discuss the results and with your permission, we will provide you with a referral to an appropriate health professional. If you wish, you will have the opportunity to discuss any concerns you may have about your child as well as the outcome of the assessment with the psychologist.

The appointment should not cause any distress to your child. If your child does become distressed during the assessment, we will have the opportunity to take small breaks between the assessments or you will be offered the opportunity to return and complete the assessment at a later date.

This follow-up will help us to understand the long-term influence of Omega 3 supplementation during pregnancy on children's development at 4 years, although you may not directly benefit from participating in this study.

The study investigators (Prof Maria Makrides, Dr Lisa Smithers, Prof Robert Gibson and Dr Peter Anderson) plan to publish the results of the study and this may enhance their scientific reputation. The investigators will not receive any direct financial benefit from this study

Your rights

If you decide to participate you are free to withdraw from the follow-up study at any time without any explanation of why you have chosen to do so and without prejudice to you or your child's future care.

All information gathered will be treated with confidence and no information that could identify you or your child will be released to any person who is not directly associated with the study except in the case of a legal requirement to pass on personal information to authorised third parties. This requirement is standard and applies to information collected both in research and non-research situations. Such requests to access information are rare; however we have an obligation to inform you of this possibility. Results from the study will be published in medical journals and at professional meetings, but neither you nor your child will be identified in any way. If you would like further information about the study please contact our research staff on (08) 8204 5007.

If you or your child suffer injury as a result of participation in this study, compensation might be paid without litigation. However, such compensation is not automatic and you may have to take legal action to determine whether you should be paid.

The DOMInO trial and this follow up study have been funded by the National Health and Medical Research Council of Australia (NH&MRC).

To better understand the long term influence of omega 3 supplementation during pregnancy on child development and growth, our research staff may contact you to discuss the possibility of your child being involved in future follow-up studies.

How to take part

One of our research staff will contact you shortly to answer any questions you may have about the *'DOMInO trial development follow-up study'*. If you decide that you would be interested in participating with your child we will organise an appointment closer to their 4 year mark.

Any Questions?

If at any time during the study you have any problems regarding appointments or have any other queries, please ring on main office phone number 8204 5007 and leave a message on our answering machine. One of our staff will return your call as soon as possible. If you have a problem and would like to talk to us immediately please ring 8204 5511 and ask for pager 23517 and one of our research nurses will answer your call.

This study has been reviewed by the Flinders Clinical Research Ethics Committee. Should you wish to discuss the study with someone not directly involved, in particular in relation to matters

concerning policies, your rights as a participant, or should you wish to make a confidential complaint, you may contact Executive Officer FCREC- Research, at the Flinders Medical Centre on 8204 4507 or email 'research.ethics@fmc.sa.gov.au'.

Yours Sincerely

The DOMInO team

Child Nutrition Research Centre
Flinders Medical Centre
3 Flinders Drive
Bedford Park SA 5042
Tel: 8204 5007

APPENDIX 4: CONSENT FORM (WOMEN'S AND CHILDREN'S HOSPITAL)

I

Government of South Australia
Children, Youth and Women's Health Service

Women's & Children's Health Research Institute Inc.

Women's & Children's Hospital, Research Ethics Committee

CONSENT FORM

Follow-up of children whose mothers participated in the DOMINO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

(Scientific title: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood?)

I _____

hereby consent to my own and my child's involvement in the research project entitled:

Follow-up of children whose mothers participated in the DOMINO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

1. The nature and purpose of the research project described on the attached Information Sheet has been explained to me. I understand it, and agree to my child and I taking part.
2. I understand that my child may not directly benefit by taking part in this study.
3. I acknowledge that the possible risks and/or side effects, discomforts and inconveniences, as outlined in the Information Sheet, have been explained to me.
4. I understand that I can withdraw my child from the study at any stage and that this will not affect medical care or any other aspects of my child's relationship with this healthcare service.
5. I understand that I will be reimbursed a total of $20 for attendance at the follow up clinic for the developmental assessment when my child is 4 years of age.
6. I have had the opportunity to discuss this research project with a family member or friend, and/or have had the opportunity to have a family member or friend present whilst the research project was being explained by the researcher.
7. I am aware that I should retain a copy of the Consent Form, when completed, and the Information Sheet.
8. I understand that while information gained in the study may be published, neither my child nor I will be identified and information will be confidential.

9. I consent to my child having the following procedures:
   - An assessment with a psychologist that will include a number of activities measuring general development (called the Differential Abilities Scale II), mental flexibility, and language development.
   - Measurements of weight, height and head circumference. The whole appointment will take about 2 hours.

10. I consent to answering the questionnaires as explained in the information sheet and am aware that these will take approximately 30 minutes to complete.

11. I understand that study personnel may review my child's medical records at the Women's and Children's Hospital and any other hospital my child may be transferred to and from.

12. I understand that I may be contacted about possible involvement in future follow-up studies conducted by the Child Nutrition Research Centre.

13. I understand that all information provided will be kept confidential as explained in the attached Information Sheet except where there is a requirement by law for it to be divulged.

Signed: .....

Relationship to participant: .....

Full name of participant: .....

Dated: .....

I certify that I have explained the study and that child's parent/carer understands what is involved.

Signed: ..... Title: .....

Dated: .....

APPENDIX 5: CONSENT FORM (FLINDERS MEDICAL CENTRE)

SOUTHERN ADELAIDE HEALTH SERVICE / FLINDERS UNIVERSITY

CONSENT BY A THIRD PARTY TO PARTICIPATION IN RESEARCH

I, ... (first or given names) ..... (last name) ..... request and give consent to

..... (first or given names) ..... (last name) .....'s involvement in the research

project: **Scientific title: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood?**

Follow-up of children whose mothers participated in the DOMINO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

I acknowledge the nature, purpose and contemplated effects of the research project, especially as far as they affect ..... (first or given names) ..... (last name) .....

have been fully explained to my satisfaction by ..... (first or given names) ..... (last name) .....

and my consent is given voluntarily.

I acknowledge that the detail(s) of the following has/have been explained to me, including indications of risks; any discomfort involved; anticipation of length of time; and the frequency with which they will be performed:

1. I consent to my child having the following procedures:
   - An assessment with a psychologist that will include a number of activities measuring general development (called the Differential Abilities Scale II), mental flexibility, and language development.
   - Measurements of weight, height and head circumference. The whole appointment will take about 2 hours.

2. I consent to answering the questionnaires as explained in the information sheet and am aware that these will take approximately 30 minutes to complete.

3. I understand that all information provided will be kept confidential as explained in the attached Information Sheet except where there is a requirement by law for it to be divulged.

4. I understand that while information gained in the study may be published, neither my child nor I will be identified and information will be confidential.

5. I understand that I will be reimbursed a total of $20 for attendance at the follow up clinic for the developmental assessment when my child is 4 years of age.

I have understood and am satisfied with the explanations that I have been given.

I have been provided with a written information sheet.

I understand that ..... (first or given names) ..... (last name) .....'s involvement in this research project may not be of any direct benefit to him/her and that I may withdraw my consent at any stage without affecting his/her rights or the responsibilities of the researchers in any respect.

I declare that I am over the age of 18 years.

I understand that I may be contacted about possible involvement in future follow-up studies conducted by the Child Nutrition Research Centre.

I acknowledge that I have been informed that should he/she receive an injury as a result of taking part in this study, legal action may need to be taken to determine whether he/she should be paid.

Signature of parent, legal guardian or authorised person: ..... Date: .....

Relationship to subject: .....

FOR OFFICE USE

.....
.....
.....

I, ... have described to ..... the research project and nature and effects of procedure(s) involved. In my opinion he/she understands the explanation and has freely given his/her consent.

Signature: ..... Date: .....

Status in Project: .....

APPENDIX 6: UPDATED CONTACT DETAILS FORM (WOMEN'S AND CHILDREN'S HOSPITAL)

Updated Contact Details – 4 Year follow up of Children who participated in the DOMInO Study

To enable us to keep in contact with you please complete this form with your current details and return to the address below (no stamp required):

DOMInO 4 Study

Child Nutrition Research Centre

Women's & Children's Hospital

Reply Paid 60668

NORTH ADELAIDE SA 5006

DATE _____ **STUDY I.D._____**

Child's Details

Full Name:

Mother's Details

Full Name:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Father's Details

Full Name:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Alternative Contact Details (*i.e. Grandparent, Aunty, friend)*

Full Name:

Relationship to child:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Alternative Contact Details (*i.e. Grandparent, Aunty, friend)*

Full Name:

Relationship to child:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Thank you for your time

APPENDIX 7: UPDATED CONTACT DETAILS FORM (FLINDERS MEDICAL CENTRE)

Updated Contact Details – 4 Year follow up of Children who participated in the DOMInO Study

To enable us to keep in contact with you please complete this form with your current details and return to the address below (no stamp required):

DOMInO 4 Study

Child Nutrition Research Centre

Flinders Medical Centre

Reply Paid 60085

BEDFORD PARK SA 5042

DATE _____ **STUDY I.D. _____**

Child's Details

Full Name:

Mother's Details

Full Name:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Father's Details

Full Name:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Alternative Contact Details (*i.e. Grandparent, Aunty, friend)*

Full Name:

Relationship to child:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Alternative Contact Details (*i.e. Grandparent, Aunty, friend)*

Full Name:

Relationship to child:

Address:

Post Code:

Home Ph: Work Ph: Mobile:

E-mail:

Thank you for your time ☺

APPENDIX 8: 4 YEAR CRF QUESTIONS

The DOMInO STUDY (DHA to Optimise Mother Infant Outcomes)

Follow-up of children whose mothers participated in the DOMInO trial: does fish oil supplementation in pregnancy influence child development at 4 years?

CASE REPORT FORM

Study ID: _____ ___ ___ ___ ___

Coordinating Centre
Child Nutrition Research Centre
Women's and Children's Health Research Institute
Women's and Children's Hospital
72 King William Road
North Adelaide, South Australia 5006
Phone: (08) 8161 8045, Facsimile (08) 8161 8228

DMAC MANAGEMENT INFORMATION SYSTEM: http://www.dmac.adelaide.edu.au/choir

ENROLMENT CENTRE INFORMATION:

☐ **WCH – Women's & Children's Hospital**

☐ **FMC – Flinders Medical Centre**

Investigator Statement:
I confirm that the data recorded in this Case Report Form accurately and completely represent the results of the examinations, tests, and evaluations performed on the dates specified.

_____
Investigator Signature

_____
Investigator Name (please print)

__ __ / __ __ / __ __ __ __

TABLE OF CONTENTS

SECTION A: 4 YEAR FOLLOW-UP FAMILY INFORMATION

A1 Family Structure - Who does the child live with? *(cross one only)*

- ❑ Intact family (Natural parents living together)
- ❑ Separated Parents (Divided Care)
- ❑ Sole Parent - Mother
- ❑ Sole Parent - Father
- ❑ Fostered
- ❑ Adopted
- ❑ Other, *specify* _____

A2 Who is the primary carer of the child? *(cross one only)*

- ❑ Mother
- ❑ Father
- ❑ Other, *specify* _____

A3 How many days per fortnight is the child in the care of the primary carer?

❑ Full-time or __ __ days per fortnight

A4 What is the primary carer's date of birth?

__ __ / __ __ / __ __ __ __ *(dd/mm/yyyy)*

A5 Did the primary carer complete secondary school?

- ❑ Yes
- ❑ No
- ❑ Unknown

A6 Has the primary carer completed any further study?

- ❑ Yes
- ❑ No (*go to Question A7)*
- ❑ Unknown (*go to Question A7)*

SECTION A: 4 YEAR FOLLOW-UP FAMILY INFORMATION

A6.1 What is the highest qualification that the primary carer has completed?
(cross one only)

- ❑ Certificate/Diploma
- ❑ Degree
- ❑ Higher Degree
- ❑ Other, *specify* _____
- ❑ Unknown

A7 How many years has the primary carer spent in full time education from Year 1?
(including primary and secondary school and any further study, even if not completed)

__ __ years or ❑ Unknown

A8 Has the primary carer been in the workforce at any time in the last 12 months?

- ❑ Yes (Go to Question A8.1)
- ❑ No (Go to Question A8.4)

A8.1 What is the primary carer's usual or regular occupation?

_____

A8.2 List main tasks

_____

_____

A8.3 Is the primary carer currently employed?

- ❑ Yes *(go to A10)*
- ❑ No *(go to A10)*

A8.4 What has been the *main* activity of the primary carer during this time?
(*cross one only*)

- ❑ Actively seeking work
- ❑ Home duties (and *not* actively seeking work)
- ❑ Student (and *not* actively seeking work)
- ❑ Disability Pension
- ❑ Carer Pension
- ❑ Other, specify_____

A9 *Not required at this assessment*

For DMAC Office Use Only

For data entry ANZSCO Code _____

SECTION A: 4 YEAR FOLLOW-UP FAMILY INFORMATION

A10 Who is the secondary carer of the child?

- ❑ Mother
- ❑ Father
- ❑ Other, *specify* _____
- ❑ Not applicable *(go to Question B1)*

A11 How many days per fortnight is the child in the care of the secondary carer?

❑ Full-time or __ __ days per fortnight

A12 What is the secondary carer's date of birth?

__ __ / __ __ / __ __ __ __ *(dd/mm/yyyy)*

A13 Did the secondary carer complete secondary school?

- ❑ Yes
- ❑ No
- ❑ Unknown

A14 Has the secondary carer completed any further study?

- ❑ Yes
- ❑ No *(go to Question A15)*
- ❑ Unknown *(go to Question A15)*

A14.1 What is the highest qualification that the secondary carer has completed?
(cross one only)

- ❑ Certificate/Diploma
- ❑ Degree
- ❑ Higher Degree
- ❑ Other, *specify* _____
- ❑ Unknown

SECTION A: 4 YEAR FOLLOW-UP FAMILY INFORMATION

A15 How many years has the secondary carer spent in full time education since Year 1?
(including primary and secondary school and any further study, even if not completed)

__ __.__ years or ❑ Unknown

A16 Has the secondary carer been in the workforce at any time in the last 12 months?

❑ Yes (Go to Question A16.1)
❑ No (Go to Question A16.4)

A16.1 What is the secondary carer's usual or regular occupation?

_____

A16.2 List main tasks

_____

_____

A16.3 Is the secondary carer currently employed?

❑ Yes (go to section B)
❑ No (go to section B)

A16.4 What has been the *main* activity of the secondary carer during this time?
(cross one only)

❑ Actively seeking work
❑ Home duties (and *not* actively seeking work)
❑ Student (and *not* actively seeking work)
❑ Disability Pension
❑ Carer Pension
❑ Other, specify_____

A17 *Not required at this assessment*

For DMAC Office Use Only
ANZSCO Code _____

SECTION B: 4 YEAR FOLLOW-UP ENVIRONMENT

B1 **How many adults (≥ 16 years) live in the home of the primary carer?**

__ __

B2 **How many children (< 16 years) other than the study child live in the home of the primary carer?**

__ __

B3 **What is the primary language spoken in the home of the primary carer?**

- ❑ English
- ❑ Other, *specify*_____

B4 **Does anyone living in the home of the primary carer smoke cigarettes?**

- ❑ Yes
- ❑ No *(go to Question B5)*
- ❑ Unknown *(go to Question B5)*

B4.1 If yes, specify *(tick all that apply)*

- ❑ Primary Carer
- ❑ 1 person (other than primary carer)
- ❑ 2 people (other than primary carer)
- ❑ 3 or more people (other than primary carer)

B5 **Does the child attend child care/child minding (not pre-school) outside the home at least once per week?**

- ❑ Yes
- ❑ No
- ❑ Unknown

B6 **Does the child attend a structured Kindergarten/Pre-School facility (not day-care) at least once per week?**

- ❑ Yes
- ❑ No
- ❑ Unknown

SECTION C: 4 YEAR FOLLOW-UP PARENTAL HEALTH DATA

C1 *Not required at this assessment.*

C2 Since we last saw you, has the biological mother been medically diagnosed with any of the following conditions?

	Condition	YES	NO	Unknown
C2.1	Type 1 diabetes	❑	❑	❑
C2.2	Type 2 diabetes	❑	❑	❑
C2.3	High blood pressure	❑	❑	❑
C2.4	Angina	❑	❑	❑
C2.5	Asthma	❑	❑	❑
C2.6	Sleep apnoea	❑	❑	❑
C2.7	Peripheral vascular disease	❑	❑	❑
C2.8	Depression	❑	❑	❑
C2.9	Other chronic health condition(s) If yes, *specify*_____	❑	❑	❑
C2.10	Have there been any maternal SAEs (ICU admission or death)? If Yes, complete SAE form for each SAE	❑	❑	❑

SECTION D: 4 YEAR FOLLOW-UP CHILD HEALTH DATA

D1 **Since we last saw you, has the child been diagnosed by a doctor with any of the following conditions?**

	Condition	YES	NO	Unknown
D1.1	Type 1 diabetes	❑	❑	❑
D1.2	Type 2 diabetes	❑	❑	❑
D1.3	Renal disease	❑	❑	❑
D1.4	Asthma	❑	❑	❑
D1.5	Eczema	❑	❑	❑
D1.6	Attention Deficit Hyperactivity Disorder (ADHD)	❑	❑	❑
D1.7	Autism spectrum disorder (including Asperger's Syndrome)	❑	❑	❑
D1.8	Other learning/behavioral disorder If yes, *specify*_____	❑	❑	❑
D1.9	Other chronic health condition(s) **If yes, *specify***_____ **If no or unknown**, *go to Question D2*	❑	❑	❑

D1.9.1 Is an SAE form required (major congenital condition)?

❑ Yes
❑ No (Go to Question D2)

If Yes, complete SAE form for each SAE

SECTION D: 4 YEAR FOLLOW-UP CHILD HEALTH DATA

D2 Since we last saw you, has the child had a hospital admission >24 hours duration?

❑ Yes
❑ No *(go to Question E1)*

D2.1 If yes, please complete table, listing each admission >24 hours:

Event	Primary Reason	Secondary Reason
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

(If more than 10 hospital admissions, download extra form from MIS)

SECTION D: 4 YEAR FOLLOW-UP CHILD HEALTH DATA

D2.2 Were any of these admissions to an Intensive Care Unit?

- ❑ Yes
- ❑ No *(go to Question E1)*
- ❑ Unknown *(go to Question E1)*

If Yes, complete a SAE form for each ICU admission

SECTION E: 4 YEAR FOLLOW-UP PHYSICAL ASSESSMENT

E1 HEIGHT

E1.1 Who took this measurement?

- ❑ CNRC Staff, *please state name* _____
- ❑ Other Health Professional
- ❑ Parent/Carer at a CNRC clinic
- ❑ Parent/Carer at another location
- ❑ Other, *specify* _____
- ❑ Unknown

E1.2	Date measured	__ __/__ __/__ __ __ __
E1.3	1st measure	__ __ __ . __ cm
E1.4	2nd measure	__ __ __ . __ cm ❑ Not Done
If difference between 1st and 2nd measure is ≥0.5cm, then perform 3rd measurement		
E1.5	3rd measure	__ __ __ . __ cm ❑ Not Done

SECTION E: 4 YEAR FOLLOW-UP PHYSICAL ASSESSMENT

E2 WEIGHT

E2.1 Who took this measurement?

- ❑ CNRC Staff, *please state name*_____
- ❑ Other Health Professional
- ❑ Parent/Carer at a CNRC clinic
- ❑ Parent/Carer at another location
- ❑ Other, *specify*_____
- ❑ Unknown

E2.2	Date measured	__ __/__ __/__ __ __ __
E2.3	1st measure	__ __ __ . __ cm
E2.4	2nd measure	__ __ __ . __ cm ❑ Not Done
If difference between 1st and 2nd measure is ≥100grams, then perform 3rd measurement		
E2.5	3rd measure	__ __ __ . __ cm ❑ Not Done

SECTION E: 4 YEAR FOLLOW-UP PHYSICAL ASSESSMENT

E3 HEAD CIRCUMFERENCE

E3.1 Who took this measurement?

- ❑ CNRC Staff, *please state name* _____
- ❑ Other Health Professional
- ❑ Parent/Carer at a CNRC clinic
- ❑ Parent/Carer at another location
- ❑ Other, *specify* _____
- ❑ Unknown

E3.2	Date measured	__ __/__ __/__ __ __ __
E3.3	1st measure	__ __ __ . __ cm
E3.4	2nd measure	__ __ __ . __ cm ❑ Not Done
If difference between 1st and 2nd measure is >0.5cm, then perform 3rd measurement		
E3.5	3rd measure	__ __ __ . __ cm ❑ Not Done

E4 ***Not required at this assessment.***

E5 ***Not required at this assessment.***

E6 ***Not required at this assessment.***

SECTION F: 4 YEAR FOLLOW-UP DIETARY INFORMATION

F1 Diet – Fish Meals

How many fish meals (60 to 80 grams of fish, equivalent to one small can of tuna or 4 fish fingers) did the child consume within the last month?

___ ___

- ❑ No fish
- ❑ Unknown

F2 Diet – DHA Enriched Foods

How many DHA enriched foods did the child consume within the last month?
(Refer to list of fish oil enriched foods)

___ ___

- ❑ No DHA enriched foods
- ❑ Unknown

F3 Dietary Supplements

Does the child take any dietary (vitamin/mineral) supplements?

- ❑ Yes
- ❑ No (*Go to Question G1*)

F3.1 If Yes, Specify_____

F3.2 Please specify frequency

- ❑ Less than once per week
- ❑ Once per week
- ❑ About 3 times per week
- ❑ Everyday
- ❑ Unknown

F3.3 Is this dietary supplement DHA enriched?
(Refer to Supplement List)

- ❑ Yes
- ❑ No

SECTION G: 4 YEAR FOLLOW-UP PSYCHOLOGICAL ASSESSMENT

G1 Assessment Date __ __/__ __/__ __ __ __
dd mm yyyy

G2 Age of Child at Time of Assessment years ___ months __ __

G3 Was the DASII assessment completed?

- ❑ Yes
- ❑ No (*Go to Question G4*)

G3.1 Who administered the DASII assessment?

_____

G3.2 Where was the DASII assessment completed?

- ❑ Study Clinic
- ❑ Other Clinic
- ❑ Child Care Centre
- ❑ Home
- ❑ Other, *specify*_____

G4 Was the Day-Night Stroop assessment completed?

- ❑ Yes
- ❑ No (*Go to Question G5*)

G4.1 Who administered the Day-Night Stroop assessment?

_____

G4.2 Where was the Day-Night Stroop assessment completed?

- ❑ Study Clinic
- ❑ Other Clinic _____
- ❑ Child Care Centre
- ❑ Home
- ❑ Other, *specify*_____

SECTION G: 4 YEAR FOLLOW-UP PSYCHOLOGICAL ASSESSMENT

G5 Was the CELF-P2 assessment completed?

❑ Yes
❑ No (*Go to Question G6*)

G5.1 Who administered the CELF-P2 assessment?

_____

G5.2 Where was the CELF-P2 assessment completed?

❑ Study Clinic
❑ Other Clinic _____
❑ Child Care Centre
❑ Home
❑ Other, *specify*_____

G6 Was the Parent/Carer present during the assessment?

❑ Yes
❑ No

SECTION H: 4 YEAR FOLLOW-UP PARENT QUESTIONNAIRES

H1 Have the Parent Questionnaires been completed?

	Parent Questionnaire	YES	NO
H1.1	BRIEF-P	❑	❑
H1.2	SDQ	❑	❑
H1.3	RLE	❑	❑
H1.4	FAD	❑	❑
H1.5	HSQ	❑	❑

4 YEAR Section completed by: Name (please print): Signature:	**Date**	**Checked by:** Name (please print): Initials:	**Date**

APPENDIX 9: ‘QUESTIONNAIRE PACK’ AND BRIEF-P

DOMInO Trial Randomisation Number: __ __ __ __ __ __

Version2_01/07/2011 Date: __ __ / __ __ / __ __ __ __

Questionnaire Completed by *(Please circle response)*: Mother / Father / Other:.....

How questionnaires were completed *(Please circle response)*: on paper / by phone call / by email / Other, specify.....

Strengths and Difficulties Questionnaire

For each item, please mark the box for Not True, Somewhat True or Certainly True. It would help us if you answered all items as best you can even if you are not absolutely certain. Please give your answers on the basis of your child's behaviour **over the last six months**.

	Not True	Somewhat True	Certainly True
Considerate of other people's feelings	☐	☐	☐
Restless, overactive, cannot stay still for long	☐	☐	☐
Often complains of headaches, stomach-aches or sickness	☐	☐	☐
Shares readily with other children, for example toys, treats, pencils	☐	☐	☐
Often loses temper	☐	☐	☐
Rather solitary, prefers to play alone	☐	☐	☐
Generally well behaved, usually does what adults request	☐	☐	☐
Many worries or often seems worried	☐	☐	☐
Helpful if someone is hurt, upset or feeling ill	☐	☐	☐
Constantly fidgeting or squirming	☐	☐	☐
Has at least one good friend	☐	☐	☐
Often fights with other children or bullies them	☐	☐	☐
Often unhappy, depressed or tearful	☐	☐	☐
Generally liked by other children	☐	☐	☐
Easily distracted, concentration wanders	☐	☐	☐
Nervous or clingy in new situations, easily loses confidence	☐	☐	☐
Kind to younger children	☐	☐	☐
Often lies or cheats	☐	☐	☐
Picked on or bullied by other children	☐	☐	☐
Often volunteers to help others (parents, teachers, other children)	☐	☐	☐
Think things out before acting	☐	☐	☐
Steals from home, school or elsewhere	☐	☐	☐
Gets along better with adults than with other children	☐	☐	☐
Many fears, easily scared	☐	☐	☐
Good attention span, sees chores or homework through to the end	☐	☐	☐

Do you have any other comments or concerns? Yes / No
If yes, please specify in the space below:

_____

_____

_____

_____

Please turn over

Overall, do you think that your child has difficulties in one or more of the following areas: emotions, concentration, behaviour or being able to get along with other people?

No	Yes - minor difficulties	Yes - definite difficulties	Yes - severe difficulties
☐	☐	☐	☐

If you have answered "Yes", please answer the following questions about these difficulties:

• How long have these difficulties been present?

Less than a month	1-5 months	6-12 months	Over a year
☐	☐	☐	☐

• Do the difficulties upset or distress your child?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

• Do the difficulties interfere with your child's everyday life in the following areas?

	Not at all	Only a little	Quite a lot	A great deal
HOME LIFE	☐	☐	☐	☐
FRIENDSHIPS	☐	☐	☐	☐
CLASSROOM LEARNING	☐	☐	☐	☐
LEISURE ACTIVITIES	☐	☐	☐	☐

• Do the difficulties put a burden on you or the family as a whole?

Not at all	Only a little	Quite a lot	A great deal
☐	☐	☐	☐

Recent Life Events

Listed below are a number of events. Please read each item carefully and then indicate whether or not each event has happened to you in the past year.
Please tick the **NO** box if the event has not occurred.
Please tick the **YES** box if the event has occurred.
Please tick the **Still affects me** box if the event is still having an effect on your life.

EVENT	No	Yes	Still affects me
Have you had a serious illness or been seriously injured?	☐	☐	☐
Has one of your immediate family* been seriously ill or injured? * immediate family includes mother, father, sister, brother, partner, child.	☐	☐	☐
Have any of your close friends or other close relatives been seriously ill or injured?	☐	☐	☐
Has any of your immediate family died?	☐	☐	☐
Have any of your other close relatives or friends died?	☐	☐	☐
Have you separated from your partner (not including death)?	☐	☐	☐
Have you had any serious problem with a close friend, neighbour or relative?	☐	☐	☐
Have you, or an immediate family member been subject to serious racial abuse, attack or threats?	☐	☐	☐
Have you, or an immediate family member been subject to any abuse, attack or threat, perhaps due to you or someone close to you having a disability of any kind (i.e. a mental health problem, a learning disability or a physical problem)?	☐	☐	☐
Have you, or an immediate family member been subject to any other form of serious abuse, attack or threat?	☐	☐	☐
Have you or your partner been unemployed or seeking work for more than one month?	☐	☐	☐
Have you or your partner been sacked from your job or made redundant?	☐	☐	☐
Have you had any major financial difficulties (e.g. debts, difficulty paying bills)?	☐	☐	☐
Have you, or an immediate family member had any Police contact or been in a court appearance?	☐	☐	☐
Have you or an immediate member of your family been burgled or mugged?	☐	☐	☐
Have you or another individual who lives with you given birth?	☐	☐	☐
Have you or another individual who lives with you suffered from a miscarriage or had a stillbirth?	☐	☐	☐
Have you moved house (through choice)?	☐	☐	☐
Have you moved house (not through choice)?	☐	☐	☐
Have you had any housing difficulties?	☐	☐	☐
Have you had any other significant event? If yes, please specify _____	☐	☐	☐

Please turn over

Family Assessment Device: General Functioning

This questionnaire contains a number of statements about families. Please read each statement carefully and decide how well it describes your own family.
Try not to spend too much time thinking about each statement, but respond as quickly and as honestly as you can. If you have trouble with one, answer with your first reaction. Please be sure to answer every statement.

1 = Strongly agree 2 = Agree 3 = Disagree 4 = Strongly disagree

	1	2	3	4
1. Planning family activities is difficult because we misunderstand each other.	☐	☐	☐	☐
2. In times of crisis we can turn to each other for support.	☐	☐	☐	☐
3. We cannot talk to each other about the sadness we feel	☐	☐	☐	☐
4. Individuals are accepted for what they are.	☐	☐	☐	☐
5. We avoid discussing our fears and concerns	☐	☐	☐	☐
6. We can express feelings to each other.	☐	☐	☐	☐
7. There are lots of bad feelings in this family.	☐	☐	☐	☐
8. We feel accepted for what we are.	☐	☐	☐	☐
9. Making decisions is a problem for our family.	☐	☐	☐	☐
10. We are able to make decisions about how to solve problems.	☐	☐	☐	☐
11. We don't get along well together.	☐	☐	☐	☐
12. We confide in each other.	☐	☐	☐	☐

Home Environment – Version 2

Please answer all of the following questions about how your child's time is spent and some of the activities of your family. On some questions, you may want to tick more than one square.

1. Do you get any magazines in the mail?
   YES ☐ NO ☐
   If YES, what kind?
   ☐ home and family magazines
   ☐ news magazines
   ☐ children's magazines
   ☐ other

2. Does your child have a toy box or other special place where he/she keeps his/her toys?
   YES ☐ NO ☐

3. How many children's books does your family own?
   ☐ 0 to 2
   ☐ 3 to 9
   ☐ 10 or more

4. How many books do you have besides children's books?
   ☐ 0 to 9
   ☐ 10 to 20
   ☐ more than 20
   And, where do you keep them?
   ☐ in boxes (packed)
   ☐ on a bookcase
   ☐ other - explain _____

5. How often does someone take your child into a supermarket?
   ☐ hardly ever, I prefer to go alone
   ☐ at least once a month
   ☐ at least twice a month
   ☐ at least once a week

6. About how many times in the past week did you have to spank your child? _____

7. Do you have a TV?
   YES ☐ NO ☐
   If YES, about how many hours is the TV/DVD/Video on each day? _____

8. How often does someone get a chance to read stories to your child?
   ☐ hardly ever
   ☐ at least once a week
   ☐ at least 3 times a week
   ☐ at least 5 times a week

9. Do you ever sing to your child or sing when he/she is nearby?
   YES ☐ NO ☐

10. Does your child put away his/her toys by himself/herself most of the time?
    YES ☐ NO ☐

11. Is your child allowed to walk or ride his/her tricycle by himself/herself to the house of a friend or relative?
    YES ☐ NO ☐

12. What do you do with your child's art work?
    ☐ let him/her keep it
    ☐ put it away
    ☐ hang it somewhere in the house

13. In the space below write what you might say if your child said, "Look at that big truck."
    _____
    _____

14. What do you usually do when a friend is visiting you in your home and your child has nothing to do?
    ☐ suggest something for him/her to do
    ☐ offer him/her a toy
    ☐ give him/her a biscuit or something to eat
    ☐ put him/her to bed for a nap
    ☐ play with him/her

15. How often does your child eat a meal at the table with both mother and father (or other adult male)?
    ☐ never
    ☐ at least once a month
    ☐ at least once a week
    ☐ at least twice a week
    ☐ at least 3 or 4 times a week
    ☐ at least once a day

16. How often does your child spend time playing or "working" with his/her father (or other adult male)?
    ☐ at least 4 times a week
    ☐ at least twice a week
    ☐ at least once a week
    ☐ at least once a month
    ☐ never

Please turn over

17. How often does someone get a chance to take your child out of the house for an outing (shopping, park, zoo, restaurant, museum, car trip, library, etc.)?
- ☐ at least 6 times a year
- ☐ at least once a month
- ☐ at least twice a month
- ☐ at least once a week

18. Tick the things which you (or other adult or older child) are helping or have helped your child to learn:
- ☐ colours (like naming colours of things)
- ☐ alphabet
- ☐ numbers
- ☐ understanding of time (like morning-afternoon and now-later)
- ☐ shapes (like drawing circles or squares)
- ☐ reading new words or writing her/his name

19. Has your child learned any songs, prayers, or nursery rhymes?
YES ☐ NO ☐
If "yes", where did he/she learn them?
- ☐ at day care or preschool
- ☐ from a sister or brother
- ☐ at church or Sunday school
- ☐ from mother or father
- ☐ from television

20. It is 30 minutes before dinner and your child is hungry. Most of the time you would:
- ☐ give him/her a snack
- ☐ have him/her wait for dinner

21. Which items do you sometimes let your child choose for himself/herself?
- ☐ part of what to have for breakfast or lunch
- ☐ favourite food in the supermarket (fruit, cereal, biscuits, etc.)
- ☐ the clothes he/she wants to put on
- ☐ none of the above

22. What would you do if your child got angry and hit you?
- ☐ hit him/her to show him/her it hurts
- ☐ send him/her to him/her room
- ☐ spank him/her
- ☐ talk to him/her
- ☐ ignore it

23. Do you have any pets?
YES ☐ NO ☐

24. Do you have any live plants in your house?
YES ☐ NO ☐

25. Which of the following best describes your neighbourhood:
- ☐ it is not as clean as I would like it
- ☐ the houses are not well cared for
- ☐ it is well cared for
- ☐ it is well cared for and attractive

26. How many bedrooms does your house have?
_____
How many people are living in your house?
_____

27. Do you occasionally try new recipes that you find in the newspaper or in magazines?
YES ☐ NO ☐

28. Is anyone in the family presently taking a class at a university or TAFE college?
YES ☐ NO ☐

29. Who does the supermarket shopping for your family?

	Sometimes	Often
Mother	☐	☐
Father	☐	☐
Grandparent	☐	☐
Older child	☐	☐
Other	☐	☐

30. Most of the decisions about how the family income is to be spent are made by:
- ☐ Mother
- ☐ Father
- ☐ Grandparent
- ☐ Friend

31. How often do you and your child get a chance to play together (like pretend games, dolls, house, cars and trucks, or table games)?
- ☐ hardly ever, too young
- ☐ at least once a week
- ☐ at least 3-4 times a week
- ☐ everyday

32. Do you have any friends or relatives with children about the same age as your child?
YES ☐ NO ☐

33. When your child asks if he/she can do something you think he/she is too young to do, would you be more likely to say
- ☐ no, I don't want you to
- ☐ no
- ☐ not now
- ☐ no – you're too young now, but when you're older you will be able to do it.

34. What would happen if your child spilled his/her milk?
- ☐ he/she would be spanked
- ☐ he/she would have to clean it up
- ☐ someone else would have to clean it up
- ☐ he/she would be sent to his/her ro

Please turn over

We are interested in finding out what kinds of toys children have in their homes. The items listed below are for children of different ages.

Please tick any of the following that you have in your home and that your child is allowed to play with. Do not tick the ones that you do not have now or ones that are broken.

We do not expect a child to have all of these things.

1. ☐ Dolls with clothes or paper dolls
2. ☐ Stuffed animals, animal toys or animal books
3. ☐ Dress-up clothes or costumes
4. ☐ Tricycle, bicycle or scooter
5. ☐ Stroller or walker
6. ☐ Pull-cart
7. ☐ Child-size car
8. ☐ Pull or push toy
9. ☐ Hanging mobile
10. ☐ Child-size furniture
11. ☐ High chair
12. ☐ Playpen
13. ☐ Puzzles – at least three
14. ☐ Alphabet toy, alphabet game or alphabet book
15. ☐ Number toy, number game or number book
16. ☐ Colouring book
17. ☐ Dot-to-dot or colour-by-number book
18. ☐ Scissors
19. ☐ Pegboard
20. ☐ Toy telephone or toy mobile phone
21. ☐ Plastic snap together beads
22. ☐ Musical box or music toy
23. ☐ Children's books
24. ☐ Ball
25. ☐ Shape ball or box
26. ☐ Kindy gym
27. ☐ Bouncinette or door swing
28. ☐ Squeeze toys
29. ☐ Rattles
30. ☐ TV
31. ☐ Busy Box
32. ☐ Gun
33. ☐ Clay or play dough
34. ☐ Real or toy musical instruments
35. ☐ Sand box
36. ☐ Homemade building toys
37. ☐ Blocks
38. ☐ Lego or building blocks
39. ☐ Record player/CD player/cassette player
40. ☐ Children's records/CD's/cassettes
41. ☐ Blackboard
42. ☐ Swings
43. ☐ Jungle gym
44. ☐ Car, truck or train
45. ☐ Measuring cups
46. ☐ Pots and pans
47. ☐ Toy dishes
48. ☐ Doll carriage
49. ☐ Plastic tools and workbench
50. ☐ Crayons, paints or pencils

Thank you for your help ☺

Gioia, G.A., Espy. K.A. & Isquith, P.K. (1996). *Brief-P Behavior Rating Inventory of Executive Function Preschool*. Florida, U.S.A: Psychological Assessment Resources.

APPENDIX 10: STANDARD OPERATING PROCEDURES - HEAD

Title: Head Circumference Measurement

Document ID: CNRC_006

Version: Version 2

Author: Karen Best

Author Signature: _____ Date:

Effective Date: 12th March 2009

Review Before: 12th March 2011

Department/institution name: *Child Nutrition Research Centre*

Reviewed and Approved by:

Signature: _____ Date:

Departmental Head*: Maria Makrides*

Signature: _____ Date:

1. INTRODUCTION AND PURPOSE

The objective of this SOP is to detail the procedures required when performing head circumference measurements on study participants involved in clinical trials within the Child Nutrition Research Centre (CNRC) or in collaboration with the CNRC.

2. SCOPE/ APPLICABILITY

This SOP applies to all clinical research staff within the CNRC who are expected to perform or assist with anthropometric measurements. The anthropometry procedures outlined are a guide for measurements performed in relation to CNRC clinical trials, there may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

3. PROCEDURE

3.1 Preparation

- Use a single use paper tape marked in centimetres to measure head circumference.
- Inform the mother and or participant as to what measurement is being taken.
- Braids or hair ornaments should be removed if they hinder they interfere with positioning the head measurement.
- Position for measurement will depend upon the age the child. Head circumference may be measured while the child is lying down, in their mother's arms or standing for an older child.

3.2 Head Circumference measurement

- Place the tape around the child head across the frontal bones just above the eyebrows. The tape should lie above the ears on each side, and over the occipital prominence at the back of the head.
- Hold the tape snugly around the head and moved it up and down over the back of the head to locate the maximal circumference of the head. The tape should be perpendicular to the long axis of the face and should be pulled gently to compress the hair and underlying soft tissues.

3. PROCEDURE cont.

- Record the measurement to the nearest 0.1 cm.
- Remove the tape and reposition to obtain a second measurement.
- Record both measurements.
- Measurement differences greater than 5mm should be repeated a third time. The two measures in closest agreement are recorded.

N.B. Number of measurements required may differ depending upon your trial protocol.

4. CARE FOR MEASUREMENT EQUIPMENT

- Dispose of paper tape measure.

5. REFERENCES

[1] WHO MGRS protocol de Onis and Food and Nutrition Bulletin, Vol 25, no 1, page 5

6. APPENDICES

Appendix 1: SOP Change Log

END OF DOCUMENT

APPENDIX 1 : SOP CHANGE LOG

Version No.	*Reason for Issue*
1	First Version
2	Update of SOP format.

APPENDIX 11: STANDARD OPERATING PROCEDURES - HEIGHT

Title: Height Measurement

Document ID: CNRC_015

Version: Version 2

Author: Karen Best

Author Signature: _____ Date:

Effective Date: 11th March 2009

Review Before: 11th March 2011

Department/institution name: *Child Nutrition Research Centre*

Reviewed and Approved by:

Signature: _____ Date:

Departmental Head*: Maria Makrides*

Signature: _____ Date:

1. INTRODUCTION AND PURPOSE

The objective of this SOP is to detail the procedures required when performing height measurements on study participants involved in clinical trials within the Child Nutrition Research Centre (CNRC) or in collaboration with the CNRC. Standing height is performed on participants >2years of age.

The anthropometry procedures outlined in this SOP are based on the WHO Multi-centre Reference Growth Study and are a guide for measurements performed within the CNRC. There may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

2. SCOPE/ APPLICABILITY

This SOP applies to all clinical research staff within the CNRC who are expected to perform or assist with anthropometric measurements. The anthropometry procedures outlined are a guide for measurements performed in relation to CNRC clinical trials, there may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

3. PROCEDURE

3.1 Preparation

- Assemble equipment required;
    - Standing height will be measured with a potable or fixed stadiometer.
- Inform the participant as to what measurement is being taken and request that they present themselves without shoes and heavy outer clothing.
- Remove any hair ornaments including pony tails.

3. PROCEDURE

3.2 Height Measurement

- Working with the mother, and kneeling in order to get down to the level of the child. Help the child to stand on the baseboard or floor with feet slightly apart.

- The back of the head, shoulder blades, buttocks, calves, and heels should all touch the vertical board (or wall) with arms hanging freely against the trunk and palms facing the thighs. (Refer figure 5.1)

- Ask the mother to hold the child's knees and ankles to help keep the legs straight and feet flat, with heels and calves touching the vertical board. Ask her to focus the child's attention, soothe the child as needed, and inform you if the child moves out of position.

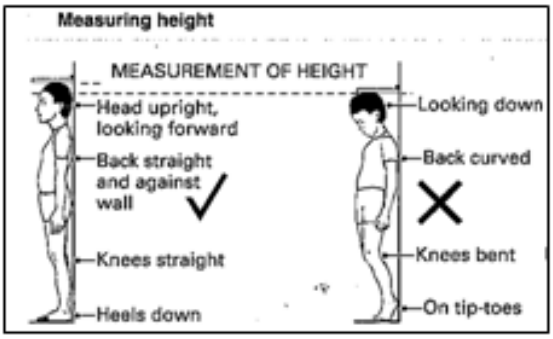
- Position the child's head so that a horizontal line from the ear canal to the lower border of the eye socket runs parallel to the base board or floor. To keep the head in this position, gently cup the child's chin with your hand.

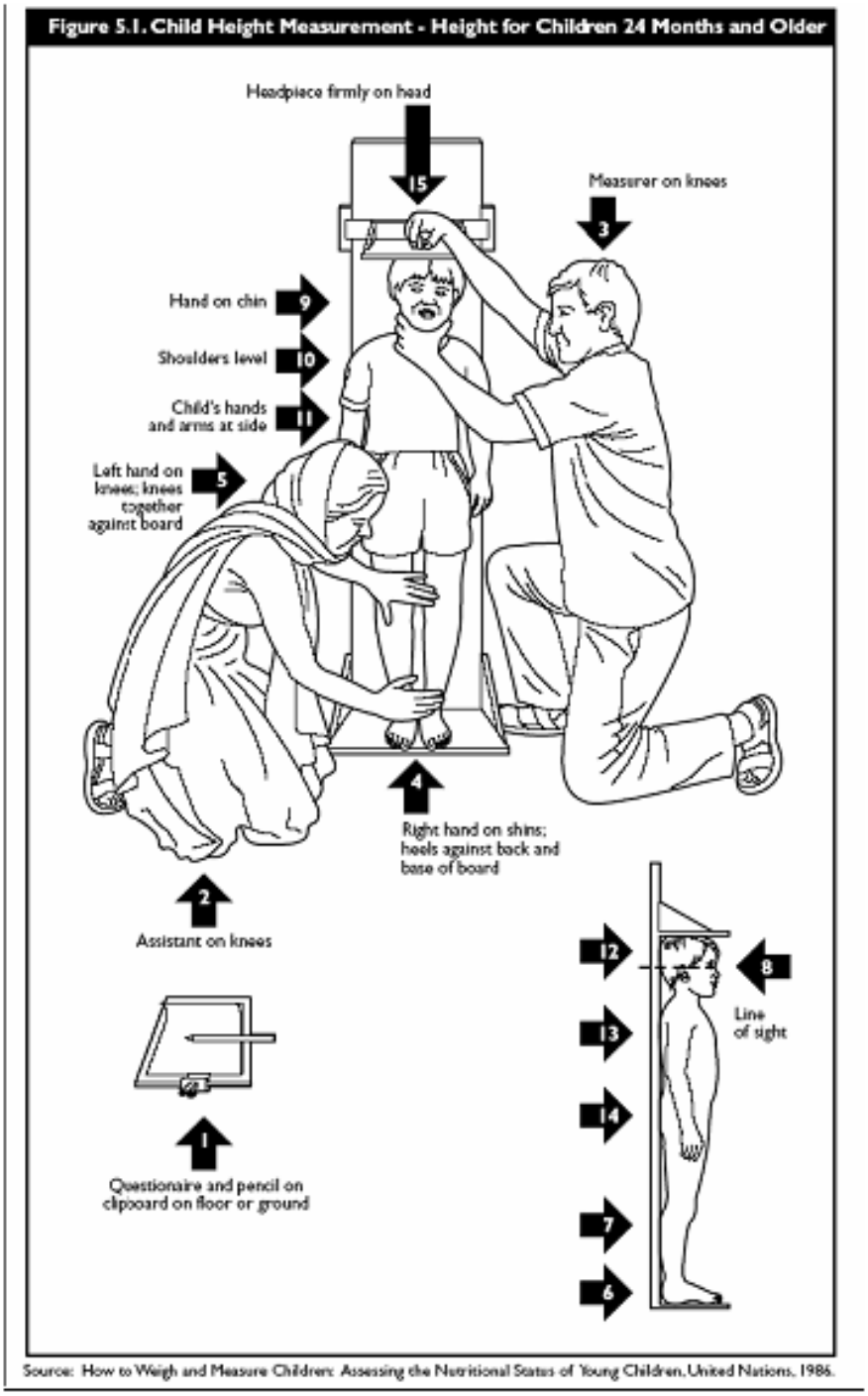
- If necessary, place gentle pressure on the child's abdomen to help the child stand to full height.

- Still keeping the head in position use your other hand to pull down the headboard to rest firmly on top of the head and compress the hair.

- Read the measurement just before the child exhales and record the child's height in centimetres to the last completed 0.1 cm.

- If more than on measurement is required, have the child step completely OFF the stadiometer and reposition EACH TIME.





(Figure adapted from WHO MGRS protocol de Onis and Food and Nutrition Bulletin*)*

4. CARE FOR MEASUREMENT EQUIPMENT

Proper care of the stadiometer is important to ensure that measurements are as accurate as possible. The equipment must be kept clean and stored at normal indoor temperature, protected from humidity and wetness.

5. REFERENCES

[i] WHO MGRS protocol de Onis and Food and Nutrition Bulletin, Vol 25, no 1, page 5

6. APPENDICES

Appendix 1: SOP Change Log

END OF DOCUMENT

APPENDIX 1 : SOP CHANGE LOG

Version No.	*Reason for Issue*
2	Separate SOP for Height. Originally was combined with Length SOP.

APPENDIX 12: STANDARD OPERATING PROCEDURES – WEIGHT

Title: Weight Measurement

Document ID: CNRC_004

Version: Version 2

Author: Karen Best

Author Signature: _____ Date:

Effective Date: 13th March 2009

Review Before: 13th March 2011

Department/institution name: *Child Nutrition Research Centre*

Reviewed and Approved by:

Signature: _____ Date:

Departmental Head*: Maria Makrides*

Signature: _____ Date:

1. INTRODUCTION AND PURPOSE

The objective of this SOP is to detail the procedures required when performing weight measurements on study participants involved in clinical trials within the Child Nutrition Research Centre (CNRC) or in collaboration with the CNRC.

The anthropometry procedures outlined in this SOP are based on the WHO Multi-centre Reference Growth Study and are a guide for measurements performed within the CNRC. There may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

2. SCOPE/ APPLICABILITY

This SOP applies to all clinical research staff within the CNRC who are expected to perform or assist with anthropometric measurements. The anthropometry procedures outlined are a guide for measurements performed in relation to CNRC clinical trials, there may be study specific requirements for individual clinical trials which require additional procedures, please refer to your trial protocol.

3. PROCEDURE

3.1 Preparation

- Body weight needs to be measured with a direct reading electronic balance. The balances should be accurate to at least the nearest 5 grams. The accuracy of each balance needs to be checked at least two or three times yearly. Calibrated or standard weights will be available for this purpose.
- Inform the participant/parent as to what measurement is being taken and request that they present themselves without shoes and heavy outer clothing.
- Weight will be measured in kilograms to the nearest 10g.

Recumbent Electronic Scales

- Place disposable paper sheeting on the scales prior to weighing the child.
- Children under 2 years of age should be weighed naked.
- Make sure the scale is zeroed before the child is placed on the scale.

Floor scales

- Ensure the scales are placed on a hard flat surface and the scale is zeroed before the participant stands on them.

3. PROCEDURES

3.2 Weight - *If the child is less than 2 years old*

- Ask the parent to lie the infant on the infant scales
- Wait until the child has ceased movement, then record the weight.
- Remove the child from the scales and re-zero before performing further measurements.

In Mothers arms

For children unwilling to cooperate with the recumbent electronic scale, the child can be weighed in mother's arms as follows.

- Ask the mother to remove her shoes and any heavy clothing and stand on the zeroed electronic floor scale.
- Make sure her feet are centered in the middle of the scale.
- Mother's weight is recorded and the scale is tared (zeroed with mother still on scale).
- Return the child to the mother's arms while the mother is still standing on the electronic floor scales.

3. PROCEDURES

3.2 Weight - *If the child is 2 years or older*

If the child is 2 years or older and will stand still on the electronic floor scales, weigh the child alone.

- Ask the mother to help the child remove shoes and outer clothing (anything easily removable without causing discomfort).
- Ensure the scale is zeroed before the child steps on the scale.
- Ask the child to stand in the middle of the scale, feet slightly apart (on the footprints if marked) and to remain still until the weight appears on the display.
- The child's hands should be hanging loosely at his/her side.
- Record the child's weight to the nearest 0.1kg.

4. CARE FOR MEASUREMENT EQUIPMENT

Proper care of the scales is important to ensure that measurements are as accurate as possible. The equipment must be kept clean and stored at normal indoor temperature, protected from humidity and wetness.

Cleaning

- Disposable paper towelling should be used to protect the scales whilst weighing and disposed after each use.
- Scales should be wiped with a detergent and water solution at the end of use.
- Any body fluids should be cleaned immediately with a detergent and water solution.

Calibration

- Accuracy of the scales should be checked monthly using calibrating weights and recorded on the equipment checklist.

5. REFERENCES

[1] WHO MGRS protocol de Onis and Food and Nutrition Bulletin, Vol 25, no 1, page 5

6. APPENDICES

Appendix 1: SOP Change Log

END OF DOCUMENT

APPENDIX 1 : SOP CHANGE LOG

Version No.	*Reason for Issue*
2	SOP format updated

APPENDIX 13: ETHICS APPROVAL (WOMEN'S AND CHILDREN'S HOSPITAL)

Government of South Australia
SA Health

Women's & Children's Hospital

Research Secretariat
72 King William Road
North Adelaide SA 5006
Tel 08 8161 6521
Tel 08 8161 6390
Fax 08 8161 8177
www.cywhs.sa.gov.au

25th March 2010

Prof M Makrides
CNRC
CYWHS

Dear Maria

Re: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood? REC2242/12/12

I refer to your letter dated 16th February 2010 and an email from Dr L Smithers dated 22nd March 2010 in response to matters raised by the CYWHS Human Research Ethics Committee at its December 2010 meeting. I am pleased to advise that your protocol has been granted full ethics approval and meets the requirements of the *National Statement on Ethical Conduct in Human Research*.

I note that you have provided signed Confidentiality Agreements and advised that National Police Certificates are held or non-CYWHS staff involved in the study. If in the future, the study involves other non CYWHS staff or students, a signed Confidentiality Agreement will be required and, if they visit any CYWHS site or access identifiable patient information, a National Police Certificate provided to the Ethics Committee and the Human Resources Department. The study may proceed on this proviso.

I remind you approval is given subject to:
- immediate notification of any serious or unexpected adverse events to subjects;
- immediate notification of any unforeseen events that might affect continued ethical acceptability of the project;
- submission of any proposed changes to the original protocol. Changes must be approved by the Committee before they are implemented;
- immediate advice, giving reasons, if the protocol is discontinued before its completion;
- submission of an annual report on the progress of the study, and a final report when it is completed. It is your responsibility to provide these reports – without reminder from the Ethics Committee.

Approval is given for three years only. If the study is more prolonged than this, an extension request should be submitted unless there are significant modifications, in which case a new submission may be required. Please note the approval number above indicates the month and year in which approval expires and it should be used in any future communication.

If University of Adelaide personnel are involved in this project, you, as chief investigator must submit a Human Research Approval notification form online at http://www.adelaide.edu.au/ethics/human/guidelines/ within 14 days of receiving this ethical clearance to ensure compliance with University requirements and appropriate indemnification.

TAMARA ZUTLEVICS (DR)
CHAIR
CYWHS HUMAN RESEARCH ETHICS COMMITTEE

APPENDIX 14: ETHICS APPROVAL (FLINDERS MEDICAL CENTRE)

From: Randhawa, Harry (Health)
Sent: Wednesday, 9 June 2010 11:48
To: 'lisa.smithers@unisa.edu.au'; 'peter.anderson@mcri.edu.au'; 'Robert.gibson@adelaide.edu.au'; Makrides, Maria (Health)
Subject: RE: 129/10 - Final Ethical Approval Granted
Importance: High

Dear Prof Maria Makrides

This is a formal correspondence from the Southern Adelaide Health Service / Flinders University Human Research Ethics Committee. This committee was renamed to reflect the regional nature of the committee and the fact that the committee is jointly hosted by the Flinders University. This committee used to be known as the Flinders Clinical Research Ethics Committee. Whilst this official title of the committee has changed the committee is still properly constituted under AHEC requirements with the registration number EC00188. This committee operates in accordance with the "National Statement on Ethical Conduct in Human Research (2007)." This department only uses email correspondence for all documents unless prior arrangements have been made with the manager. No hard copy correspondence will be issued.

Application Number: 129/10

Title: Does maternal supplementation with n-3 long-chain PUFA in pregnancy influence cognitive development in childhood?

Chief investigator: Prof Maria Makrides

The Issue: The Southern Adelaide Health Service / Flinders University Human Research Ethics Committee (SAFUHREC) have reviewed and approved the above application. Your project may now commence. The approval extends to the following documents received via email dated 25 March 2010:

- Cover letter
- FMC General Ethics Application
- Information Sheet and Consent Form
- Notification of indemnification by the Department of Health representative
- Email support from the Director of the Division of Women's & Babies
- Attachments including questionnaires used in the project
- Study Protocol

Your response to committee concerns received via email dated 19 may 2010 including the following documents were also approved:

- The response to the queries
- The letter to caregivers
- The BRIEF-P questionnaire including guidance notes on it's use
- Recent life events questionnaire
- Strengths and difficulties questionnaire
- Section A: 4 Year Follow-Up Family Information

Approval Period: 9 June 2010 to 9 June 2013

Please retain a copy of this approval for your records.

TERMS AND CONDITIONS OF ETHICAL APPROVAL

Final ethical approval is granted subject to the researcher agreeing to meet the following terms and conditions:

1. Compliance with the *National Statement on Ethical Conduct in Human Research* (2007) & the *Australian Code for the Responsible Conduct of Research* (2007)

2. To immediately report to FCREC anything that may change the ethical or scientific integrity of the project.
3. To regularly review the FCREC website and comply with all submission requirements as they change from time to time.
4. Submit an annual report on each anniversary of the date of final approval and in the correct template from the FCREC website
5. Confidentiality of research participants MUST be maintained at all times.
6. A copy of the signed consent form must be given to the participant unless the project is an audit
7. Any reports or publications derived from the research should be submitted to the Committee at the completion of the project.
8. Report Significant Adverse events (SAE's) as per SAE requirements available at our website.
9. The researchers agree to use electronic format for all correspondence with this department.
10. All requests for access to medical records at any SAHS site must be accompanied by this approval email.

Cheers Harry

Dr Harry Randhawa MB BS, LLB/LP.
Regional Manager - Human Research Ethics Department
Southern Adelaide Health Service
SA Health
Room 2A221 - Inside Human Resources
Flinders Medical Centre, Bedford Park SA 5042

Tel: 08 8204 6453
Fax: 08 8204 4586
Mobile: 0422 687 087
Email: Harry.Randhawa@health.sa.gov.au

Website: http://www.flinders.sa.gov.au/research/pages/ethics

APPENDIX 15: VALIDITY OF CELF P-2 SUBTESTS AND COMPOSITES

TABLE 22. CELF P-2 Australian intercorrelations of norm-referenced subtests and composite scores

	Sentence Structure	**Word Structure**	**Expressive Vocabulary**
Sentence Structure			
Word Structure	0.56		
Expressive Vocabulary	0.58	0.66	
Core Language Score	0.83	0.87	0.88

APPENDIX 16: INTERPRETATION OF SDQ SCORES

TABLE 23. Interpretation of SDQ scores

	Close to average unlikely to be clinically significant	Slightly raised may reflect clinically significant problems	High substantial risk of clinically significant problems
Total difficulties score (/40)	0-13	14-16	17-40
Emotional symptoms score (/10)	0-3	4	5-10
Conduct problems score (/10)	0-2	3	4-10
Hyperactivity score (/10)	0-5	6	7-10
Peer problem score (/10)	0-2	3	4-10
	Close to average not clinically significant	Slightly low may be clinically significant	Low substantial risk of clinically significant problems
Prosocial behaviour score (/10)	6-10	5	0-4
Impact score (/10)	0	1	2-10

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