LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN BREAST MILK AND INFANT FORMULA, MATERNAL PERINATAL MENTAL HEALTH, AND INFANT DEVELOPMENT

Sarah Ann Keim

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2009

Approved by:

Julie Daniels

Anna Maria Siega-Riz

Nancy Dole

Amy Herring

Peter Scheidt

ABSTRACT

SARAH ANN KEIM: Long-chain Polyunsaturated Fatty Acids in Breast Milk and Infant Formula, Maternal Perinatal Mental Health, and Infant Development
(Under the direction of Julie Daniels)

This study examined the associations between maternal perinatal mental health or exposure to long-chain polyunsaturated fatty acids (LCPUFAs) in relation to infant development in the Pregnancy, Infection, and Nutrition Study (2002-2006) (n=358). Certain LCPUFAs have been shown to benefit visual acuity, while maternal mental health may negatively affect child development. Women completed questionnaires during pregnancy to assess trait anxiety and depressive symptoms. A home visit in the fourth postpartum month assessed perceived stress and depressive symptoms, collected infant feeding data, and obtained breast milk samples. Infant development was assessed at 12 months using the Mullen Scales of Early Learning. Multivariable linear regression was used to examine the associations between trait anxiety, perceived stress, and depressive symptoms in relation to Mullen scores. Similar techniques were used to examine LCPUFA exposure in relation to Mullen scores and whether women with elevated depressive symptoms had lower breast milk docosahexaenoic acid (DHA) concentration.

High levels of trait anxiety were associated with lower Receptive Language (adjusted β=-2.9, 95% confidence interval: -5.6, -0.3) and Early Learning Composite (adjusted β =-4.5, CI: -8.9, 0.0) scores. No associations were observed between anxiety and other sub-scale scores or between perceived stress or depressive symptoms and Mullen scores. Mean DHA content of breast milk samples was 0.28% of fatty acids (standard deviation=0.22); mean

arachidonic acid (AA) content 0.57% (SD=0.20). Women with elevated depressive symptoms before 20 weeks gestation had 25% lower breast milk DHA than women with few symptoms. Upon adjustment for preterm birth, smoking, race and ethnicity, and education, no differences in development were observed in relation to breastfeeding exclusivity. No association was observed between the LCPUFA content of breast milk and formula and development.

Maternal anxiety may influence overall infant cognitive development and the ability to process verbal input. Women who experienced elevated depressive symptoms in early pregnancy may have less DHA available to their infants. However, this study found no evidence of enhanced development related to LCPUFAs. Given the conflicting results among previous studies and this study's limitations, no actions are currently warranted to change infant feeding practices except to note that infant LCPUFA supplementation deserves further study.

To Mark and Claire

ACKNOWLEDGEMENTS

The PIN Study, PIN Postpartum and PIN Babies were supported by grants from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (HD37584, HD39373), the National Institute of Diabetes and Digestive and Kidney Diseases (DK61981, DK56350), and the National Institute of Environmental Health Sciences (P30ES10126) of the National Institutes of Health. I thank the Carolina Population Center for their support including Kathryn Carrier for technical assistance and Diane Kaczor for programming support. I thank the women and children in the PIN studies.

I thank my dissertation committee, Julie Daniels, Nancy Dole, Amy Herring, Peter Scheidt, and Anna Maria Siega-Riz, for their encouragement and wisdom throughout. I especially thank Julie Daniels, my chair and advisor, who dedicated considerable time, attention, and guidance throughout this process. I thank her for her patience and willingness to work with me from two states away. A thank you to the NICHD which granted me an alternative work schedule and partial funding and to Peter Scheidt for making it happen.

Several people took time to provide assistance or comments on draft manuscripts (Barbara Goldman, Ann Keim). Finally, I thank my husband, Mark, who inspires and challenges me to be a better epidemiologist; my parents, siblings, and siblings-in-law who encouraged me along the way and set good examples with their graduate work; my classmates; and my work colleagues who patiently tolerated my schedule for four years.

TABLE OF CONTENTS

LIST OF TABLES

Table

LIST OF FIGURES

Figure

ABBREVIATIONS

AA	arachidonic acid
AIMS	Alberta Infant Motor Scale
ALA	α-linoleic acid
BMI	body mass index
CAT	Clinical Adaptive Test
CES-D	Centers for Epidemiologic Studies Depression Scale
CLAMS	Clinical Linguistic and Auditory Milestone Scale
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
EPDS	Edinburgh Postnatal Depression Scale
ERG	electroretinographic
FA	fatty acid
FDA	Food and Drug Administration
FFQ	Food frequency questionnaire
GM	General Movements
LCPUFA	long-chain polyunsaturated fatty acid
LA	linoleic acid
MDI	Mental Development Index
n-6	omega-6
n-3	omega-3
PDI	Psychomotor Development Index
PIN	Pregnancy, Infection, and Nutrition Study

RCT	Randomized Controlled Trial
STAI	State-Trait Anxiety Inventory
UNC	University of North Carolina
VEP	visual evoked potential

CHAPTER 1

INTRODUCTION

Healthy neurodevelopment during gestation and infancy can provide a foundation for positive behavioral and cognitive outcomes later in childhood and beyond. Infants who are born at term and at a healthy weight, unexposed to substances like tobacco and alcohol, to a mother with a healthy diet, and into a supportive home environment are likely to see these advantages reflected in their intelligence and behavior, school performance, and mental health later in life.[1-6] Meanwhile, infants born without these advantages may not attain their full developmental potential. Identifying and better understanding the factors that promote or impede healthy development during gestation and infancy can help inform obstetric and pediatric practice as well as public health policy to optimize outcomes.

The particular role of certain dietary long-chain polyunsaturated fatty acids (LCPUFAs) in healthy brain development has been of interest for several decades. Fatty acids are important constituents of cell membranes, and LCPUFAs accumulate in large concentration in the developing brain, particularly in the gray matter (especially the synaptic membranes) and in the rod photoreceptors of the retina.[7-10] Docosahexaenoic acid (DHA) has been of primary interest for these reasons. DHA and other LCPUFAs like arachidonic acid (AA) are present in human breast milk and are also added to enhance some commercially-available infant formula products. A number of studies have found that DHA intake is associated with positive outcomes like improved visual acuity particularly in preterm infants, but other studies have found no association.[11-15] A beneficial effect on

development has been suggested but remains uncertain as well.[16-19] Lack of clear associations may be because of the variation in the type of formulas or supplements fed to participants or differences in the outcome measurements used; also, many previous studies have been hampered by sample size constraints, limited length of follow-up, or poor control of confounding. Most studies in this area have been feeding or supplementation trials, which have the advantage of a randomized design but may have limited generalizability. Observational studies have been challenged by issues of confounding since choices about breastfeeding and formula feeding are intertwined with factors like socioeconomic status. Improved understanding of the role of LCPUFAs in development could help refine guidance about infant feeding practices and the content of infant formulas. The present study will examine the association between feeding method, infant exposure to DHA and AA during the first 4 months of life and developmental outcomes at 12 months of age, using detailed information about feeding patterns and breast milk content and accounting for key confounders.

Depression affects between 6 and 17 percent of women at some point during their lifetime.[20-22] The life stages corresponding to pregnancy and new motherhood may be particularly susceptible periods; postpartum depression affects up to 19 percent of new mothers, and an estimated 12 to 18 percent of women are depressed during pregnancy.[23, 24] Anxiety, stress, and depressive symptoms are inter-related phenomena; women who perceive their circumstances as highly stressful are also likely to experience more depressive symptoms or anxiety.[25] Numerous studies have observed an association between maternal postpartum or chronic depressive symptoms and poorer child performance on cognitive and behavioral assessments.[26-35] Studies noted that greater maternal stress and anxiety interfere

with mother-child interaction, with consequences for infant temperament and cognition.[36-39] However, few studies have reported negative effects as early as infancy, and those results have been inconsistent.[29, 40, 41]

Additionally, depressed adults have been shown to have lower circulating levels of DHA, and depressed women may have inadequate supplies of fatty acids to pass transplacentally to their fetus or to feed them via breast milk.[42-44] The present study will explore the association between maternal depressive symptoms, trait anxiety, and perceived stress during pregnancy and postpartum and infant developmental outcomes at 12 months of age. Whether women with elevated depressive symptoms have lower concentrations of DHA in their breastmilk will also be examined.

The present study is an opportunity to address questions about the role of LCPUFA exposure and maternal psychological health in infant development. Women and their children (n=358 children) who were followed prospectively from early pregnancy to one year of age and completed assessments of maternal depressive symptoms, trait anxiety, and perceived stress; infant development; and infant feeding practices will be included in the present study. The study has several advantages over previous research. First, no other observational studies of infant development have incorporated detailed information about the fatty acid content of both breast milk and infant formula (both formulas with LCPUFAs added and those without LCPUFAs). Second, few studies assess women for depressive symptoms during pregnancy and postpartum, and this study assessed depressive symptoms at three time points. Third, this study uses the Mullen, an assessment of multiple developmental domains. Fourth, the study population for this study more closely resembles the general population of women and infants than some studies which drew samples of

women who were clinically depressed. Finally, the study incorporates data on the potential confounders that often plague research on breastfeeding or LCPUFA exposure and brain development. As a result, the present study can help address some of the shortcomings of previous studies and build upon the present understanding of the role of LCPUFAs and maternal psychological health in early child development. The study is based on prospective data from the Pregnancy, Infection, and Nutrition (PIN) Study of pregnant women in central North Carolina and its postpartum component involving their infants.[45]

CHAPTER 2

REVIEW OF THE LITERATURE

Introduction

For several decades intense research efforts have been placed on uncovering the factors that underlie healthy development of the human brain during gestation and infancy. Likewise substantial effort has gone toward identifying exposures that are harmful to neurodevelopment with the goal of eliminating those exposures. It is clear that children who experience a healthy start to life and live in a nurturing early environment are more likely to achieve educationally, be productive adults, bear healthy children themselves, and live free of mental illness.

This healthy start may begin even before conception with the health of the parents, but it certainly can be marked with the earliest signs of fetal brain development. The first few weeks of gestation are marked by the development of the notochord and the neural tube. Cell proliferation, neuronal migration and synaptic formation begin to at least some extent during the first trimester, and the total complement of adult neurons is present in the fetal brain by the time the brain growth spurt starts in the third trimester.[46-48] From the third trimester of *in utero* development to approximately 18 months of age, the brain undergoes dramatic growth in terms of overall size and cell number.[49] Myelination occurs as well as a large increase in dendritic complexity and synapse formation.[47, 48] At the time of birth, the human brain has already attained 27 percent of its final adult weight.[46] The brain growth spurt has been identified as a critical window where a wide range of exposures have been

shown to be harmful to the developing brain. For instance, during the third trimester, fetal exposure to alcohol has been associated with cerebral growth restriction and neuronal loss and exposure to lead with reduced intelligence.[5, 50] From a psychosocial perspective, it is apparent from studies like the Infant Health and Development Program that positive influences like presence of caregivers and interventions like home visiting during infancy result in better cognitive and behavioral outcomes.[2, 51]

Long-chain polyunsaturated fatty acids and infant development

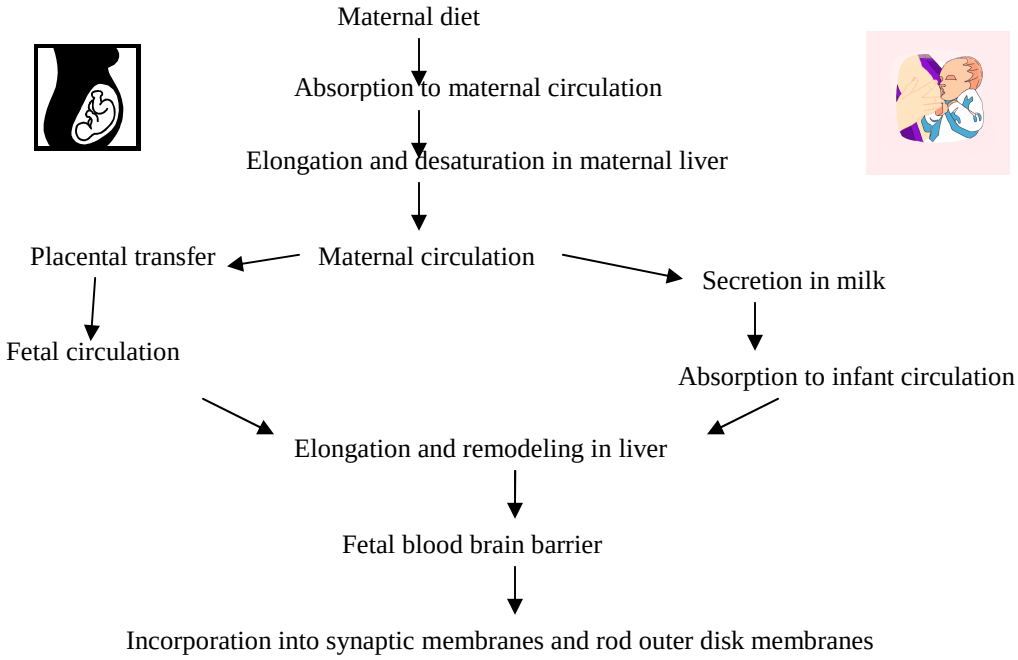
Background and Significance

Various aspects of diet play important roles in brain development, both during pregnancy and in infancy. Appropriate maternal weight gain during pregnancy has been shown to be important for fetal growth which is directly related to brain development.[52] Iron deficiency during pregnancy and in childhood has been shown to adversely effect behavior in childhood.[53-55] And, inadequate supplies of choline and folate during fetal development can result in neural tube defects.[56, 57] Iodine seems important for cognition and motor development in childhood.[58] Over the past two decades much research has focused on the role of dietary omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids in infant neurodevelopment. Some members of these classes of fatty acids can be produced endogenously, but dietary sources are essential for certain fatty acids. Linoleic acid (LA) and α-linoleic acid (ALA) are the predominant forms present in the human diet and are considered essential fatty acids since they cannot be produced endogenously.[49] Most fatty acid metabolism is performed by the liver where fatty acids are elongated and desaturated to other forms, including DHA (22:6n-3) and AA (20:4n-6).[49] DHA and AA are members of a

group of fatty acids known as LCPUFAs because of their structure consisting of more than 18 carbon atoms.[49, 59] DHA and AA are themselves present in a range of food sources as well as produced endogenously from ALA and LA.[49] DHA is found in abundance in seafood like atlantic salmon, pacific cod, and tuna and also in certain oils and nuts; AA is found particularly in egg yolks and animal fats and organ meats.[60, 61] Human breast milk also contains DHA and AA, and many infant formulas are enhanced with DHA and AA.[62-65]

In the human body, LCPUFAs play a number of roles including as components of membrane phospholipids and as precursors of prostaglandins, thromboxanes, and leukotrienes.[59, 66, 67] AA can be found in most tissues in the human body, while DHA tends to be more highly concentrated in the brain, retina, and testis.[49] Within the retina and brain tissues, DHA concentrates in the rod photoreceptors and synaptic membranes in the gray matter.[8, 9, 68] During the brain growth spurt, the amount of DHA in the brain increases substantially in both an absolute and relative sense.[49] Between 25 weeks gestation and 2 years of age, the total amount of DHA in the brain increases from 3,000 nmol per gram brain to 10,000 nmol per gram brain.[69] Also during the third trimester the liver and adipose tissues of the developing fetus build up stores of DHA to fuel postnatal neurodevelopment.[70] During pregnancy, maternal DHA from the diet or endogenous production crosses the placenta and is the major contributor to the DHA in the fetal brain (Figure 2.1).[49]

Figure 2.1. DHA accretion in the fetal and infant brain (Adapted from Lauritzen [49])



During gestation the fetal liver and adipose tissues store DHA, to provide a postnatal source for ongoing brain development.[70, 71] Thus, the main reason why infants born preterm might require more dietary DHA than term infants is because they have lower internal stores to draw from.[49] Lauritzen et al. estimates the difference in the DHA stores of an infant born at 30 weeks versus a term infant to be 4.5 g DHA.[49] Clandinin et al. estimated that term infants have approximately 3.6 grams of total fatty acids in total brain tissue at birth, while preterm infants have an average of 1.1 grams.[70] Both preterm and term infants can transform LA to AA and ALA to DHA.[72] However, rates of transformation vary across individual infants and preterm infants may be unable to acquire or produce enough DHA and AA during infancy to make up for their deficit.[73, 74]

Several factors affect the amount of LCPUFAs that the developing fetus obtains via the placenta or breast milk, and this may affect the amount of LCPUFAs available to the

developing brain. First, the third trimester of pregnancy generally corresponds to a natural increase in the amount of circulating fatty acids so more DHA is available during the period of rapid brain development. Second, birth order plays a role. Maternal stores of DHA are not completely replenished between pregnancies, so later children may receive less DHA than first births.[75] While internal stores of DHA can help fuel postnatal brain development, dietary sources of DHA are likely the larger source for the infant.[73] Human breast milk contains DHA in varying amounts depending on maternal diet, smoking, body mass index (BMI) and weight gain, parity, duration of lactation, adipose stores, and hepatic metabolism of fatty acids. AA levels tend to be more consistent than DHA levels.[73] Estimates of DHA concentration in the breast milk of women from the U.S. range from 0.06 to 0.29 percent of total fatty acids (FA%), and are lower on average than for other populations with high seafood intake.[62, 63] For instance, Idota et al. estimated the DHA concentration in breast milk from a sample of Japanese women to be 1.00 FA%, and Jørgensen et al. found the concentration to be 0.43 wt% for a sample of Danish women.[76, 77] Vegetarian and vegan women have lower levels of DHA in their breast milk than women who consume animal products; Sanders and Reddy estimated DHA in their sample to be 0.14 FA%.[78] Breast milk fatty acid concentrations can fluctuate slightly day-to-day, but it has been estimated that preterm infants ingest approximately 24 mg DHA and 36 mg AA each day, while a 4-month old infant takes in 100 mg.[49, 70] Jørgensen et al. estimate that fish intake could explain 55 percent of the variability in breast milk DHA content.[79] The LCPUFA concentration of breast milk does not seem to vary significantly across lactation, however. Studies by Mitoulas et al. and Ribeiro et al. collected frequent samples over at least the first 4 months postpartum and observed no significant changes in DHA or AA content over time.[80, 81] This

is in contrast to a few older studies that suggested a possible decrease over time, but these studies relied on samples collected at fewer time points.[82, 83] Among women who smoke during lactation, breast milk LCPUFA levels tend to be lower than for non-smoking women.[84] Maternal BMI and weight gain during pregnancy are also positively associated with the concentration of fatty acids in breast milk.[85, 86] Maternal hepatic activity also contributes to breast milk fatty acid concentration, since the amount in breast milk exceeds the amount found in adipose stores and diet combined.[49]

Infant formula manufacturers have strived to produce formulas as similar as possible to breast milk in how infants digest them and in their composition.[87] Current recommendations for the content of essential fatty acids for infant formulas vary. The U.S. Food and Drug Administration (FDA) issued nutrient requirements in 1985 including a requirement for at least 2.7 percent of total fatty acids to be from LA.[88] The FDA convened an expert panel via the Life Sciences Research Office that provided recommendations for at least 8 percent of total fatty acids from LA, 1.75 percent from ALA, and a LA to ALA ratio of greater than 6 but less than 16.[89] The 1991 report from the European Society for Paediatric Gastroenterology and Nutrition contained recommendations for formula LA content of 4.5 percent up to 10.8 percent.[90] There is no consensus about the necessity of adding LCPUFAs to formula products. However, a workshop involving many of the leading investigators in this field recommended that formulas for preterm infants contain 0.35 FA% as DHA and 0.4 FA% as AA, 0.2 FA% as DHA and 0.35 FA% as AA for term infants.[91] In 2002, the first infant formula product with DHA and AA added became available to consumers in the U.S.[92] Formulas with LCPUFAs added had been available in Europe and some Asian countries since at least the early 1990s.[93] The multiple studies carried out in the

1990s that suggested neurodevelopmental benefits of LCPUFAs were influential in manufacturers' decisions to add DHA and AA to many of their products. Most of the new formulas were based on existing products but with DHA and AA added, and in most cases both versions co-existed on the market for several years. By 2007, many infant formulas were available in the enhanced version only. The amount of DHA and AA added varies slightly by manufacturer (Appendix 1).

Until formulas were modified to contain DHA and AA, formula-fed infants obtained DHA and AA by metabolizing LA and ALA.[94] Studies based on postmortem examinations of formula or breastfed infants found that breastfed infants had a higher proportion of cerebral cortex fatty acids as DHA than infants fed formula with no added DHA and AA.[94] Also, the concentration of DHA in the brain increased across infancy for the breastfed infants but was constant for infants fed traditional formulas, suggesting that without a dietary source of DHA, the infant is not able to meet the needs of its rapidly developing brain.[94] However, DHA concentrations in the infant retina did not vary between the groups nor did AA concentrations in the brain and retina.[94]. The similarity in AA concentrations may be due to the composition of the formulas used at the time, where the specific LA and ALA levels resulted in metabolism of AA at levels close to those observed in breastfed infants.[94] The results pertaining to concentrations in the retina may be because the amount of DHA in the retina plateaus at 40 weeks gestation, and the retina has mechanisms to conserve DHA in times of undersupply.[94] A study by Farquharson et al. found that formula fed infants experienced a more rapid decrease in the concentration of DHA in liver and adipose tissue over the first ten postnatal weeks as compared to breastfed infants, indicating that formula fed infants tended to draw down DHA stores in the absence of dietary DHA.[95, 96] Despite the

noted differences in LCPUFA concentrations in various infant tissues when comparing breastfed to formula fed infants, there remains uncertainty about whether differences in daily functioning (e.g., cognition, motor skills) result.

Critical Review of the Literature

Numerous studies have examined associations between various aspects of breastfeeding or LCPUFA supplementation and visual development, cognition and behavior ever since the first report by Hoefer and Hardy in 1929.[97] Much of the evidence for a beneficial effect on neurodevelopment comes from studies comparing preterm infants fed formulas containing LCPUFAs to infants fed a control formula or breastfed.[11, 12, 98] Many of these studies found a positive association between LCPUFA formulas or breast milk and plasma phospholipid or red blood cell DHA content and performance on tests of visual acuity. Faldella et al. compared infants who received a formula with LCPUFAs to infants who received a traditional formula for preterm infants and infants who were breastfed.[98] They found that infants received the traditional formula lagged in the development of visual evoked potentials (VEPs), a measure of optic and brain visual processing maturation.[98] A study by Uauy et al. measured electroretinographic (ERG) responses in very low birthweight infants to assess retinal maturity. The infants were assigned to either human milk, formula low in n-3 fatty acids, formula with ALA, or formula with ALA and marine oils.[99] They found that rod function was similar for the infants who received the formula containing DHA from marine oils or the human milk, and the other infants showed less well-developed function.[99] Extending upon that work, the same team reported that VEP and forced-choice preferential looking at 36 and 57 weeks post-conception reflected comparable visual acuity

development between infants fed human milk and infants fed formula containing marine oils.[11] Infants fed a traditional LA-containing formula performed the worst, while infants on a formula with ALA and LA performed in the intermediate range.[11] In similar work led by Carlson, 33 preterm infants fed a marine-oil supplemented formula containing eicosapentaenoic acid (EPA), a LCPUFA, demonstrated better visual acuity as measured by the Teller Acuity Card test up until at least 6 months of age as compared to 34 infants on a traditional formula.[12] However, no statistically significant differences were detected at subsequent ages.[12] Also, it was found that the marine oil formula impeded infant growth and AA status, possibly due to the presence of EPA.[74, 100] The DHA-formula fed infants had better visual acuity at term and 2 months (but not beyond 4 months) as measured by the Teller Acuity Card test.[13] All of these studies were limited in size to approximately 10 to 35 infants per experimental group, but all found some positive effects of DHA supplementation on visual development of preterm infants, even if the effect was transient.

A number of other studies found no difference between supplemented and unsupplemented infants. These include a study by Innis et al. that employed Teller cards at 2 and 4 months.[14] A study by O'Connor et al. found no effect on acuity using Teller cards at 2, 4 and 6 months, but did see an advantage using swept-parameter VEP at 6 months.[15]

A common finding for some of these studies is that effects seen at very young ages are no longer seen beyond 4 months of age.[12, 13] It seems that LCPUFAs may accelerate very early visual development but unexposed infants eventually catch up. One outstanding question is whether this short-lived advantage in visual acuity results in any longer-term advantage in terms of cognition and other aspects of development that matter for daily

functioning. Further studies have attempted to answer this question by following children into later infancy and assessing global development.

Tables 2.1-2.3 include studies from developed countries that assessed cognition (including language development), visual development (except acuity) or motor skills in children 18 months of age or younger. This includes randomized and observational studies involving infants born at term or preterm.

Researchers have taken several approaches to addressing the question of whether infants benefit from either breastfeeding or LCPUFA supplementation in terms of development of cognitive, visual or motor skills. A number of studies have employed randomized designs while others have been observational. Based on early indications that preterm infants might benefit more than term infants from breastfeeding or LCPUFA supplementation, some studies have continued to study preterm infants. Others have attempted to detect effects in term infants. Meanwhile, a small number of studies have tested supplements in pregnant or lactating women and looked for effects in their breastfed children.

1. Randomized studies

The results of randomized studies have been mixed. A Cochrane review on this topic concluded that "there is little evidence from randomized trials of LCPUFA supplementation to support the hypothesis that LCPUFA supplementation confers a benefit for…general development of term infants….A beneficial effect on information processing is possible but larger studies over longer periods are required to conclude that LCPUFA supplementation provides a benefit when compared with standard formula."[101] These studies randomized

formula fed infants to one of one or more LCPUFA formulas or a control formula. Most studies also included a breastfed comparison group (non-randomized). Studies (n=20) that assessed infant development at 18 months of age or younger are listed in Table 2.1, Of the studies reviewed, 15 studies chose the Bayley Scales of Infant Development, 3 the Fagan Test of Infant Intelligence, 3 the MacArthur Communicative Development Inventories, 2 the Brunet-Lezine test, 1 the Infant Planning Test, 2 the Knobloch, Passamanick, and Sherrards test, 1 used gross motor milestones, and 1 an assessment of general movements. Of the largest studies, only a few found that infants fed LCPUFA formulas performed significantly better than infants fed control formulas on the tests used. A few of the small trials found a benefit of LCPUFA supplementation, but results remained inconsistent. Studies that involved only preterm infants were more likely to show a benefit from LCPUFA supplementation than studies of term infants.

Table 2.1. Previous studies of LCPUFA supplementation and infant development

Author (year)	*Study design (trial, observational) n*	*Exposure*	*Outcome measure(s) (age in months)*	*Results*
Agostoni (1995)[16]	Trial, n=90	LCPUFA formula, control formula, breastfed	Brunet-Lezine (4m)	LCPUFA group scored higher than control formula group (105.3 vs 96.5). Breastfed group scored higher than control group (102.2 vs 96.5). F=4.93, p=0.009.
Agostoni (2000)[102]	Trial, n=90	LCPUFA formula, control formula, breastfed	Brunet-Lezine (4m, 12m)	Breastfed (25.4) and LCPUFA (26.0) groups scored higher on eye-hand coordination than control formula group (22.0), p<0.001. No differences on other scales or at 12 months
Agostoni (2009)[103]	Trial, n=1160	20 mg DHA supplement or placebo	Time to achievement of 4 gross motor milestones	Sitting without support achieved sooner among supplemented group (26 weeks vs 27 weeks, p<0.001)
Auestad (2001)[104]	Trial, n=404	LCPUFA formulas, control formula, breastfed	Fagan (6m, 9m), Bayley (6m, 12m), MacArthur (9m, 14m)	Fagan – no difference in novelty preference, look duration between control and LCPUFA groups at 6 and 9 mos. Bayley – no difference between groups at 6 and 12 mos. MacArthur – no differences except mean vocabulary expression score higher for fish-LCPUFA formula than egg-LCPUFA formula (104±13 vs 96±18) at 14 mos.
Ben (2004)[105]	Trial, n=271	LCPUFA formula, control formula, breastfed	Bayley (3m, 6m)	LCPUFA group scored 2.4 pts higher on MDI, 3.6 pts higher on PDI compared to control formula group at 3 months. No other differences observed.
Birch (2000)[17]	Trial, n=56	DHA, DHA+AA, control formula	Bayley (18m)	DHA+AA group had higher MDI than DHA group (105.6 vs 102.4) or control group (98.3) F=3.18, p<0.05. No difference seen for PDI (101.7, 99.4, 98.6, H=4.05, p=0.13).
Bouwstra (2003, 2005)[106-108]	Trial, n=397	LCPUFA formula, control formula, breastfed	General movements (GM) (3m), Bayley (18m)	Breastfeeding for >6 wks associated with less mildly abnormal GM (p<0.0015) and more normal-optimal GM (p,0.0025). Control infants had mildly abnormal movements more often than breastfed or LCPUFA groups (31 vs 19 vs 20%). Bayley – no significant differences between control, LCPUFA, and breastfed groups (mean MDI=105.4±15.0, 102.7±15.4, 107.5±16.0; PDI=100.9±13.6, 99.4±13.4, 103.2±14.5)
Carlson (1993)[109]	Trial, n=56	LCPUFA formula, control formula	Bayley (12m)	LCPUFA group scored lower on PDI (82.2 vs 92.6) than control group (p<0.03)

Clandinin (2005)[110]	Trial, n=361	LCPUFA formula (DHA, AA), LCPUFA formula (plus EPA), control formula	Bayley (18m)	LCPUFA groups 6-10 points higher on MDI ($p \approx 0.05$) 6 points on PDI than controls ($p<0.05$)
Fang (2005)[111]	Trial, n=27	LCPUFA formula, control formula	Bayley (6m, 12m)	LCPUFA group scored higher on MDI (mean=96.1±8.6) then control group (91.7±10.4) at 6 mos. And at 12 mos (98.7±80, 90.5±6.9). For PDI at 6 mos (102.2±10.5, 95.4±13.2) and 12 mos (98.0±5.8, 86.7±11.1). Repeated measures ANOVA for MDI p=0.02, PDI p=0.008.
Fewtrell (2002)[112]	Trial, n=240	LCPUFA formula, control formula, breastfed	Knobloch (9m), Bayley (18m)	No difference between LCPUFA and control groups on Knobloch (1.5 pts higher for LCPUFA group, CI: -1.8-4.7) or Bayley MDI (control group 2.6 pts lower, CI: -7.39-2.28) or PDI (2.1 pts, CI: -6.8-2.67). Breastfed group performed better on Knobloch than formula groups (5.7 to 7.2 pts higher, $p<0.005$) and on Bayley MDI (8.9 to 11.5 pts, $p<0.005$) and PDI (4.6-6.6 pts, $p<0.005$)
Fewtrell (2004)[113]	Trial, n=238	LCPUFA formula, control formula	Bayley (18m)	No difference between LCPUFA and control group on Bayley except for boys on MDI (control group 5.7 pts lower, CI: 0.3-11.1)
Lucas (1999)[18]	Trial, n=241 to 250	LCPUFA formula, control formula	Bayley (18m), Knobloch (9m)	Bayley – no difference between LCPUFA group and control group (difference in MDI=0.5, CI: -2.7-3.8; PDI=0.6, CI: -1.8-3.0). Knobloch – no difference overall (difference=0.5, CI: -1.6-2.7) or on subscales
Makrides (2000)[19]	Trial, n=68	DHA, DHA+AA, control formula, breastfed	Bayley (12m)	No differences between breastfed (mean MDI=114+-13, mean PDI=101+-21) and DHA+AA group (MDI=108+-16, PDI=103+-22) or any other group
Makrides (2009)[114]	Trial, n=657	High DHA formula, standard DHA formula	Bayley (18m)	No difference in MDI scores except for girls (high DHA MDI 4.5 pts higher (CI: 0.5-8.5) than standard DHA group)

O'Connor (2001, 2003)[15, 115]	2001 – trial, 2003 - cohort from RCT, n=470	2001 – LCPUFA fish oil formula, LCPUFA egg formula, breast milk, control formula. 2003 - predominantly breast milk, ≥50%, <50%, predominantly formula fed	2001 - Fagan (6m, 9m), Bayley (12m), MacArthur (9m, 14m); 2003 - Bayley (12m)	Fagan – LCPUFA egg group scored higher (59.4±7.7) than LCPUFA fish group (57.0±7.5) and control group (57.5±7.4) on novelty preference (6), no difference at 9 mos. Bayley - No difference between groups in trial. Increasing breastfeeding duration associated with higher MDI at 12 mos. MacArthur – no differences at 9 mos. in the trial, LCPUFA infants had better Vocabulary Comprehension than control infants (14) and increasing duration associated with higher Vocabulary Comphrension (9)
Scott (1998)[116]	Trial, n=197	DHA formula, DHA+AA formula, control formula, breastfed	Bayley (12m), MacArthur (14m)	Bayley – no difference, Vocabulary Comprehension lower in DHA group than breastfed (92 vs 101, p=0.017), Vocabulary Production lower in DHA group than control formula group (91 vs 101, p=0.052), no differences between DHA+AA group and breastfed group
Van Wezel-Meijler (2002)[117]	Trial, n=42	LCPUFA formula, control formula	Bayley (3m, 6m, 12m)	No difference in Bayley MDI or PDI at any time point
Werkman (1996), Carlson (1996)[118, 119]	Trial, n=51-67	DHA formula, control formula	Fagan (6.5m, 9m, 12m)	No difference in novelty preference but greater number of looks and shorter duration in DHA infants
Willats (1998)[120]	Trial, n=44	LCPUFA formula, control formula	Infant Planning Test (10m)	LCPUFA infants had more intentional solutions (median=2.0 vs 0.0, p=0.02) and higher intention scores (14.4 vs 11.5, p=0.04)

2. Observational studies

Twenty-one observational studies are detailed in Table 2.2. These studies varied widely in how they defined their exposure groups. Some compared a breastfed group to formula fed groups, while others made comparisons among breastfed infants only. Outcome measures likewise varied widely; 15 studies used the Bayley, 1 used the Denver Developmental Screening Test, 1 the Fagan, 1 used the Knobloch, 1 used the Alberta Infant Motor Scale, 1 used a measure of parent concern about the developmental progress of their child, 1 used a neurobehavioral score, and 1 used a customized measure of gross and fine motor skills and language. Because of the varying definitions of the exposure groups and choice of outcome measures, it is difficult to compare results across studies. The largest of these studies found that the odds of gross motor delay decreased with increasing duration of breastfeeding in a representative sample of infants from the United Kingdom, after adjusting for maternal age, birth weight, gestational age, maternal smoking, social class, maternal education, maternal employment, single parenthood, Malaise Inventory, postnatal attachment, parenting views, number of siblings, child care, and hours cared for by others [121]. Many of the other studies found some benefit to longer breastfeeding. However, the studies varied widely in the ability to control for important confounders, and the results are inconsistent.

Table 2.2. Previous studies of breastfeeding and infant development

Author (year)	*Study design (trial, observational) n*	*Exposure*	*Outcome measure(s) (age in months)*	*Results*
Agostoni (2001)[122]	Obs, n=44	Breastfeeding duration	Bayley (12m)	No significant differences between groups. Group breastfed ≥ 6 mos scored higher on PDI (difference=6.6, CI:-0.6-13.8) and MDI (2.0, CI: -3.2-7.3) than group breastfed < 6 mos
Angelsen (2001)[123]	Obs, n=345	Breastfed <3 mos., 3-6 mos., ≥ 6 mos.	Bayley (13m)	Infants breastfed ≥ 6 mos had higher MDI than infants < 3 mos (mean MDI=117.7(SD 11.7) vs 109.9 (13.1)). No other differences among groups.
Dee (2007)[124]	Obs cross-sectional, n=22,399	Breastfeeding initiation, duration	Parent concern about language and motor skills	Concerns about development inversely related to breastfeeding initiation, duration for those breastfed 3 or more months
Bier (2002)[125]	Obs, n=39	Partially breast milk, formula fed	Alberta Infant Motor Scale (3m, 7m, 12m), Bayley (7m, 12m)	AIMS – milk group scored higher than formula group (adj mean=48 (SD=20) vs 35 (12), p=0.05) at 3 mos and 12 mos (62 (20) vs 47 (15) p<0.05) but not 7 mos. Bayley – milk group scored higher on MDI (mean=100 (12)) than formula group (91 (10)) at 12 mos, but no difference at 7 mos.
Florey (1995)[126]	Obs, n=592	Breastfed, formula fed	Bayley (18m)	Breastfed infants scored 3.7 to 5.7 points higher on MDI than formula fed infants. No differences for PDI.
Gomez-Sanchiz (2003)[127]	Obs, n=249	Breastfed partially or exclusively > 4 mos., ≤ 4mos., formula fed	Bayley (18m)	Group breastfed ≤ 4 mos group scored higher than formula group on MDI but not PDI (MDI β=4.7 CI: 1.7-7.7, PDI β=3.6, CI: -0.2-7.3). Group breastfed > 4 mos scored higher than group breastfed ≤ 4 mos. on MDI but not PDI (MDI β=7.2 CI: 4.3-10.1, PDI β=0.8, CI: -2.7-4.4).
Innis (1996)[128]	Obs, n=433	Breastfeeding duration, exclusivity	Fagan (10m)	No difference in novelty preference found by duration or exclusivity.
Eidelman (2004), Feldman (2003)[129, 130]	Obs, n=86	>75%, 25-75%, <25% breast milk	Bayley (6m)	MDI and PDI scores were highest for >75% group (MDI=94.2±8.8, PDI=85.8±11.5) compared to 25-75% group (91.7±7.2 p<0.05, 78.6±12.6 p<0.01). No difference between 25-75% group and <25% group
Jacobson (2008)[131]	Obs, n=109	Maternal plasma phospholipid levels * weeks exclusive breastfeeding	Bayley (11m)	No association between exposure and Bayley MDI or PDI

Lucas (1994)[132]	Trial, n=114	Donor breast milk, formula fed	Bayley (18m)	Breast milk group scored lower than formula group on MDI (0.5 pts, CI: -6.2-7.1) and PDI (1.2 pts, CI: -4.4-6.8)
Lucas (1989)[133]	Trial, n=502	Donor breast milk, formula fed	Knobloch (9m)	Formula group scored higher on Knobloch than donor breast milk group (3.0 pts, CI: 0.5-5.6).
Morley (1988)[134]	Obs, n=771	Breastfed, formula fed	Bayley (18m)	Breast milk group scored higher on MDI (7.9 pts, $p<0.01$) and PDI (6.8 pts, $p>0.001$).
Pinelli (2003)[135]	Cohort from RCT, n=148	Exclusively breastfed, partially breastfed, formula fed	Bayley (6m, 12m)	Infants >80% breastfed scored 1 points higher on MDI and PDI at 6 mos. and 1 point higher on PDI and MDI at 12 mos. (all $p<0.05$)
Rogan (1993)[136]	Obs, n=855	Breastfed ≥ 20 wks + weaned ≥ 50 wks, 5-19 or 20 wks + weaned 19-49 wks, ≤ 4 or 5-19 wks + weaned 9-19 wks, ≤ 4 wks + weaned ≤ 9 wks, formula fed	Bayley (6, 12, 18)	No significant differences at 6, 12, 18 mos
Sacker (2006)[121]	Obs, n=14,660	Breastfeeding duration, exclusivity	Denver (9m)	Odds of gross motor delay lower for prolonged exclusive breastfed group vs never breastfed group (OR=0.67, CI: 0.54-0.84), fine motor delay (OR=0.93, CI: 0.74-1.16)
Strain (2008)[137]	Obs, n=229	Maternal serum fatty acids	Bayley (9m)	PDI positively associated with total n-3 levels.(beta=28.26, $p=0.03$)
Vohr (2006)[138]	Prospective cohort from RCT, n=1,035	Some breast milk, no breast milk	Bayley (18m)	Breast milk group scored higher on MDI and PDI (MDI=79.9±18, PDI=84.6±19) than no breast milk group (75.8±16, 81.3±17) ($p=0.07$, 0.02)
Morrow-Tlucak (1988)[139]	Obs, n=219	Breastfed >4 mos, ≤ 4 mos, formula fed	Bayley (6m, 12m)	Breastfeeding duration positively associated with Bayley score at 12 mos. (>4 mos: 121.3, ≤4 mos: 116.0. formula: 111.2, $F=3.24$, $p=0.04$), No significant differences at 6 mos. (114.0, 113.1, 110.8, $F=0.55$, NS).
Ounsted (1988)[140]	Obs, n=307	Breastfed 2, 6, 12 months, bottlefed	Neurobehavioral score (2m, 6m, 12m)	No differences at 2 mos. Breastfed babies had higher neurobehavioral scores at 6 mos ($F=4.5$, $p<0.05$) and 12 mos (4.3, $p<0.05$). Breastfed babies had higher motor scores at 12 mos ($F=4.8$, $p<0.01$) but not social scores ($F=1.5$).
Temboury (1994)[141]	Obs, n=229	Breastfed ≤ 3 mos, formula fed or breastfed ≤ 1 mo	Bayley (18m)	Comparing bottle-fed to breastfeeding, (odds of lower score) OR=1.86, $p=0.044$ for MDI. OR=1.73, $p=0.06$ for PDI.

Vestergaard (1999)[142]	Obs, n=1656	Breastfeeding duration, exclusivity	General motor skills, fine motor skills, language (8m)	Highest vs lowest breastfeeding category: crawling (RR=1.3, CI:1.0-1.6), pincer grip (1.2, 1.1-1.3), polysyllable babbling (1.5, 1.3-1.8)

3. Maternal supplementation studies

Another way to examine the association between LCPUFA intake and infant development is to randomize pregnant or lactating women to dietary supplements containing LCPUFAs or a placebo. While biologic measures like red blood cell DHA levels were often associated with supplementation, developmental outcomes tended to reflect no benefit from supplementation.

Table 2.3. Previous studies of LCPUFA supplementation in pregnant and lactating women and infant development

Author (year)	*Study design (trial, observational) n*	*Exposure*	*Outcome measure(s) (age)*	*Results*
Helland (2001)[143]	Trial, n=262	Cod liver oil, corn oil	Fagan (6m, 9m)	Cod liver oil group did not differ from the corn oil group on novelty preference at 6 mos (mean=55.2 (SD=4.5) vs 55.4 (3.7) or 9 mos (55.5 (3.8) vs 56.2 (3.5))
Gibson (1997)[144]	Trial, n=52	4 different doses of DHA algal oil, placebo	Bayley (12m)	Breast milk DHA content positively associated with MDI (r=0.29, p=0.04) but not PDI.
Lauritzen (2005)[145]	Trial, n=175	Fish oil, olive oil, high fish intake	Infant Planning Test (9m), MacArthur (12m), gross motor	Fish oil group no better on problem solving than olive oil group, except for girls (mean=5.7±2.7 vs 3.7±2.5, p=0.024). Fish oil group performed worse on Vocabulary Comprehension (mean=54±37) than olive oil group (71±45, p=0.045), and on sentence complexity (boys) (mean=0.05 (25^{th}-75^{th} pct 0-3 vs 4.5 (0-10), p=0.043). No differences in gross motor function.
Jensen (2005)[146]	Trial, n=227	DHA capsules, vegetable oil capsules	Gesell Developmental Inventory (12m), Clinical Linguistic and Auditory Milestone Scale (CLAMS) (12m), Clinical Adaptive Test (CAT) (12m)	DHA group did not perform better than control group on Gesell (mean=101.8±13.8 vs 99.5±13.3), CLAMS (100.6±14.6 vs 102.5±13.2) or CAT (109.0±10.7 vs 110.0±10.8)
Judge (2007)[147]	Trial, n=29	DHA bars, placebo bars	Fagan (9m), infant problem solving (9m)	DHA group had better problem solving but not recognition memory
Tofail (2006)[148]	Trial, n=400	Fish oil, soy oil	Bayley (10m)	Fish oil group scored no higher on MDI (102.5 vs 101.5) or PDI (101.7 vs 100.5) than soy oil group

It remains an unresolved question whether breastfeeding duration or supplementation with LCPUFAs has a beneficial effect on infant neurodevelopment, despite the large number of studies that have addressed this issue. A range of challenges confront studies in this area, including issues of study design, exposure measurement, outcome measurement, and appropriate consideration of confounders.

Several aspects of study design may be partly responsible for the equivocal results of previous studies. One challenge for the randomized studies in particular is generalizability. The vast majority of the research on the potential benefits of LCPUFA intake has adopted a randomized design at least in part to eliminate issues of confounding. Many of these studies tested formulas that were specially developed for research purposes and not for wide consumption. Infants who were compliant with the study protocols would have been fed exclusively the assigned formula product and not breast milk or other formulas. Also, studies that include a breastfed comparison group do not randomize infants into breastfed or formula-fed groups due to ethical concerns. Instead, the breastfed group is generally made up of infants whose mothers intend to breastfeed while the formula-fed groups are infants whose mothers intend to formula feed.[104, 116] The infants who participated in these studies are not representative of formula-fed infants in the general population who often switch formula products one or more times during infancy. Also, many infants in the general population are fed some breast milk and some formula, and their experience is generally not reflected in these studies. Second, many studies have been very small, perhaps too small to detect an effect. It is helpful to consider that smaller studies in this area more often find a positive association, while large studies tend to find no association.[149] Third, some studies have been of limited duration, so an effect might not be observed because infants did not

consume the supplemented formulas long enough or were not followed long enough. Given that fetuses accrue fatty acids through the placenta over a period of months during gestation and then most continue to take in fatty acids through breast milk or formula over the first weeks and months postnatally, it may not be surprising that supplement studies of short duration are unable to detect an effect.

In terms of exposure, studies have varied widely in their choice of formulas fed to study infants, leading to incomparability across studies. Formulas with low fatty acid content have been used in some studies, possibly limiting their ability to observe an effect. Also, the balance of ingredients in the formulas used can affect how much of the LCPUFAs are available to the infant since n-3 and n-6 fatty acids compete for the same enzyme substrates.[150] Overabundance of one fatty acid can suppress production of another. As a result, the DHA content must be considered along with the content of other fatty acids when comparing studies. Previous observational studies have generally employed fairly crude measures of exposure. They have not thoroughly taken into account the wide variation in the extent to which infants are breastfed or formula fed and the differences in LCPUFA content. By extension, no previous studies have had the benefit of very detailed information about breastfeeding and formula use (formula with and without LCPUFAs) to examine dose-response relationships. Many randomized studies employed intent-to-treat analysis, normally a strength but which may obscure the variation in LCPUFA levels infants to which infants were exposed.[15, 112] Observational studies of breastfeeding duration and exclusivity use varying exposure classification schemes, and this may explain some of the inconsistency in results.

Previous studies have been challenged in terms of outcome measurement. The choice of outcome measures has varied widely across studies, so it is difficult to compare them. Studies have focused variously on mental or motor development, language development, visual attention, problem-solving, behavior, or intelligence and employed a variety of instruments to measure each. In some studies, developmental outcomes were not measured using validated and reliable instruments. Assessments of especially young infants are often not predictive of later outcomes like IQ or behavior, and some instruments are not designed to pinpoint variations within the normal range of cognition or behavior[151, 152]; low predictive value is one drawback of using an instrument like the Bayley Scales of Infant Development [153-155]. Another challenge is the timing of assessment. Some instruments are only valid when applied to a narrow age window, and sometimes they are administered outside that window. Also, some previous studies employed tests of global development (e.g., Bayley) which may be insensitive to specific aspects of development that may be affected by LCPUFA intake or breastfeeding.

A major challenge for previous studies has been control of confounding factors. Studies that compare breastfed children to formula-fed children are subject to criticism because of inadequate control of important confounding variables. And, this is not just a problem for the observational studies. Because it is generally considered unethical to randomize infants to breastfeeding versus formula feeding, most randomized studies only randomize within the formula fed group (to a LCPUFA formula or a control formula). Thus, those studies are subject to some of the same confounding issues as observational studies.

Many factors distinguish breastfed infants and formula fed infants aside from the amount of various fatty acids they may ingest. First, breast milk and infant formulas differ in

their composition in more ways than just the fatty acid content. For instance, human breast milk contains a variety of hormones, immunoglobulins, antibodies, antioxidants, proteins, and other substances.[156] Infant formulas are designed to mimic some of these components but do not contain some of them or contain them in different form.[156] It is possible that many of these substances could contribute to healthy neurodevelopment. Second, the practice of breastfeeding itself involves enhanced opportunities for mother-infant bonding, not just a transfer of nutrients to the infant. Strong bonds between mother and infant are important for optimal neurodevelopment. Therefore, an association between the LCPUFA content of breast milk and enhanced development could be explained by bonding. Third are the potential confounding factors underlying whether and how long an infant is breastfed versus formula-fed. Whether an infant is exclusively breastfed, exclusively formula fed, or partially breastfed is determined by a number of factors, only some of which may be under the mother's direct control. These include attitudes about breastfeeding held by the woman and her family, counseling by health care providers, workplace policies, availability of information and help for feeding problems, and the ability for the woman and infant to be together.[156, 157] Women who breastfeed tend to be of higher socioeconomic status including educational attainment and they tend to be older.[158, 159] In the U.S., whites tend to choose breastfeeding more than other racial and ethnic groups.[157, 158] Other factors can affect whether a woman chooses to feed her infant a formula with DHA and AA or one without, although little research has examined these factors. These could include the cost (formulas with LCPUFAs cost more), knowledge about the purported benefits of LCPUFAs, whether she receives support from the Women, Infants and Children program which subsidizes enhanced formulas, and advice obtained from a health care provider or other trusted person.

These factors do influence breastfeeding initiation.[158, 160-162] These psychosocial factors might be associated with the development of the infant as well and so could confound the association between LCPUFA intake and infant development. And, a range of other factors (e.g., maternal diet, parity, depression) might be related to the composition of breast milk. These can confound an association between LCPUFA intake and developmental outcomes if they are associated with development as well.

Opportunities for Future Research

The limitations in previous studies leave a number of opportunities for future research. Most previous studies of LCPUFA intake and infant neurodevelopment employed measures of visual acuity as the main outcome measures of interest. However, recent studies have focused more on measures of global development. This can help assess whether the effects observed on visual acuity translate into lasting effects on cognition, but this approach cannot ascertain whether specific aspects of development are differently affected. Studies that assessed cognitive development most often employed the Bayley Scales of Infant Development even though the Bayley is a global measure of development and has limited predictive value. No studies have examined the effect of fatty acid intake on the development of cognitive, visual and motor development using the Mullen Scales of Early Learning (Mullen). Future studies should employ developmental measures like the Mullen that assess specific aspects of development, not global development, to help pinpoint what aspects of development may be affected.

Well-designed observational studies that can account for the role of key confounders can help clarify whether there is a positive effect on child development due to LCPUFA

intake or from breastfeeding. Addtionally, studies should be large enough to be able to detect an effect if one exists. Exposure measurement methods should be enhanced to account for the variability in breast milk concentrations of LCPUFAs and the variety of infant formulas available.

These opportunities are taken by the present study (Specific Aim 1). The present study employs a prospective design and includes over 350 infants in follow-up to 12 months of age. It employs very detailed exposure measurement techniques that incorporate breast milk LCPUFA content as well as formula LCPUFA content on a month-by-month basis. Finally, the developmental exams measure a number of specific developmental domains (e.g., language, motor, cognitive development).

Maternal perinatal depressive symptoms, trait anxiety, and perceived stress and infant development

Background and Significance

A wide range of biological, environmental, and psychosocial factors can influence child development. Maternal anxiety, perceived stress, and depression are several inter-related psychosocial factors that have been found to negatively influence development. Many women experience significant psychological distress during pregnancy, with rates of mood disorders, post-traumatic stress disorders, and anxiety disorders reaching prevalence rates of 14%, 7.7%, and 6.6%, according to some studies.[24, 163, 164] Approximately 10 to 15 percent of women experience elevated postpartum depressive symptoms during at least some portion of the six months after delivery, although prevalence estimates vary widely from 5 to 60 percent across studies.[23, 165, 166] Pregnancy and the birth of a new infant are

psychoemotional milestones, and women may be more susceptible to psychological distress if they lack supportive influences or are experiencing other major life stresses at the same time. Some of the strongest risk factors for elevated psychological distress during or after pregnancy include: low self-esteem, life stress, social support, marital relationship/status, history of depression, socioeconomic status, unplanned pregnancy, and infant temperament.[167]

Anxiety and stress during pregnancy have been associated with more somatic complaints, low birth weight, preterm birth, and impaired infant orientation and self-regulation.[168-171] The physiologic pathways that connect these have not yet been well-characterized but likely involve the interaction of cortisol and other hormones and the activity of the hypothalamic-pituitary-adrenal axis.[172]

A number of studies have addressed the association between maternal perinatal psychological health and infant developmental outcomes. The impetus for this area of research is the recognition that infant development is sensitive to the psychosocial environment and that these maternal symptoms may interfere with optimal care-giving. Several mechanisms could underlie this association and each is discussed below. First, common genetic factors could underlie both psychological health and abnormal development, although research has yet to uncover any specific genetic factors. Second, depressive symptoms, anxiety or stress can serve as a direct, negative psychosocial exposure for the infant.

First, it is possible that common genetic factors underlie mood and stress responses and abnormal development. Mother and child may share particular genotypes that predispose them to depression and poor neurodevelopment. A number of studies have

identified possible genetic contributors to depression, although it is thought that environmental factors play a larger role; thus, gene-environment interactions make research in this area challenging.[173] As examples, studies of depression have focused on the polymorphisms *HTTPLR* and *BDNF* V66M, and on a mutation for *TPH2*.[174-176] Progress in understanding the genetic underpinnings of depressive symptoms has been disappointing; results of most studies have not been reproducible. Nevertheless, it is conceivable that genotypes that affect neurotransmitter function, for instance, could contribute to the risk of mood disorders or elevated anxiety in adulthood as well as behavior during childhood.

Second, it is possible that maternal affect and anxiety inhibit healthy bonding between mother and child which in turn inhibits healthy development. The postpartum period is marked with major life changes and stresses for the new mother, most of which are offset by the great satisfactions a new baby also brings. But some women experience difficulty with this transition due to a lack of or misplaced expectations, other stressful life events, or lack of social support.[177-179] Conversely, women who exhibit many symptoms of depression are more likely to have low self-efficacy which can inhibit their ability to successfully serve in the maternal role.[180, 181] In addition, a difficult or unhealthy child can exacerbate these circumstances.[182, 183] Women who have trouble adapting to the maternal role may not interact optimally with their infant. Previous studies have found that mothers who were depressed or anxious tended to be more irritable and hostile, display less engagement with their child, and to interact and play less with their child.[36-39, 184] Infants of depressed mothers are more likely to be fussy, show fewer positive facial expressions, and are less vocal.[185] It has been well established that positive parent or caregiver relationships are critical for healthy development during infancy.[186]

Critical review of the literature

A handful number of studies have examined the association between maternal anxiety, depressive symptoms or stress (during pregnancy, postpartum) and developmental outcomes in very young children (i.e., global development, cognition). Outcomes during infancy are of primary interest in this proposal, but there are relatively few studies in this age group. The following inventory of the literature includes studies that assessed infants at up to 24 months of age. (Table 2.4).

Table 2.4. Previous studies of maternal depressive symptoms, anxiety, and stress and infant development

Author (year), n	*Exposure*	*Outcome measure(s), age*	*Results*
Brouwers (2001)[187], n=105	State-Trait Anxiety Inventory (32w)	Bayley (12m, 24m)	High anxiety group had lower PDI at 12 m (89 vs 97; t=2.35, p=0.02) than low anxiety group but not at 24m (98 vs 100; t=0.47, p=0.64). High anxiety group had lower MDI at 24m (95 vs 106) than low anxiety group but not at 12m (97 vs 103; t=1.85, p=0.07).
Carvalho (2008)[188], n=36	State-Trait Anxiety Inventory, Beck Depression Inventory	Bayley (12m)	No significant correlation between anxiety or depression and Bayley scores (no details provided).
Cornish (2005)[189], n=112	Composite International Diagnostic Interview (4m, 12m), CES-D (4m, 12m, 15m)	Bayley (15m), Receptive-Expressive Emergent Language Test (12m)	Chronic but not brief depression associated with lower MDI scores (t=6.61, p<0.025), lower PDI (t=9.60, p<0.025), and proportion not yet walking (t=1.79, p<0.025) than never depressed. No association between depression and total language quotient (F=0.33, p>0.10).
DiPietro (2006)[190], n=94	Profile of Moods Scale (24w gestation), State-Trait Anxiety Inventory (28w gestation), Daily Stress Inventory (24w gestation), Perceived Stress Scale (28w), Center for Epidemiologic Studies Depression Scale (32w), Pregnancy Experience Scale (32w)	Bayley (24m)	Pregnancy-specific distress associated with lower PDI (beta=-14.89, p<0.05). Prenatal depression and anxiety associated with better MDI and PDI scores (betas ranged 2.05-2.59, p<0.05).
Galler (2000)[38], n=226	General Adjustment and Morale Scale, Zung Depression and Anxiety Scale (7w, 6m)	Griffiths Mental Development Scales (7w, 3m, 6m)	Depressive symptoms at 7w associated with lower infant social and performance at 3m. Maternal mood at 6m associated with lower motor development at 6m
Huizink (2003) [ref]	Everyday Problem List, Pregnancy Related Anxieties Questionnaire-Revised (15-17w, 27-28w, 37-38w)	Bayley (3m, 18m)	Daily hassles (espec at 15-17w) associated with lower MDI at 8 mos
Kurstjens (2001) [191], n=1329	Research Diagnostic Criteria, Schedule of Affective Disorders and Schizophrenia	Griffiths Scales of Babies' Abilities (20m)	No association between postpartum depression or later depression or chronicity, severity or recency with cognitive outcomes.

Lyons-Ruth (1986)[192], n=NA	Center for Epidemiologic Studies Depression Scale (between 0-12m, 18m)	Bayley (12m)	CES-D associated with MDI (r=-0.32) and PDI (r=-0.30)
Murray (1993, 1996, 1999)[29, 31, 40], n=59	Edinburgh Postnatal Depression Scale (6w), Research Diagnostic Criteria, Schedule of Affective Disorders and Schizophrenia (18m), psychiatric interview (2m)	Piaget object concept (9m, 18m), Reynell (18m), Bayley (18m)	Depression associated with Bayley score among boys (interaction with gender R^2=0.04, p<0.04). Depression associated with object concept (18m, not 9m) (χ^2=6.20, p<0.05)

Many previous studies support the conclusion that perinatal depressive symptoms negatively affect cognition in children at least up to age 7, although whether effects can be seen during infancy remains unclear. Also, it seems that chronic depressive symptoms continuing well into childhood may affect cognition concurrently, but perinatal symptoms may continue to exert a detectable effect itself as the child grows older. Another theme apparent in previous research is that depressive symptoms may interact with other psychosocial contextual factors like socioeconomic status to produce the observed cognitive deficits. A common theme in several studies is the interaction between postpartum depressive symptoms and infant sex; boys might be more vulnerable to the effects of postpartum depression.[27, 29, 40, 191] However, large studies and more representative samples could help clarify whether this interaction is real.

In contrast with depressive symptoms which often increase and subside over time, trait anxiety is a fairly stable characteristic of the individual.[193] This is in contrast to state anxiety which is situational. As a result, it is difficult to separate out the critical exposure period for trait anxiety since it may continue to affect the infant from before birth into childhood.

It is important to consider the potential confounding role of the other maternal psychological factors (trait anxiety, perceived stress, depressive symptoms) in addition to factors like infant temperament, maternal self-esteem, and presence of the father or partner in studies of maternal psychological health and infant development. Psychosocial factors like anxiety and stress have some shared biologic underpinnings with depressive symptoms. Some studies have taken a thorough look at the role of contextual factors like marital relationships and the family environment and have found that these factors appear to explain

at least part of what appears to be the long-term effect of perinatal depressive symptoms.[194] Some studies also looking for long-term effects have found that the effects of perinatal depressive symptoms are mediated by attachment during infancy—those who were insecure infants tended to become children with cognitive deficits or behavioral problems.[194] And, the potential effect of anxiety seems to operate through parent-child interaction.[37, 38]

Several gaps remain in understanding the effect of maternal perinatal psychological health on child development. First, very few published studies conducted developmental assessments during infancy. As a result, it is unclear whether there is an observable effect during the first year of life. Second, many studies have been limited in size; several of the key studies in this area involved 120 infants or fewer and may have been underpowered since they have tended to find larger effects than large studies have found. Third, previous studies have been inconsistent in their ability to control for potential confounding factors like the presence of a supportive partner or the other exposures (perceived stress, trait anxiety, depressive symptoms, accordingly). Another shortcoming is that some past studies employed a cross-sectional design, making the temporal sequence between exposure and outcome uncertain. Also, the instruments and the timing of their administration have varied widely across studies, making it difficult to compare results. It is also possible that improved understanding of the specific aspects of development that are affected could be found if instruments were chosen to target those specific aspects and not broad assessments of development.

Opportunities for Future Research

The limitations in previous research leave a number of opportunities for future research. First, new studies should focus on development during infancy to determine whether there are observable effects during this early stage and should involve a sample size large enough to be able to detect an effect if one exists. They should consider potential confounders like partner support and anxiety, which some previous studies did not consider. Another opportunity is to use developmental tests like the Mullen which examine specific aspects of development and have not been applied previously in this context. The present study employs a prospective design and measures maternal psychological health during pregnancy and postpartum and follows infants to 12 months of age. Finally, the study assesses infants using the Mullen to explore aspects of development that have not been previously examined in this context.

Perinatal depressive symptoms and docosahexaenoic acid status

Background and Significance

Depression has been associated with low levels of n-3 fatty acids, particularly DHA, in red blood cell membranes and brain orbitofrontal cortex tissue of adults.[43, 44, 195] Pregnancy may exacerbate low DHA status because maternal stores of DHA are mobilized to support the rapid development of the fetal brain.[49] If depressed women have lower circulating DHA levels, this could potentially limit the amount of fatty acids available to the infant via breast milk. Women with elevated depressive symptoms postpartum have been shown to have lower plasma phospholipid levels of DHA than women who have few symptoms.[196] The precise relationship between n-3 fatty acids and depressive symptoms is

only recently becoming better understood; DHA and EPA are involved in neural function (phospholipase A_2 activity, neurotransmitter function, regulation of enzyme activity, inflammatory response, oxidative stress, and gene expression).[197] The direction of the association between fatty acid levels and depressive symptoms is debatable. It could be explained by diet (depressed individuals may consume a less healthy diet), by underlying abnormalities in how fatty acids are produced or utilized that both reduce fatty acid levels and induce depressive symptoms, by reduced synthesis or increased elimination of fatty acids among depressed individuals, or by deficiencies in fatty acid levels due to pregnancy or lactation that could trigger depressive episodes.[197, 198] Ecologic studies have shown that populations with high levels of fish consumption tend to have lower rates of depressive symptoms, and within populations those with higher fish intake may be at lower risk.[199-201] The fatty acid content of breast milk is correlated with the content in the maternal diet.[202] Whether women who experience elevated perinatal depressive symptoms also have lower breast milk DHA levels has been examined in only one study; an ecologic study by Hibbeln found the average DHA content of breast milk to be strongly associated with national postpartum depression prevalence rates.[42] In the postpartum period, maternal DHA stores are a major source of DHA via breast milk for the infant. Approximately 73% of U.S. infants are breastfed for at least some time, and 12% are exclusively breastfed for the first 6 months.[203] If women suffering from perinatal depressive symptoms have lower circulating fatty acids levels or produce an inadequate supply of fatty acids in their breast milk, their ability to provide adequate LCPUFAs to their fetus or infant for brain development may be inhibited.

Critical Review of the Literature

A handful of studies have noted lower DHA levels among women with postpartum depression, but the results have been inconsistent and none have examined DHA levels in the breast milk (Table 2.5).[196, 204-206] A few clinical trials have evaluated whether n-3 fatty acid supplements might prevent perinatal depression or reduce symptoms, with mostly negative findings.[207-211] Most have had very small sample sizes.

Table 2.5. Previous Observational Studies of Perinatal Depressive Symptoms and DHA Status

Author (year), n	*Depressive symptoms measure*	*DHA measurement*	*Results*
Browne (2006)[206], n=80	Composite International Diagnostic Interview	Plasma phosphatidylcholine	Percent of total n-3, EPA, DHA, n-6:n-3 ratio all unassociated with being in the Diagnosis, Screened High, or control group
De Vriese (2003)[204], n=48	Diagnostic and Statistical Manual IV	Serum phospholipids and cholesterol esters	DHA lower in women who developed postpartum depression compared to controls (phospholipid, 3.11% vs 4.22%, p=0.006) (cholesterol esters, 0.38% vs 0.61, p=0.02)
Makrides (2003)[205], n=380	Edinburgh Postnatal Depression Scale	Plasma phosphatidylcholine	A 1% increase in plasma DHA associated with 59% reduction in depressive symptoms
Otto (2003)[196], n=112	Edinburgh Postnatal Depression Scale	Plasma phosphatidylcholine	No association between depressive symptoms and DHA levels at delivery or for change in DHA postpartum. Increase in DHA:EPA ratio was higher in non-depressed than depressed group (OR=0.90, p=0.04)

Opportunities for Future Research

If women who experience elevated perinatal depressive symptoms also have lower DHA levels in their breast milk, then it would appear that perinatal depression could have both a psychosocial influence as well as a nutritional one on infants. Future studies should examine depressive symptoms at multiple time points during pregnancy and postpartum in relation to breast milk DHA content.

CHAPTER 3

SPECIFIC AIMS, HYPOTHESES, AND CONCEPTUAL MODEL

The following Specific Aims and Hypotheses build on previous research in this area and are presented with the goal of advancing understanding about the roles of LCPUFAs and maternal psychological health in infant development. The Specific Aims and their corresponding hypotheses are reflected in the Conceptual Model in Figure 3.1:

1- Examine the association between exposure to LCPUFAs, whether from breast milk or formula, and visual reception, language, and motor skills, and overall cognitive development at 12 months of age.

Hypothesis – Infants exposed to low levels of certain LCPUFAs (i.e., DHA, AA) exhibit less advanced visual reception, language, and motor skills, and overall cognitive development at 12 months of age than infants exposed to high levels.

2- Examine the association between:

a. maternal trait anxiety
b. perceived stress
c. perinatal depressive symptoms

and visual reception, language, and motor skills, and overall cognitive development at 12 months of age.

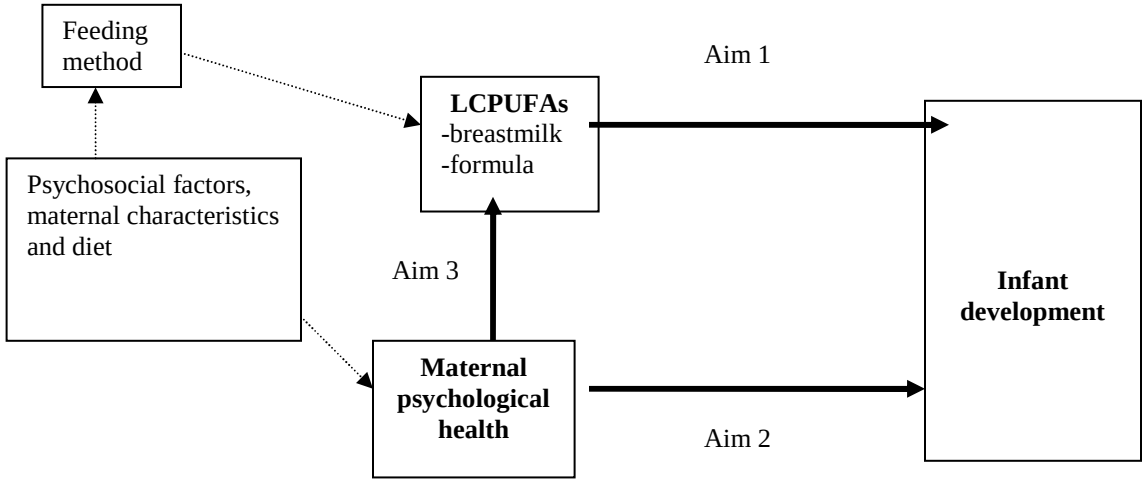
Hypothesis – Infants of women who demonstrate high levels of trait anxiety, perceived stress, or depressive symptoms perinatally exhibit less advanced visual reception, language, and motor skills, and overall cognitive development at 12

months of age than infants of women who demonstrate low levels of trait anxiety, perceived stress, or depressive symptoms.

3- Examine whether women who experience elevated perinatal depressive symptoms have lower levels of DHA in their breast milk.

Hypothesis – Women who have elevated depressive symptoms have lower DHA breast milk concentrations than women with fewer depressive symptoms.

Figure 3.1. Conceptual Model for Each Specific Aim and the Hypothesized Relationships Between the Factors



CHAPTER 4

METHODS

Study Design

Participant identification

The study population is derived from the PIN Study and the offspring of a portion of the women participants (Appendix 2).[45] The goal of the PIN Study was to identify factors associated with preterm birth. The goal of PIN Postpartum is to identify modifiable behaviors associated with high gestational weight gain and postpartum weight retention. The present study employs a prospective cohort design. (However, a component of Specific Aim 3 is cross-sectional in design.) PIN Babies focuses on child developmental outcomes in relation to the prenatal and early childhood environment.

All protocols were approved by the University of North Carolina (UNC) Biomedical Institutional Review Board; all participants provided written informed consent. The dataset is property of the University of North Carolina at Chapel Hill Carolina Population Center and is provided for analysis only in de-identified form. The University of North Carolina at Chapel Hill Public Health and Nursing Institutional Review Board and the National Institutes of Health Office of Human Subjects Research govern human subjects aspects of the present study. An exemption from full IRB review was obtained from both entities.

For the PIN Study, women were recruited from among pregnant patients at less than 20 weeks gestation seeking prenatal care at UNC Hospitals. Women were ineligible

if they were pregnant with multiple fetuses, could not communicate in English, were under age 16, had no access to a telephone, or were intending to go elsewhere for future care or delivery. Since the present study focuses largely on the infants resulting from pregnancies of women participating in PIN and because only the infants from the third wave of PIN enrollment (PIN3) were followed, the present study includes only women from PIN3.

Women (n=1,169) were eligible for PIN Postpartum if they completed the third wave of the PIN Study, agreed to be contacted after delivery and lived in the study area. Medical constraints (n=24), inability to recontact (n=207) or schedule (n=62), and refusal (n=187) resulted in 689 women who participated in a visit scheduled in the fourth postpartum month. The 305 women who provided a breast milk sample at this visit were eligible for the analysis pertaining to Specific Aim 3. Before the 12-month data collection visit, 45 became pregnant again and were ineligible to continue, 62 were unreachable, 29 moved from the study area, and 20 requested to leave the study. The PIN Babies protocol began after PIN Postpartum began and 125 infants were ineligible to complete the protocol because they reached 12 months of age before the study began conducting the infant assessments in homes.

Among the remaining 408 eligible maternal-infant pairs, some did not participate in the Mullen because the child was not present during the data collection (n=11, e.g. the mother completed the interview at her workplace), the child was asleep, sick, or fussy (n=21), the mother refused the child's participation (n=8), there was not enough time during the visit (n=3), or various other reasons (n= 7). The 358 infants who completed at least part of the Mullen were eligible for analyses pertaining to Specific Aims 1 and 2.

Methods for Study

Data collection

During pregnancy, information about depressive symptoms was collected via self-administered questionnaires before 20 weeks gestation and at 24-29 weeks gestation using the Centers for Epidemiologic Studies Depression (CES-D) scale.[212] The CES-D is intended to measure depressive symptoms in general population samples. The CES-D is designed as a short 20-item self-report tool applicable to the general population and has been shown to be useful in detecting depression in a variety of groups including pregnant women. It is not designed for clinical diagnosis of major depressive disorder. Components of depressive symptomology included in the CES-D include: depressed mood, feelings of guilt and worthlessness, retardation of psychomotor activity, appetite loss, and disturbed sleep.[212]

The questionnaire before 20 weeks gestation included the State-Trait Anxiety Inventory (STAI), the Rosenberg Self-Esteem Scale, and the MOS Social Support scale.[193, 213, 214] The trait score of the STAI is a measure of an individual's general feelings of anxiety and is relatively stable over time, in contrast to state anxiety which is situational.[193] Age, education, income and other demographic and lifestyle covariates were obtained via telephone at 17-22 weeks gestation. Delivery information was obtained from the hospital record. Preterm birth was defined as less than 37 completed weeks gestation at delivery, based on ultrasound if done before 20 weeks gestation or the date of the last menstrual period.

A home visit in the fourth postpartum month collected information on maternal and infant health and nutrition and updated information about family circumstances and maternal psychosocial health. The interview included the Edinburgh Postnatal Depression Scale (EPDS), a screening tool for depressive symptoms, and the 10-item Perceived Stress Scale to measure the extent to which one's circumstances are perceived as stressful.[215, 216] Information was collected about the number of feedings per day the infant was breastfed or fed formula or other foods for each month since birth. The specific names of up to two formulas were recorded for each month.

Women who were still breastfeeding were asked to use a breast pump at around 10 AM on the day of the postpartum visit to provide three 1.5 ml tubes of milk for storage at -80 degrees Celsius at the study office. Samples were analyzed for fatty acid content by two laboratories. Samples collected before April 1, 2005, were analyzed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, Minnesota). This lab was unable to complete the analysis for the second batch of samples, so those collected after April 1, 2005, were analyzed by the Clinical Nutrition Research Center, UNC (Chapel Hill, North Carolina).

When infants reached 12 months of age, trained PIN Babies study staff scheduled a visit to administer the Mullen Scales of Early Learning (Mullen) following procedures outlined in the Mullen manual.[217] The Mullen assesses cognitive functioning in children up to 68 months of age.

Exposure Measurement

1. Breastfeeding and LCPUFAs

Infants were classified as to the method by which they were fed: exclusively breastfed, almost exclusively breastfed, partially breastfed, or formula fed for each of the first 4 postnatal months individually and combined:

Exclusively breastfeeding:

Breastfeeding only, neither other liquids nor solids

Frequency of breastfeeding at the *m*th month of age >= 1 time/day and frequency of feeding other liquids or solids at the *m*th month of age = 0 times/day

Almost exclusive breastfeeding:

Breastfeeding, plus 1 or fewer times per day feeding any other liquids or solids

Frequency of breastfeeding at the *m*th month of age >= 1 time/day and frequency of feeding formula at the *m*th month of age <= 1 time/day

Partial breastfeeding:

Breastfeeding, plus feeding other liquids or solids

Frequency of breastfeeding at the *m*th month of age >= 1 time/day and frequency of feeding formula at the *m*th month of age > 1 time/day

Formula feeding:

Formula feeding only

Frequency of breastfeeding at the *m*th month of age = 0 times/day and frequency of feeding formula at the *m*th month of age > 1 time/day

It is ideal to use a categorization scheme that differentiates among infants who are not exclusively breastfed, and similar schemes are used in many observational studies that have detailed breastfeeding information available.[121, 218] It also parallels the recommendations of the World Health Organization and the Interagency Group for Action on Breastfeeding.[219, 220]

The concentration of fatty acids in commercially available infant formulas was obtained from the U.S. Department of Agriculture Nutrient Database for Standard Reference (and for one formula, from the manufacturer's website).[221, 222] Forty-one women responded to questions about formula use with information that was ambiguous as to whether they were using a product with DHA and AA added. Several answer choices on the data collection form did not distinguish between traditional or enhanced versions of the same formula, so it was not possible to determine which product was fed. Twenty-seven of the 41 women were successfully recontacted to clarify their formula choices, and 26 women were able to clarify which formula they had used.

The concentration of fatty acids in breast milk was obtained via laboratory analysis previously described. (In addition to serving as an exposure measure for Specific Aim 1, the concentration of DHA in breast milk also serves as the dependent variable for Specific Aim 3).

In addition to the laboratory values for the concentration of DHA and AA in breast milk, we calculated variables for exposure to DHA and AA using a composite of the breast milk values and the concentrations in the formulas fed during the 4 months. To

account for variation in feeding method across months, the breast milk and formula values were weighted by the feeding method for each month:

$$exposure = \sum_{m=1}^{4} \left(\mu_{b_{(i)}} k_{m_{(i)}} + \left(1 - k_{m_{(i)}}\right) \sum_{f=1}^{F_i} \frac{1}{F_i} \mu_{f_{(i)}} \right)$$

Where,

m=month since birth

μ=concentration of the fatty acid of interest in breast milk (*b*) or infant formula (*f*) (units - % of total fatty acids)

k=feeding pattern in *m*

1, if exclusive or almost exclusive breastfeeding

0.5, if partial breastfeeding

0, if formula feeding

f=formula product ($F_i \leq 2$)

For each month of exclusive breastfeeding or almost exclusive breastfeeding, the month was assigned the value for the DHA or AA concentration from the breast milk laboratory analysis. Each month of formula feeding was assigned a fatty acid value based on the concentration in the particular formula product fed that month. If more than one formula was fed that month, an average of the values for the 2 most often fed formulas served as the fatty acid value for that month. Each month of partial breastfeeding was assigned a value that is one-half the laboratory value for the breast milk plus one-half the average formula value (up to 2 formulas) for that month. Continuous DHA and AA exposure variables for all 4 months combined were calculated by summing the monthly values.

2. Maternal perinatal depressive symptoms, trait anxiety, and perceived stress

Continuous scores from the trait anxiety scale of the STAI were categorized into tertiles based on their observed distribution in the study participants. The trait anxiety scale measures the individual's general approach to stressful circumstances, which is considered stable over time.[193] The scale consists of 20 items, scored on a scale of 1 to 4 (Not At All to Very Much So) to form a composite. Cronbach's alpha ranged 0.89 to 0.92.[193]

Continuous scores from the Perceived Stress Scale (PSS) were categorized into tertiles based on their observed distribution in the study participants. The PSS is intended to measure the degree to which an individual perceives life situations to be stressful.[216] It contains 10 items which are scored by summing scores from 0 to 4 (Never to Very Often) for the negative items and reversing the scoring for the positive items.[223] The PSS has been found to correlate moderately with counts of life events, social anxiety, and depressive symptoms, although the PSS and the CES-D do not measure the same constructs.[216] Test-retest correlations ranged 0.55 to 0.85, depending on the time between assessments.[216]

Each item on the CES-D is scored on a four-point scale that ranges from "rarely or none of the time" to "most or all of the time" the respondent felt or experienced the particular item during the previous week.[224] The early work done to evaluate the instrument found moderate test-retest correlations (range: 0.48-0.67) between administrations of the instrument, which might be expected since the instrument is focused on feelings over the previous week which would shift over time.[212] The CES-D

has been found to discriminate well between individuals under care for depression and those in the general population: 70 percent of patients and 21 percent of a general population samples scored above 16.[212] The 16/17 cutpoint for the CES-D was chosen for the present study because several studies have reported reduced specificity of the CES-D using the 15/16 cutpoint for pregnant women because some symptoms of pregnancy (e.g., fatigue) are similar to CES-D components.[225-227]

Similar to the CES-D, the EPDS is designed to screen for depression but some of its 10 items are focused on circumstances related to pregnancy and motherhood. It is not intended to substitute for a thorough psychiatric evaluation and a high score does not necessarily equate with clinical diagnosis of Major Depressive Disorder. Instead, the EPDS in intended to be administered by research or clinical staff with minimal training and in a variety of settings, and it is quick to administer.[228] In its initial validation study, at a score of 13 or above, the EPDS was able to identify all women with Definite Major Depressive Illness and two-thirds of the women with Probable Major Depressive Illness.[228] Sensitivity for detection or major or minor depressive illness was 86 percent and the positive predictive value was 73 percent. Specificity was 78 percent. At the cutpoint of 9/10, the EPDS was estimated to miss only 10 percent of depression cases.[228] Expanding on the initial validation work by Cox et al., several others have validated this cutpoint in previous studies. It detects minor depression or increases the sensitivity of the instrument for depression and has been shown to identify cases of clinical depression with more than 90% sensitivity.[228-231]

For the purposes of Specific Aim 2, a variable was constructed to examine depressive symptoms across both the antepartum and postpartum periods based on scores on the CES-D and EPDS. Women were classified as having few depressive symptoms

postpartum (EPDS=0-9) (regardless of antepartum symptoms), minor or major depressive symptoms only during the postpartum period (EPDS > 9 and both CES-D scores=0-16), and minor or major depressive symptoms during both the postpartum and antepartum periods (EPDS > 9 and at least one CES-D score > 16). For the purposes of Specific Aim 3, each CES-D measure and the EPDS were used as individual exposure measures, and a composite of the three measures (a count of the number of times a woman scored above the cutpoint on an assessment) was also used.

Outcome Measurement – Infant Development

Raw scores from the five Mullen sub-scales were converted to age-specific t-scores for the four cognitive sub-scales (Visual Reception, Fine Motor, Receptive Language, Expressive Language) and the Gross Motor sub-scale of the Mullen. The cognitive sub-scale scores were summed and converted to a standard Early Learning Composite (Composite) score following procedures outlined in the manual.[217] All analyses adjusted for preterm status; however scores were not directly adjusted for gestational age at birth because we found the procedure recommended in the manual potentially overcorrected scores for preterm infants. The age-adjusted mean score for preterm infants was higher than the mean score for term infants for the Composite and all of the Mullen sub-scales except the Fine Motor scale, although the difference was statistically significant for the Receptive Language sub-scale only (age-adjusted mean Receptive Language t-score for preterm infants=49.2, term infants=45.8, t=2.5, p=0.01).

Test-retest reliability for each subscale has been found to be high, ranging r=0.82-0.85 for children under 24 months of age.[217] The Mullen subscales have been found to

have high correlations with the Bayley MDI (range=0.53-0.59) and less so with the PDI (range=0.21-0.52), indicating that the Mullen is more focused on cognitive abilities than psychomotor.[217] Since the Mullen is intended to be an assessment of specific abilities and not a test of global development, it makes sense that the Mullen and Bayley MDI have between 28 and 35 percent of the variance between the measures overlapping based on the squared intercorrelations.[217] The predictive value of the Mullen remains unclear, but it has been used to identify language delays in children who later were diagnosed with Autism Spectrum Disorders.[232] In another study, the Mullen was able to identify approximately two-thirds of 4-year-old children who went on to fail kindergarten, first, or second grade.[233]

Covariates

A range of possible covariates were identified based on previous studies and evaluated initially through the use of Directed Acyclic Graphs (DAG). DAGs are causal graphs that can be used to represent relationships between exposure, outcome and confounding factors, based on underlying assumptions about the biological mechanisms involved [234, 235]. The very basic concepts needed to interpret the DAGs for the present study are described here. (Articles by Robins and Greenland et al. provide additional discussion about the use of DAGs since this topic cannot be adequately covered here.[234, 235]) The exposure and outcome are connected by a single-headed arrow with a question mark, indicating the potential association under study. Variables represented in the diagram are connected by arrows to indicate the direction of various associations. A variable connecting the exposure and outcome via a series of arrows consistently pointing from the direction of

the exposure toward the outcome is considered to be on the causal path (they are mediators). A confounder is represented by a variable with arrows leading from it to both the exposure and outcome (either directly or through one or more other variables). A collider is a variable that has two or more arrows pointing into it. DAGs can be helpful to identify the minimally sufficient set of confounders that could be controlled for in an analysis and to avoid inducing confounding by adjusting for variables on the causal path. Variables that are not measured in the present study are represented by the notation "U" for "unmeasured." DAGs 1-3 pertain to Specific Aims 1-3, respectively

The presence of effect measure modification was assessed using multiple partial F-tests. Where no notable effect measure modification was detected, the change-in-estimate approach was used to refine the list to the variables to be included as confounders in regression models. The confounders included in the final models are presented in Table 4.1, along with their source and classification scheme.

Table 4.1. Potential covariates for each Specific Aim, sources, classification schemes

Covariate	*Description*	*Specific Aim*	*Source*	*Classification scheme*
Race and ethnicity	Maternal race and ethnicity	1	Phone interview 17-22 weeks gestation	0= All others 1= White, non-Hispanic
Maternal education	Maternal education	1, 2, 3	Phone interview 17-22 weeks gestation	0=0-12 years 1=13-16 years 2=17+ years
Maternal age	Maternal age	3	Phone interview 17-22 weeks gestation	0=17-20 1=21-30 2=31-47
Preterm birth	Preterm birth was defined as less than 37 completed weeks gestation at delivery, based on ultrasound if done before 22 weeks gestation or the date of the last menstrual period.	1, 2	Obstetric record	0=<37 completed weeks 1= >= 37 completed weeks
Feeding method	Degree of breastfeeding or formula feeding, first 4 post-natal months combined	1	Interview 4th month postpartum	2=Exclusive or almost exclusive breastfeeding 1=Partial breastfeeding 0=Formula feeding
Maternal anxiety	Trait anxiety	2	Self-administered questionnaire at <20 weeks gestation	1=0-<31 2=31-<40 3=40+
Maternal stress	Cohen's Perceived Stress Scale	2	Interview 4th month postpartum	1=0-<17 2=17-<23 3=23+

Maternal smoking	Ever smoked cigarettes during specified time period	1, 3	Phone interview 27-30 weeks gestation Interview 4th month postpartum	0=never smoked during pregnancy or to 4 months postpartum 1=ever smoked during pregnancy or to 4 months postpartum
Parity	The number of prior live or stillbirths	1	Phone interview 17-22 weeks gestation	0=none 1=1 or more
Maternal self-esteem	Rosenberg's self-esteem scale	2	Self-administered questionnaire at <20 weeks gestation	1=0-<50 2=50-<56 3=56+
Infant sex	Infant sex	1	Delivery record	0=female 1=male
Maternal n-3 intake	Estimate of daily intake of n-3 fatty acids	3	Food Frequency Questionnaire at 24-29 weeks gestation	Continuous
Presence of spouse or partner	Currently married or living with partner	3	Interview 4th month postpartum	0=no 1=yes
Laboratory	Variable for laboratory	1, 3	Laboratory record	0=University of North Carolina 1=University of Minnesota 2=University of Minnesota

Data Analysis

Overview

The conceptual model and specific aims laid out previously provide the framework for the analysis plan. Some common procedures apply to all specific aims. First, all exposure variables, outcome variables, and potential covariates were examined using univariate techniques to assess the degree of missing data and characteristics of the distribution of each (e.g., mean, median, mode, standard deviation, range, skewness, kurtosis).

Second, based on the previously constructed exposure and outcome variables, bivariate analyses (chi-square, simple linear regression, odds ratios) explored unadjusted associations between exposures and outcomes. Multiple partial f-tests checked for the presence of effect measure modification by the potential covariates included in the DAGs. Then, an assessment was done for whether there was potential confounding by each potential covariate using the change-in-estimate approach. Confounders were defined as variables identified via the DAGs and subsequently via the change-in-estimate approach. Finally, multivariable linear regression was used to examine exposure-outcome relationships. Graphical methods assisted in checking the assumptions underlying linear regression. For Specific Aim 3, the dependent variable (DHA) was log transformed to provide a better fit. Next, multiple imputation was used to fill in values for missing data for the analyses pertaining to Specific Aim 2 so that all women in the dataset had three depression scores (2 during pregnancy and 1 in the fourth postpartum month). Some infants had one or more missing sub-scale scores for the Mullen, and these were imputed as well as other covariates with a small proportion of missing values. Multiple imputation is a good approach when

more than one variable has missing data to be imputed.[236, 237] It will increase the statistical power and also decrease potential bias that might result if analyses relied on only complete case analysis, if the imputation model is well-specified. The Markov chain Monte Carlo method using SAS procedures (PROC MI) produced 10 replications of the dataset with missing values imputed. The imputed datasets were analyzed using SAS (version 9.1) linear regression procedures and combined with PROC MIANALYZE to produce the effect estimates reported.[238]

Directed Acyclic Graphs

The DAG displayed in Figure 4.1 guided the analysis for Specific Aim 1. The box labeled "LCPUFA intake" corresponds to the calculated LCPUFA variables described previously. While those calculations consider the role of feeding method, they do not adequately account for the important differences between infants who are breastfed and those who are formula fed. As a result, feeding method was treated as a confounder in this analysis. Maternal diet is an important influence on breast milk fatty acid composition, and socioeconomic status and race and ethnicity both influence breastfeeding duration and formula choice, so these all should be considered as potential confounders given their association with developmental outcomes. The other covariates noted in Figure 4.1 were handled by controlling for the potential confounders just mentioned.

Figure 4.1. Directed Acyclic Graph #1– Specific Aim 1

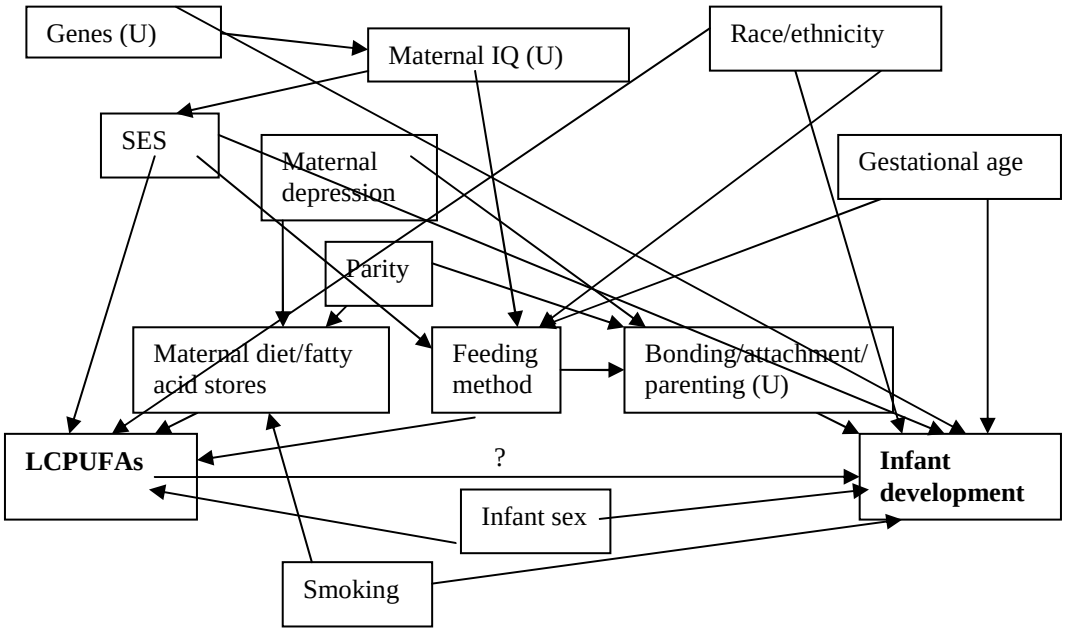
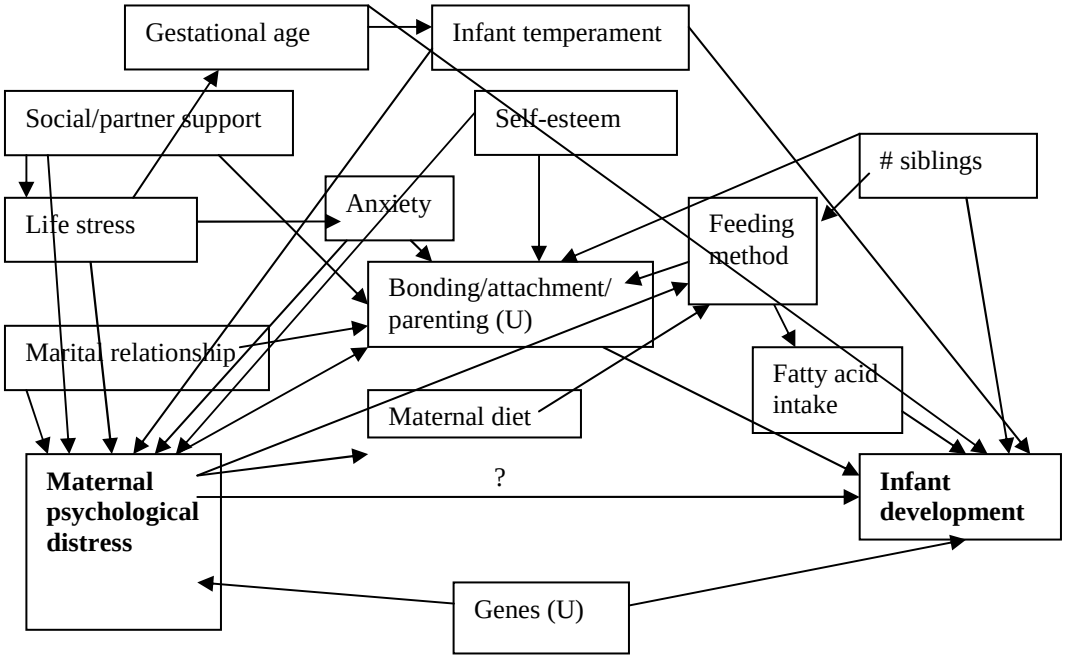


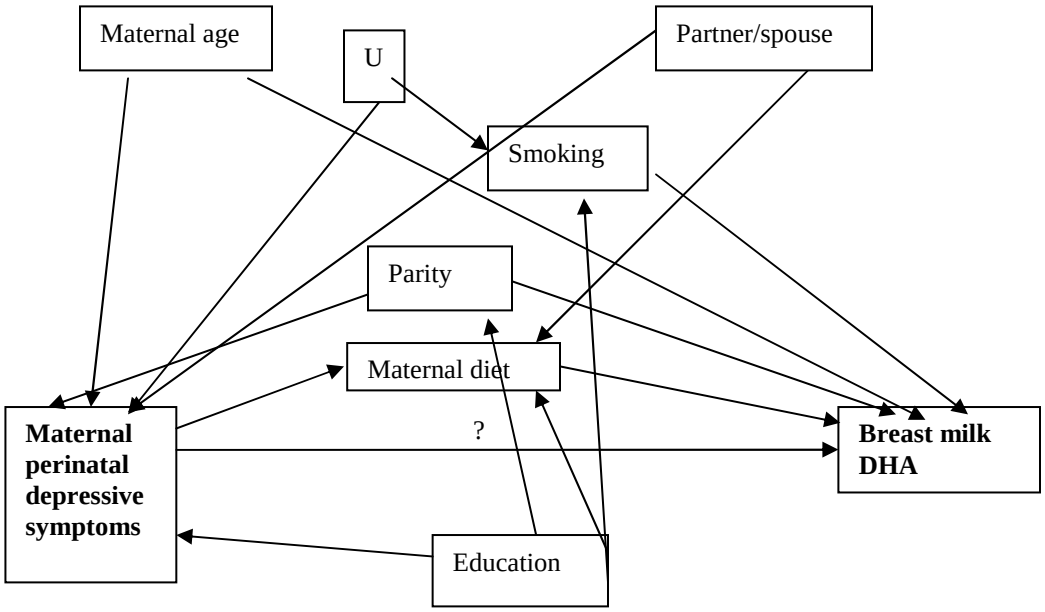
Figure 4.2 displays the DAG for Specific Aim 2. Taking into consideration all the potential paths and seeking the smallest possible set of potential covariates for adjustment, the following list of potential confounders will be considered: infant temperament, maternal stress, maternal anxiety, social support, marital relationship, and maternal self-esteem.

Figure 4.2. Directed Acyclic Graph #2– Specific Aim 2



For Specific Aim 3, the breast milk LCPUFA values only (not incorporating formula values), in continuous form, will serve as the dependent variables for this component on depressive symptoms and DHA content of breast milk (while recognizing the uncertainty about the temporal direction of this potential association). Potential confounders were identified from previous literature. The direction of the association between maternal diet and depressive symptoms is debatable. As a result, models initially included diet but were reanalyzed with diet excluded to observe whether effect estimates changed.

Figure 4.3. Directed Acyclic Graph #3– Specific Aim 3



CHAPTER 5

RESULTS

Specific Aim 1: Long-chain polyunsaturated fatty acids in breast milk and formula and infant cognitive development

Introduction

The role of certain dietary long-chain polyunsaturated fatty acids (LCPUFAs) in healthy brain development has been of interest for several decades. Fatty acids are important constituents of cell membranes, and LCPUFAs, especially docosahexaenoic acid (DHA), accumulate in large concentration in the developing brain of the infant, particularly in the gray matter and in the rod photoreceptors of the retina.[7-10] DHA and other LCPUFAs like arachidonic acid (AA) are present in human breast milk. A number of studies have found that feeding LCPUFA-containing formulas is associated with positive outcomes like improved global development; other studies have found no association with development, yet these fatty acids are added to many commercially available infant formulas.[15, 104, 110-113, 116, 117, 221, 239, 240]

Inconsistency in results may be because of variation in the formulas fed to study participants, diversity in the definition of comparison groups, differences in outcome measurements used, sample size constraints, limited length of follow-up, and poor control of confounding. Most studies in this area have been infant feeding trials often involving only preterm infants. A randomized design can be an advantage, but these studies may have

limited generalizability because they rely on intent-to-treat analysis by group rather than effective dose of LCPUFAs reaching the infant and are not based on the actual breast feeding patterns reflecting the combinations of breast milk and formulas infants are fed in the general population. Most studies used a measure of global development like the Bayley Scales of Infant Development which is unable to separately evaluate specific aspects of development, like language, to see whether some aspects of development are benefited more than others.

Because only 12% of U.S. children are exclusively breastfed at 6 months of age, most children are introduced to formula at some point in infancy.[203] As a result, both breast milk and infant formulas can be important sources of LCPUFAs in infant diets, but their relative contribution changes with shifts in feeding method across infancy. We found no studies that examined developmental outcomes in relation to the LCPUFA content of both breast milk and formula while considering changes in feeding method across time.

Improved understanding of the role of LCPUFAs in development could help refine guidance about infant feeding practices. This study addresses three distinct questions: 1) is the extent of breastfeeding during the first 4 postnatal months associated with visual reception, language, and motor skills, and overall cognitive development as measured via the Mullen Scales of Early learning (the Mullen) at 12 months of age? 2) Is the LCPUFA concentration of breast milk associated with development (Mullen scores) among infants who are exclusively breastfed? 3) Is the LCPUFA concentration of the breast milk and formulas infants are exposed to during the first 4 postnatal months associated with development (Mullen scores)?

Methods

Study population

Data were collected as part of the Pregnancy, Infection, and Nutrition Study (PIN) and its postnatal follow-up components, PIN Postpartum and PIN Babies. The goal of the PIN Study was to identify factors associated with preterm birth.[45] The goal of PIN Postpartum is to identify modifiable behaviors associated with high gestational weight gain and postpartum weight retention. PIN Babies focuses on child developmental outcomes in relation to the prenatal and early childhood environment. All protocols were approved by the University of North Carolina (UNC) Biomedical Institutional Review Board; all participants provided written informed consent.

Women (n=1,169) were eligible for PIN Postpartum if they completed the third wave of the PIN Study, agreed to be contacted after delivery and lived in the study area. Medical constraints (n=24), inability to recontact (n=207) or schedule (n=62), and refusal (n=187) resulted in 689 women who participated in a home visit in the fourth postpartum month. The PIN Babies protocol began after PIN Postpartum began; as a result, 125 infants were ineligible to continue to PIN Babies. Also, 45 women became pregnant again, 62 were unreachable, 29 moved from the study area, and 20 requested to leave the study. Among the remaining 408 eligible maternal-infant pairs, some did not participate in the Mullen assessment during a home visit at 12 months of age because the child was not present during the data collection (n=11, e.g. the mother completed the interview at her workplace), the child

was asleep, sick, or fussy (n=21), the mother refused the child's participation (n=8), there was not enough time during the visit (n=3), or various other reasons (n= 7).

Exposure measurement

The first home visit collected information for each month about the number of feedings per day the infant was breastfed or fed formula or other foods.

Women who were still breastfeeding were asked to use a breast pump at around 10 AM on the day of the postpartum visit to provide three 1.5 ml tubes of milk for storage at -80 degrees Celsius at the study office. Samples were analyzed for fatty acid content by two laboratories. Samples collected before April 1, 2005, were analyzed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, Minnesota). This lab was unable to complete the analysis for the second batch of samples, so those collected after April 1, 2005, were analyzed by the Clinical Nutrition Research Center, UNC (Chapel Hill, North Carolina). Fatty acid extraction was performed on 0.5 ml samples of breast milk mixed with 0.5 ml 0.9% saline using the method of Bligh and Dyer.[241] The chloroform phase was transferred to a clean tube and evaporated to dryness under a nitrogen flow. Residual lipids were saponified and fatty acids were transmethylated by sequentially adding 1ml 4.25% NaOH in $CHCl_3$:MeOH (2:1, v/v) and 1N HCl in saline.[242] After vigorous mixing, the samples were centrifuged at 1500g for 5 minutes. The fatty acid methyl esters in the lower phase were evaporated to dryness under nitrogen and then resuspended in 50 μl undecane and analyzed by capillary gas chromatography (injector 240°, detector 280°). To check the efficacy of

the extraction, 0.1 mg 17:0 standard (diheptadecanoyl phosphatidylcholine) was added. The individual fatty acids were identified by comparison with authentic standards (Nu Chek Prep, Elysian, MN). Data were analyzed using Perkin Elmer Totalchrom Chromatography Software, version 6.2 (Somerset, NJ).

Infants were classified as to the method by which they were fed: exclusively breastfed, almost exclusively breastfed, partially breastfed, or formula fed for each of the first 4 postnatal months individually and combined. Infants who were breastfed for all feedings per day were considered exclusively breastfed. Infants who were breastfed and also supplemented 1 formula feeding per day were considered almost exclusively breastfed. Infants who were fed infant formula for all daily feedings were considered formula fed. All other infants were partially breastfed.

The specific names of up to two formulas were recorded for each month. The concentration of fatty acids in commercially available infant formulas was obtained from the U.S. Department of Agriculture Nutrient Database for Standard Reference (and for one formula, from the manufacturer's website) which was used to assign values for the fatty acid concentrations for each formula.[221, 222]

We used a combination of the laboratory values for the concentrations of DHA and AA in breast milk and concentrations in formula to create composite DHA and AA exposure variables to reflect feeding patterns over the first 4 months. To account for variation in feeding method across months, the breast milk and formula values were weighted by the feeding method for each month:

$$exposure = \sum_{m=1}^{4} \left(\mu_{b_{(i)}} k_{m_{(i)}} + \left(1 - k_{m_{(i)}}\right) \sum_{f=1}^{F_i} \frac{1}{F_i} \mu_{f_{(i)}} \right)$$

Where,

m=month since birth

μ=concentration of the fatty acid of interest in breast milk (*b*) or infant formula (*f*) (units - % of total fatty acids)

k=feeding pattern in *m*

1, if exclusive or almost exclusive breastfeeding

0.5, if partial breastfeeding

0, if formula feeding

f=formula product ($F_i \leq 2$)

For each month of exclusive breastfeeding or almost exclusive breastfeeding, the month was assigned the value for the DHA or AA concentration from the breast milk laboratory analysis. Each month of formula feeding was assigned a fatty acid value based on the concentration in the particular formula product fed that month. If more than one formula was fed that month, an average of the values for the 2 most often fed formulas served as the fatty acid value for that month. Each month of partial breastfeeding was assigned a value that is one-half the laboratory value for the breast milk plus one-half the average formula value (up to 2 formulas) for that month. Continuous DHA and AA exposure variables for all 4 months combined were calculated by summing the monthly values.

Outcome measurement

When infants reached 12 months of age, trained PIN Babies study staff scheduled a visit to administer the Mullen Scales of Early Learning (Mullen) following procedures outlined in the Mullen manual.[217] The Mullen assesses cognitive functioning in children up to 68 months of age. Raw scores were converted to age-specific t-scores for the four cognitive sub-scales (Visual Reception, Fine Motor, Receptive Language, Expressive Language) and the Gross Motor sub-scale of the Mullen. The cognitive sub-scale scores were summed and converted to a standard Early Learning Composite (Composite) score following procedures outlined in the manual.[217] Scores were not directly adjusted for gestational age at birth because we found that the procedure recommended in the Mullen manual overcorrected scores for preterm infants.[243] However, an indicator of preterm status was included in regression models.

Covariate measurement

Several potential covariates were measured during pregnancy. Data on maternal age, education, and other demographic and lifestyle covariates were obtained via telephone at 17 to 22 weeks gestation. A self-administered questionnaire before 20 weeks gestation included the State-Trait Anxiety Inventory (STAI) to measure trait anxiety.[193] Parity was defined as the number of prior live or stillbirths. Delivery information was obtained from the hospital record. Preterm birth was defined as less than 37 completed weeks gestation at delivery, based on ultrasound if done before 22 weeks gestation or the date of the last menstrual period. At the first

home visit, women were asked about current smoking and family income and completed the Edinburgh Postnatal Depression Scale to assess depressive symptoms.[228]

Statistical analysis

We built three sets of models corresponding to the three research questions mentioned previously:

-*Breastfeeding models*: we compared infants who were in the exclusively or almost exclusively breastfed groups for the first 4 postnatal months to those who were formula fed among each Mullen sub-scale and the Composite score. Infants who were partially breastfed were also compared to formula fed infants.

-*Breast milk models*: we examined the association between the DHA and AA concentration of the breast milk samples and Mullen scores among infants who were exclusively breastfed for the first 4 postnatal months combined.

-*Combined models*: we used the constructed variables that incorporated breast milk and infant formula DHA and AA concentration according to the feeding method for each month, and these were examined in relation to Mullen scores.

Linear regression models were used to examine these associations. SAS software version 9.1 was used for all analyses.[238] The residuals were found to be approximately normally distributed using kernel density and Q-Q plots and to have constant variance using the White test.[244]

Potential covariates were identified *a priori* based on previous studies and constructed Directed Acyclic Graphs.[234, 235] Adjusted models incorporated confounders that resulted in a change of greater than 10% in the beta coefficient for the exposure variable when removed from a model. Potential covariates considered included: income (categorical variable for percentage of the federal poverty level), education (high school or less, college, more than college), maternal age (16-20, 21-30, 31+), parity (0 or 1+), race and ethnicity (white, non-Hispanic versus all others), infant sex, preterm birth, trait anxiety (tertiles), and depressive symptoms (EPDS 0-9 versus 10+). Multiple partial F-tests checked for the presence of effect measure modification.

Because some women who breastfed for at least several weeks did not provide a breast milk sample, we explored the effect on our results of imputing missing fatty acid values for those women (n=88, 24.6% of women). The Markov chain Monte Carlo method using SAS procedures (PROC MI) produced 10 dataset replications with missing values imputed, and these were combined with PROC MI ANALYZE for linear regression analysis.[236-238]

Results

To be eligible for the present analysis, infants completed at least one sub-scale of the Mullen at (n=358; 343 completed all sub-scales). All infants had information available about feeding method. Overall, 57.8% of infants were exclusively or almost exclusively breastfed, 31.3% partially breastfed, and 10.9% formula fed for the 4 months. When examined month-by-month, it is apparent that breast feeding gradually declined while formula feeding increased. By the time of the first home visit, 68 (19.0%) of the

358 women eligible for this analysis had discontinued breastfeeding, 231 (64.5%) provided a breast milk sample (230 were analyzable), 17 (4.7%) were unable to provide a sample, and 3 (0.8%) refused participation in breast milk collection.

Women who discontinued breastfeeding before the visit or were unable to refused to provide a sample were more likely to smoke (OR=7.8, 95% CI: 3.5, 17.3), have a preterm infant (OR=3.0, 95% CI: 1.5, 6.3), have a 12^{th} grade or less education (OR=7.9, 95% CI: 3.7, 17.0), and be other than white, non-Hispanic (OR=3.4, 95% CI: 1.9, 6.3), compared to women who provided a breast milk sample.

Table 5.1 shows the distribution of feeding method for each month and for all 4 months combined. Among infants not exclusively breastfed, the proportion of infants fed primarily a formula with DHA and AA added decreased from 72.4% in the first postnatal month to 61.2% in the fourth month (Table 5.1). Eight infants were fed more than one formula product in one or more months. Only 3 infants were fed only formulas with no DHA and AA added for all 4 months. A few women (n=6) were unable to provide enough information about the formula they fed in one or more months to determine whether DHA and AA were added, and they were excluded from the combined models. The percent of fatty acids in the formulas ranged from 0.30 to 0.37% for DHA and 0.50 to 0.67% for AA.

Feedings of other foods like cereal, fruit, juice or other milk substitutes (i.e., cow's milk) were not commonly seen in this cohort until infants were 3 months old. At that time the most commonly eaten foods were cereal (14% of infants fed cereal one or more times per day), fruit or vegetables (4%), and juice (4%).

Table 5.2 presents characteristics of infants and mothers in the study in relation to the concentration of DHA and AA in breast milk, feeding method, and Mullen Composite scores. Women with more years of education ($t=-2.5$, $p=0.02$) or a term infant ($t=-2.5$, $p=0.02$) had higher breast milk DHA levels on average than women with less education or an infant born preterm. Mean DHA and AA concentration also differed by laboratory (for DHA: $t=-6.3$, $p<0.01$; for AA: $t=-6.3$, $p<0.01$). Women who were white, non-Hispanic, more highly educated, did not smoke, or had an infant at term tended to breastfeed more than women of another race and ethnicity, women with less education, smokers or women with preterm infants.

. The infants had mean Mullen t-scores and Composite scores similar to expected scores for a general population sample of infants, with mean sub-scale t-scores around 50 and a mean Composite score of 99. Women with more years of education and non-smokers tended to have infants with higher Mullen Composite scores; female and term infants also tended to score higher.

Only education, race and ethnicity, smoking and preterm status were found to be important confounders in the breastfeeding models. In the unadjusted models, infants who were exclusively or almost exclusively breastfed for the first 4 months exhibited more advanced cognitive development compared to infants who were formula fed, but these differences were notably attenuated upon adjustment (Table 5.3). Infants of women who discontinued breastfeeding before breast milk collection did not score lower on the Mullen Composite than infants of women who had a breast milk sample.

The breast milk models included variables for lab batch, infant sex, parity, smoking, and preterm status as confounders. Within the group of infants exclusively

breastfed for the first 4 months, there were no notable differences in Mullen sub-scale or Composite scores in relation to the concentration of DHA in breast milk (Table 5.4). Gross motor development was positively associated with AA concentration.

In the combined models incorporating breast milk and formula DHA or AA concentrations and feeding method, there was no observed difference between infants in terms of Mullen sub-scale and Composite scores in relation to the weighted combination of DHA or AA in breast milk and formulas fed (Table 5.5). Adjusted models included laboratory, feeding method for the 4 months combined, infant sex, parity, smoking, education, race/ethnicity, and preterm status as confounders.

The results from the combined models were very similar when including imputed fatty acid values for women (n=88) who had no breast milk sample, so we report the estimates from complete case analyses.

Discussion

Our goal was to address three questions about infant feeding and fatty acid exposure in relation to developmental outcomes at 12 months of age. The breast feeding models addressed whether the extent of breastfeeding during the first 4 postnatal months is associated with cognitive development at 12 months. Infants who were exclusively or almost exclusively breastfed for the first 4 months exhibited better cognitive development on several Mullen scales than formula fed infants but estimates were imprecise and attenuated after accounting for the role of maternal smoking, education, race and ethnicity and preterm birth. These results are similar to some previous studies that found breastfeeding duration or exclusivity to be unrelated to infant development,[128, 136] but in

contrast to other studies that noted enhanced development among breastfed infants.[122, 135, 139, 141, 142] Studies vary in their ability to control for key confounders of the association between breastfeeding and infant development, and this may explain some of the variation in results. Also, studies often use tests of global development which may not reveal whether specific aspects of development are affected differently from others.

In models focused on breast milk, we found the LCPUFA concentration to be unrelated to infant development among exclusively breastfed infants. Breast milk is the sole source of LCPUFAs for some infants through at least the first few months of life. The mean DHA and AA concentrations in the breast milk samples in this study were similar to those in other studies of U.S. women; for instance, Birch et al. found mean levels of 0.29% as DHA and 0.56% as AA.[63] Studying the association between breast milk DHA and AA levels and developmental outcomes among children who were exclusively breastfed avoids the potential for confounding due to the choice of feeding method; this is a strength of the present study. Most studies that focused on breast milk as the source of DHA have been maternal supplementation trials, and results have been equivocal possibly because global tests of development are generally used.[144-146]

Because many infants are not exclusively breastfed, we built models that combined information about the DHA and AA concentration of breast milk samples and the DHA and AA concentration of formulas fed. The relative DHA and AA contribution from each source was accounted for by weighting both concentrations by the feeding method for each month. We are aware of no previous studies that incorporated information about changing feeding methods across time with measures of LCPUFA exposure from multiple sources. This method of assessing exposure may better reflect

the reality of infant feeding, where feeding methods and formula choices change over time. The results of these combined models showed no association between the constructed LCPUFA variables and developmental outcomes.

Several potential weaknesses underlie our findings. First, we had too few infants fed only formulas without DHA and AA to be able to compare them to infants fed only formulas with DHA and AA. When these data were collected, commercial formulas with DHA and AA added already comprised a substantial proportion of the formulas on the market. Second, our method of assessing LCPUFA exposure by combining breast milk and formula sources is novel. It treats LCPUFAs from breast milk and from formula as equally available to the infant; it is possible that the physiologic benefit to infants from LCPUFAs varies by source. The method assumes the concentration of DHA and AA in breast milk is constant across lactation which is supported by recent studies, but may not be true for all women.[80, 81] While our infant feeding interview was very detailed, it could not quantify amount consumed.

Some infants were fed foods other than breast milk or formula one or more times per day by the time they reached 4 months of age. These foods could be an additional source of LCPUFAs. Typical baby foods are not a significant source of DHA (baby foods with DHA added were not commercially available during the time of this study), unless mixed with breast milk or formula. Feeding baby foods could reduce overall LCPUFA exposure since the foods can displace breast milk and formula in the diet. It was not possible with the available data to assess LCPUFA exposure from baby foods.

Finally, because some women stopped breastfeeding before the study visit or refused to provide a breast milk sample, we were unable to directly measure their infants'

exposure via breast milk and so they were excluded from the combined models. We imputed breast milk DHA and AA values to be able to include them in the models and explore whether results would be altered. Results were virtually unchanged, so we opted to present results with these women excluded.

This study has several strengths; the first is generalizability. Much of the research on the potential benefits of LCPUFAs has adopted a randomized design at least in part to eliminate issues of confounding. Many of these studies tested formulas that were developed specially for research purposes and not for wide consumption. Parents who were compliant with the study protocols would have fed their infants exclusively the assigned formula product and not breast milk or other formulas. It is unclear whether the experience of the infants who participated in these studies is representative of formula-fed infants in the general population who are fed commercially available formulas. Also, many infants in the general population are fed some breast milk and some formula, and their experience is generally not reflected in these studies.

Second, we incorporated LCPUFA content from breast milk and formula with detailed, monthly information about feeding method. Most studies do not take into account the wide variation in the extent to which infants are breastfed or formula fed and the differences in LCPUFA content. Previous studies have not had the benefit of this detailed information to examine dose-response relationships. Also, we were able to include infants fed (for one to three months) formulas with no DHA or AA added. This increased the variability of exposure compared to what might be observed in a sample of infants born more recently because almost all formula products today contain DHA and AA. Most randomized studies employed intent-to-treat analysis. This is normally a

strength, but it may obscure variations in exposure that occur.[15, 112] For instance, many infants in the general population are exposed to LCPUFAs from both breast milk and formula. Measuring exposure based on the actual breast feeding patterns and combinations of breast milk and formulas infants are fed would be more applicable to the general population. A major strength of a randomized design is control of confounding factors. However, this advantage may not outweigh the disadvantage of the lack of generalizability; a non-randomized design with a closer-to-representative sample and good control of confounding factors might be more informative in this case.

Third, this study was able to account for several factors (e.g., education, smoking) that confound the association between feeding method and infant development. Studies that compare breastfed children to formula-fed children are subject to criticism because of inadequate control of important confounding variables. Because it is considered unethical to randomize infants to breastfeeding versus formula feeding, most randomized studies only randomize within the formula fed group (to a LCPUFA formula or a control formula). The breastfed group is generally made up of infants who mothers intend to breastfeed while the formula-fed groups are infants whose mothers intend to formula feed.[104, 116] Thus, those studies are subject to some of the same confounding issues as observational studies.

Finally, few studies simultaneously assessed multiple aspects of infant development, not just global development, in relation to feeding method or LCPUFA exposure. The Mullen offers sub-scales corresponding to specific developmental areas, exhibits high test-retest reliability, and correlates strongly with the Bayley MDI.[217] To our knowledge, the Mullen has not previously been used to examine the associations

between feeding method or LCPUFA exposure and infant development. This study employed trained interviewers who used standard assessment techniques with high reliability.

Conclusion

The results of our analysis suggest that differences in visual reception, language, and motor skills, and overall cognitive development among infants at 12 months may be explained by factors other than DHA and AA exposure, breastfeeding duration and exclusivity, and the socio-demographic factors that underlie choices about feeding method. It is possible that the effects of LCPUFA intake are limited to visual acuity and are transient, or that more lasting effects do not become apparent until after infancy. Continued efforts to improve LCPUFA exposure assessment in population-based studies may clarify possible associations.

Table 5.1. Infant feeding characteristics in the PIN Babies Study, 2002-2006

	1st postnatal month (n, %)	*2nd postnatal month*	*3rd postnatal month*	*4th postnatal month*	*First 4 months combined*
Feeding method[a]					
Exclusively or almost exclusively breastfed	266 (74.3)	251 (70.1)	229 (64.0)	215 (60.1)	207 (57.8)
Partially breastfed	53 (14.8)	36 (10.1)	41 (11.5)	40 (11.2)	112 (31.3)
Formula fed	39 (10.9)	71 (19.8)	88 (24.6)	103 (28.8)	39 (10.9)
Primary formula fed (for those not exclusively breastfed)					
DHA, AA added	79 (72.4)	85 (68.0)	92 (63.4)	101 (61.2)	--
No DHA, AA added	24 (22.0)	34 (27.2)	49 (33.8)	61 (37.0)	--
unknown	6 (5.5)	6 (4.8)	4 (2.8)	3 (1.8)	--

[a] Infants who were breastfed for all feedings per day for the first 4 postnatal months were considered exclusively breastfed. Infants who were breastfed plus up to 1 formula feeding per day were considered almost exclusively breastfed. Infants who were fed infant formula for all daily feedings were considered formula fed. All other infants were partially breastfed.

Table 5.2. Characteristics of Infants in the PIN Babies Study and Associations with Breast Milk DHA and AA Concentrations, Feeding Method, and Mullen Scales of Early Learning Composite Score, 2002-2006

	Mean DHA concentration of breast milk (% of total fatty acids) (SD)	*Mean AA concentration of breast milk (% of total fatty acids) (SD)*	*Feeding method (first 4 postnatal months)*[a]			*Mullen Composite (mean, SD)*
			Exclusively or almost exclusively breastfed (n, %)	Partially breastfed (n,%)	Formula fed (n,%)	
Overall	0.28 (0.22)	0.57 (0.20)	207 (57.8)	112 (31.3)	39 (10.9)	99.4 (13.6)
Race and ethnicity						
White, non-Hispanic	0.28 (0.23)	0.56 (0.18)	184 (65.0)	77 (27.2)	22 (7.8)	100.1 (13.6)
All others	0.25 (0.17)	0.60 (0.30)	23 (30.7)	35 (46.7)	17 (22.7)	97.1 (13.8)
Maternal education						
High school or less	0.20 (0.10)	0.50 (0.19)	7 (14.0)	29 (58.0)	14 (28.0)	95.7 (14.7)
Greater than high school	0.28 (0.22)	0.57 (0.20)	200 (64.9)	83 (27.0)	25 (8.1)	100.1 (13.4)
Preterm birth						
Preterm	0.21 (0.11)	0.56 (0.17)	14 (31.1)	22 (48.9)	9 (20.0)	94.1 (14.8)
Term	0.28 (0.23)	0.57 (0.21)	193 (61.7)	90 (28.8)	30 (9.6)	100.2 (13.3)
Smoking						
Yes	0.38 (0.28)	0.57 (0.19)	10 (22.7)	23 (52.3)	11 (25.0)	94.3 (12.6)
No	0.27 (0.22)	0.57 (0.20)	197 (62.7)	89 (28.3)	28 (8.9)	100.1 (13.6)
Parity						
0	0.30 (0.24)	0.58 (0.22)	104 (60.5)	55 (32.0)	13 (7.6)	99.6 (13.9)
>=1	0.25 (0.20)	0.55 (0.18)	103 (55.4)	57 (30.7)	26 (14.0)	99.3 (13.4)
Infant sex						
Female	0.30 (0.23)	0.58 (0.22)	94 (57.3)	54 (32.9)	16 (9.8)	102.3 (14.0)
Male	0.26 (0.21)	0.55 (0.18)	113 (58.6)	57 (29.5)	23 (11.9)	97.1 (12.8)
Laboratory						
Minnesota	0.20 (0.15)	0.50 (0.10)	109 (85.2)	19 (14.8)	--	100.6 (14.2)
North Carolina	0.37 (0.26)	0.65 (0.26)	86 (83.5)	17 (16.5)	--	101.7 (13.0)

[a] Infants who were breastfed for all feedings per day for the first 4 postnatal months were considered exclusively breastfed. Infants who were breastfed plus up to 1 formula feeding per day were considered almost exclusively breastfed. Infants who were fed infant formula for all daily feedings were considered formula fed. All other infants were partially breastfed.

Table 5.3. Association between feeding method for the first four postnatal months and scores on the Mullen Scales of Early Learning, PIN Babies study, 2002-2006

Mullen Scales of Early Learning		*Feeding Method (formula fed=reference, n=39)*	
		Exclusively or almost exclusively breastfed (n=207)	Partially Breastfed (n=112)
Gross motor			
	β (CI) (unadjusted model)	1.7 (-2.4, 5.8)	0.9 (-3.5, 5.3)
	β (CI) (adjusted model) [b]	1.1 (-3.4, 5.5)	0.8 (-3.6, 5.2)
Visual reception			
	β (CI) (unadjusted model)	4.4 (0.7, 8.2)	0.6 (-3.4, 4.7)
	β (CI) (adjusted model) [b]	2.5 (-1.6, 6.6)	0.0 (-4.1, 4.0)
Fine motor			
	β (CI) (unadjusted model)	4.6 (0.8, 8.3)	2.2 (-1.8, 6.3)
	β (CI) (adjusted model) [b]	2.7 (-1.4, 6.8)	1.5 (-2.6, 5.6)
Receptive language			
	β (CI) (unadjusted model)	1.2 (-1.6, 3.9)	-1.2 (-4.1, 1.7)
	β (CI) (adjusted model) [b]	0.7 (-2.3, 3.7)	-1.3 (-4.2, 1.7)
Expressive language			
	β (CI) (unadjusted model)	2.2 (-0.9, 5.3)	0.4 (-2.9, 3.7)
	β (CI) (adjusted model) [b]	1.7 (-1.7, 5.1)	0.3 (-3.1, 3.6)
Composite			
	β (CI) (unadjusted model)	6.2 (1.6, 10.8)	1.0 (-4.0, 5.9)
	β (CI) (adjusted model) [b]	4.0 (-1.1, 9.0)	0.2 (-4.8, 5.2)

[a] Infants who were breastfed for all feedings per day for the first 4 postnatal months were considered exclusively breastfed. Infants who were breastfed plus up to 1 formula feeding per day were considered almost exclusively breastfed. Infants who were fed infant formula for all daily feedings were considered formula fed. All other infants were partially breastfed.

[b] Adjusted models include education, race and ethnicity, smoking, and preterm status.

Table 5.4. Association between DHA, AA in breast milk and scores on the Mullen Scales of Early Learning among infants exclusively breastfed (n=183) the first 4 postnatal months, PIN Babies Study, 2002-2006

		Breast milk DHA (continuous)	*Breast milk AA (continuous)*
Mullen Scales of Early Learning			
Gross motor			
	β (CI) (unadjusted model) [a]	5.6 (-2.8, 13.9)	10.6 (0.5, 20.7)
	β (CI) (adjusted model) [b]	3.9 (-4.6, 12.4)	9.0 (-1.0, 19.0)
Visual reception			
	β (CI) (unadjusted model) [a]	-3.5 (-11.1, 4.1)	4.3 (-4.7, 13.3)
	β (CI) (adjusted model) [b]	-2.7 (-10.5, 5.1)	4.4 (-4.6, 13.3)
Fine motor			
	β (CI) (unadjusted model) [a]	2.8 (-4.8, 10.5)	3.8 (-5.3, 12.8)
	β (CI) (adjusted model) [b]	2.3 (-5.2, 9.9)	1.7 (-7.0, 10.4)
Receptive language			
	β (CI) (unadjusted model) [a]	0.7 (-4.5, 5.9)	3.5 (-2.8, 9.8)
	β (CI) (adjusted model) [b]	-0.7 (-5.9, 4.5)	2.2 (-4.0, 8.5)
Expressive language			
	β (CI) (unadjusted model) [a]	-0.4 (-6.3, 5.6)	4.7 (-2.4, 11.9)
	β (CI) (adjusted model) [b]	-1.6 (-7.6, 4.4)	3.6 (-3.6, 10.8)
Composite			
	β (CI) (unadjusted model) [a]	-0.3 (-9.5, 8.8)	8.2 (-2.6, 19.1)
	β (CI) (adjusted model) [b]	-1.3 (-10.3, 7.7)	6.1 (-4.3, 16.5)

[a]Unadjusted models include a variable for laboratory

[b]Adjusted models include lab batch, infant sex, parity, smoking, and preterm status

Table 5.5. Association between DHA, AA in breast milk and infant formula weighted by feeding method for the first four postnatal months and scores on the Mullen Scales of Early Learning, PIN Babies Study, 2002-2006

		DHA in breast milk and formula (continuous)	*AA in breast milk and formula (continuous)*
Mullen Scales of Early Learning			
Gross motor			
	β (CI) (unadjusted model) [a]	1.3 (-0.7, 3.2)	1.4 (-0.9, 3.6)
	β (CI) (adjusted model) [b]	1.1 (-0.9, 3.1)	1.2 (-1.1, 3.4)
Visual reception			
	β (CI) (unadjusted model) [a]	-0.2 (-2.0, 1.7)	0.8 (-1.3, 2.8)
	β (CI) (adjusted model) [b]	-0.1 (-2.0, 1.8)	0.7 (-1.3, 2.8)
Fine motor			
	β (CI) (unadjusted model) [a]	0.5 (-1.4, 2.3)	0.3 (-1.7, 2.4)
	β (CI) (adjusted model) [b]	0.2 (-1.7, 2.0)	0.0 (-2.0, 2.0)
Receptive language			
	β (CI) (unadjusted model) [a]	0.3 (-1.0, 1.5)	0.4 (-1.0, 1.9)
	β (CI) (adjusted model) [b]	-0.1 (-1.4, 1.2)	0.3 (-1.2, 1.7)
Expressive language			
	β (CI) (unadjusted model) [a]	-0.2 (-1.6, 1.2)	1.0 (-0.6, 2.7)
	β (CI) (adjusted model) [b]	-0.6 (-2.1, 0.8)	0.7 (-0.9, 2.3)
Composite			
	β (CI) (unadjusted model) [a]	-0.0 (-2.3, 2.2)	1.2 (-1.3, 3.7)
	β (CI) (adjusted model) [b]	-0.5 (-2.7, 1.7)	0.9 (-1.5, 3.4)

[a]Unadjusted models include a variable for laboratory

[b]Adjusted models include laboratory, feeding method for first 4 postnatal months, infant sex, parity, smoking, education, race/ethnicity, and preterm status

CHAPTER 6

RESULTS

Specific Aim 2: A prospective study of maternal anxiety, perceived stress, and depressive symptoms in relation to infant cognitive development

Introduction

Infancy is a period of rapid cognitive development, transitioning between the critical early brain development *in utero* and the emergence of more advanced abilities in later childhood. The early psychosocial environment of the child plays a role in mental and emotional health across the lifespan. Maternal anxiety, stress, and depression are common psychosocial influences of concern for child development. Pregnancy and new motherhood may be particularly stressful periods for women; with postpartum depression affecting up to 19% of new mothers.[23]

Anxiety, stress, and depressive symptoms are inter-related phenomena; women who perceive their circumstances as highly stressful are also likely to experience more depressive symptoms or anxiety.[25] Mothers who experience significant anxiety, stress, or depression may provide a less stimulating environment for their children's development at least in part because they may react inappropriately to their children's cues.[184, 245, 246] Numerous studies have observed an association between maternal postpartum or chronic depressive symptoms and poorer child performance on cognitive and behavioral assessments.[26-35] Studies noted

that greater maternal stress and anxiety interfere with mother-child interaction, with consequences for infant temperament and cognition.[36-39] However, few studies have reported negative effects as early as infancy, and those results have been inconsistent.[29, 40, 41] Variability across studies could be due to variation in the timing and methods of assessing maternal psychological health, the timing and challenges of assessing very young children, insufficient statistical power to detect small effects, and varying ability to control for key confounders. Additionally, previous studies have used a variety of instruments to assess child development, often assessing development globally. Some areas of development may be more affected by maternal affect than others; thus separately evaluating language, visual reception, and motor skills provide more detail about potential associations.

This study examines maternal trait anxiety, perceived stress, and depressive symptoms during the pre- and early post-natal period in relation to infant visual reception, language, and motor skills, and overall cognitive development as measured via the Mullen Scales of Early Learning (the Mullen).

Methods

Study population

The Data were collected as part of the Pregnancy, Infection, and Nutrition Study (PIN) and its postnatal follow-up components, PIN Postpartum and PIN Babies. The goal of the PIN Study is to identify factors associated with preterm birth. The goal of PIN Postpartum is to identify modifiable behaviors associated with high gestational weight gain and postpartum weight retention.[45] PIN Babies focuses on child developmental outcomes in relation to the prenatal and early childhood environment.

The University of North Carolina Biomedical Institutional Review Board approved all protocols; all participants provided informed consent.

Women (n=1,169) were eligible for PIN Postpartum if they completed the third wave of the PIN Study, agreed to be contacted after delivery, and did not become pregnant again in the first year postpartum. Medical constraints (n=24), inability to recontact (n=207) or schedule (n=62), and refusal (n=187) resulted in 689 women who participated in a visit scheduled in the fourth postpartum month. Of these, 45 became pregnant again, 62 were unreachable, 29 moved from the study area, and 20 left the study before the PIN Babies visit at 12 months could be scheduled. The PIN Babies protocol began after PIN Postpartum began, and 125 infants were ineligible to complete the protocol because they reached 12 months of age before the study began conducting the assessments in the home. Among the remaining 408 eligible maternal-infant pairs, some did not participate in the Mullen because the child was not present (n=11, e.g. the mother completed the interview from work); the child was asleep, sick, or fussy (n=21); the mother refused the child's participation (n=8); there was not enough time during the visit (n=3); or other reasons (n= 7).

Data collection

During pregnancy, information about depressive symptoms was collected via self-administered questionnaires before 20 weeks gestation and at 24-29 weeks gestation using the Centers for Epidemiologic Studies Depression (CES-D) scale.[212] The CES-D is intended to measure depressive symptoms in general population samples. The questionnaire before 20 weeks gestation included the State-Trait Anxiety Inventory

(STAI), the Rosenberg Self-Esteem Scale, and the MOS Social Support scale.[193, 213, 214] The trait score of the STAI is a measure of an individual's general feelings of anxiety and is relatively stable over time, in contrast to state anxiety which is situational.[193] Age, education, income and other demographic and lifestyle covariates were obtained via telephone at 17-22 weeks gestation. Delivery information was obtained from the hospital record. Preterm birth was defined as less than 37 completed weeks gestation at delivery, based on ultrasound if done before 20 weeks gestation or the date of the last menstrual period.

The home visit in the fourth postpartum month collected information on maternal and infant health and nutrition and updated information about family circumstances and maternal psychosocial health. The interview included the Edinburgh Postnatal Depression Scale (EPDS), a screening tool for depressive symptoms, and the 10-item Perceived Stress Scale to measure the extent to which one's circumstances are perceived as stressful.[215, 216]

At 12 months postpartum, study staff administered the Mullen following procedures outlined in the Mullen manual. The Mullen is a commonly used assessment of cognitive functioning appropriate for children up to 68 months of age. Five sub-scales separately assess visual reception, expressive and receptive language, and fine and gross motor development. All but gross motor are combined to comprise a composite score reflecting overall cognitive ability.

Statistical analysis

Continuous scores from the trait anxiety scale of the STAI and the Perceived Stress Scale were categorized into tertiles based on their observed distribution in the study participants. A variable was constructed to examine depressive symptoms across both the antepartum and postpartum periods based on scores on the CES-D and EPDS. Women were classified as having few depressive symptoms postpartum (EPDS=0-9) (regardless of antepartum symptoms), minor or major depressive symptoms only during the postpartum period (EPDS > 9 and both CES-D scores=0-16), and minor or major depressive symptoms during both the postpartum and antepartum periods (EPDS > 9 and at least one CES-D score > 16). The 9/10 cutpoint for the EPDS has been validated in previous studies to measure minor depression or increase the sensitivity of the instrument to depression, and it has been shown to identify cases of clinical depression with more than 90% sensitivity.[228-231] The 16/17 cutpoint for the CES-D was chosen because several studies have reported reduced specificity of the CES-D using the 15/16 cutpoint for pregnant women because some symptoms of pregnancy (e.g., fatigue) are similar to CES-D components.[225-227]

Raw scores were converted to age-specific t-scores for the four cognitive sub-scales (Visual Reception, Fine Motor, Receptive Language, Expressive Language) and the Gross Motor sub-scale of the Mullen. The cognitive t-scores were summed and converted to a standard Early Learning Composite (Composite) score following procedures outlined in the manual.[217]

All analyses adjusted for preterm status; however scores were not directly adjusted for gestational age at birth because we found the procedure recommended in the

manual potentially overcorrected scores for preterm infants. The age-adjusted mean score for preterm infants was higher than the mean score for term infants for the Composite and all of the Mullen sub-scales except the Fine Motor scale, although the difference was statistically significant for the Receptive Language sub-scale only (age-adjusted mean Receptive Language t-score for preterm infants=48.9, term infants=45.9, t=2.3, p=0.02).

Linear regression models were used to examine the association between maternal anxiety, perceived stress, or depressive symptoms and infant scores on each Mullen sub-scale and the Composite. The residuals were found to be approximately normally distributed using kernel density and Q-Q plots and to have constant variance using the White test and plots of the residuals versus predicted values.[244] Observations were considered to be independent because only one child per family participated. The exposure-outcome relationships appeared to be linear. Since the exposure categories have an inherent ordering, tests for linear trend for each unadjusted model were conducted by including in a regression model a continuous variable for the median value of the anxiety or stress score for each tertile of anxiety or stress. To assess a linear trend for the depressive symptoms models, linear regression models included a categorical three-level depressive symptoms variable.

Multiple partial F-tests checked for effect measure modification by poverty, maternal pre-pregnancy body mass index (BMI), education, social support, infant sex, preterm birth, presence of a spouse/partner, and one or more of trait anxiety, depressive symptoms, and perceived stress depending on the exposure of interest. Potential confounders were identified *a priori* based on previous studies and constructed Directed Acyclic Graphs.[234, 235] Poverty, maternal pre-pregnancy BMI, social support, infant sex, and presence of a spouse/partner

were considered potential confounders *a priori* but were eliminated via the change-in-estimate approach.[247] This produced a final set of covariates that resulted in a change of greater than 10% in the beta coefficient for the exposure variable when removed from a model.[247] Adjusted models incorporated confounders based on this selection process.

To include women with missing data on anxiety (5.1% of women), covariates, or depressive symptoms at 1 or 2 time points (15.8% missing 1 time point, 3.4% missing 2 time points), multiple imputation was used to impute missing data . The Markov chain Monte Carlo method using SAS procedure (PROC MI) produced 10 replications of the dataset with missing values imputed.[236-238] The imputation model included: CES-D scores during pregnancy, EPDS score, perceived stress scores (17-22 weeks gestation, 27-30 weeks gestation, fourth month postpartum), trait anxiety score, state anxiety score at less than 20 weeks gestation, Rosenberg self-esteem score, MOS social support score, maternal age, family income as a percentage of the federal poverty level, maternal pre-pregnancy BMI, education, gestational age at delivery, parity, maternal race, infant sex, each Mullen sub-scale score and Composite score, and infant scores on the MacArthur Communicative Development Inventory at 12 months.[248] (For the purposes of this publication, we focused on the Mullen; MacArthur scores were not analyzed.) The imputed datasets were analyzed using SAS (version 9.1) linear regression procedures and combined with PROC MIANALYZE to produce the effect estimates reported.[238]

Results

To be eligible for this analysis, infants must have completed at least one sub-scale of the Mullen (n=358; 343 completed all). Table 6.1 shows trait anxiety, depressive symptoms,

and perceived stress categories in relation to participant characteristics. Approximately 10% of women experienced minor or major depressive symptoms during both the ante- and postpartum periods. Higher levels of anxiety, stress, and depressive symptoms were associated with younger maternal age, low self-esteem, poverty, and fewer years of education, but not with preterm birth. Anxiety, stress, and depressive symptoms were positively correlated with each other (Spearman $\hat{\rho}$ for anxiety and stress=0.41 (p=0.04), for anxiety and depressive symptoms=0.30 (p=0.04), for stress and depressive symptoms=0.51 (p=0.03)).

Infants had mean Mullen t-scores and Composite scores similar to expected for a general population sample of infants, with mean sub-scale t-scores around 50 and a mean Composite score of 99 (Table 6.1). Older and more educated mothers tended to have infants with higher Fine Motor scores (data not shown). Infants of women with more education also tended to score higher on the Composite. Preterm infants had lower Gross Motor, Fine Motor, and Composite scores than term infants.

Maternal anxiety was inversely associated with Receptive Language t-scores; infants of mothers in the highest anxiety group scored slightly lower than those in the lowest group, in a model adjusted for preterm birth, education, and self-esteem (Table 6.2). Anxiety was also associated with lower Composite scores. Trend tests suggested a linear trend across categories of anxiety for Receptive Language and Composite scores; increasing levels of anxiety were associated with decreasing Mullen scores, although all estimates were imprecise.

Maternal antepartum and postpartum depressive symptoms were not associated with Mullen scores (Table 6.3). The results were similar for maternal perceived stress, with the

exception of a positive association with Receptive Language scores. No effect measure modification was detected for the associations evaluated in Tables 6.2-6.4.

To address the possibility that women in PIN who refused or who were unable to participate in the study through the 12-month visit might be more likely to have significant levels of anxiety, stress and depressive symptoms and an infant who would have scored poorly on the Mullen, we examined the available antepartum anxiety, stress and depressive symptoms data. Women who were eligible but refused, were unreachable or not able to be scheduled for a visit in the fourth postpartum month were more likely to score above 16 on the CES-D at either time point and to score in the highest trait anxiety tertile (OR for CES-D 17+=1.5, 95% CI: 1.1, 2.0; OR for highest anxiety tertile=1.4, 95% CI: 1.1, 1.8) compared to women who participated in the first postpartum visit. Those who participated in the first postpartum visit but not the second visit at 12 months were more likely to score in the highest perceived stress tertile than those who participated in both visits (OR for the highest tertile=1.7, 95% CI: 1.1, 2.5), but scored no differently on the EPDS or trait anxiety scales (OR for EPDS 10+=1.2, 95% CI: 0.7, 1.9; OR for highest anxiety tertile=1.0, 95% CI: 0.6, 1.5). It is not possible to determine with available data whether the children who discontinued participation would have had lower Mullen scores. However, we examined other infant characteristics that might be related to Mullen scores. Infants who were born preterm were as likely as term infants to participate in the home visits (χ_1^2 for the first visit=0.18, p=0.67; χ_1^2 for the second visit=0.20, p=0.66). Maternal anxiety, stress, or depressive symptoms were no more strongly associated with an infant being preterm among those who stopped participation after the first home visit compared to those who completed the second visit.

Discussion

Elevated maternal trait anxiety was found to be associated with lower Receptive Language and Composite scores in adjusted models. Receptive language reflects the infant's use of both visual and auditory skills to process information. It is possible that mothers with high anxiety levels participate in fewer language stimulation activities with their infants, or it could be that unmeasured factors underlying maternal anxiety could affect receptive language ability as well. Previous research has found anxious mothers to be less likely to speak to their infants and less sensitive and responsive to their needs.[249,250] The association between maternal anxiety and Composite scores suggests anxiety may exert a slight negative effect across several areas of motor and language development, if not only through receptive language.

The observed difference between infants exposed to the highest levels of maternal anxiety and those exposed to the lowest levels was small (approximately one-third of a standard deviation) and imprecise. In comparison, a study comparing "late-talking" toddlers to normally developing toddlers observed a 9.5 point difference.[251] However, even small differences within the normal range can be meaningful at the population level.[252]

High levels of perceived stress were associated with slightly higher Receptive Language t-scores. This is unexpected in light of previous studies that assessed maternal stress in relation to infant behavior and global development.[253, 254] There were no significant differences in other Mullen sub-scale or Composite scores between infants exposed to maternal trait anxiety, postpartum stress, or antepartum and postpartum depressive symptoms after adjusting for key confounders.

Several factors may have contributed to the null results observed here. First, many women in this study were well-educated, had adequate financial resources, and reported high levels of social support. These factors may buffer the child's development from detrimental effects of depressive symptoms or stressful circumstances.

Second, women who experienced significant depressive symptoms or anxiety during pregnancy or high stress levels postpartum were somewhat less likely to participate postpartum. If these children would also have scored lower on the Mullen scales than participating children, these results may underestimate the true association, but the data to evaluate this were unavailable.

Third, stress, anxiety, and depression can occur together and be easily confounded by each other and other demographic factors. This study was able to examine the role of several factors that may affect the observed associations between maternal anxiety, stress, and depressive symptoms and infant development. The lack of control for key demographic and psychosocial confounders could have contributed to spurious or exaggerated associations between depressive symptoms and infant development in previous studies.

Finally, most previous studies reporting detrimental effects of maternal anxiety, stress or depression were focused on older children. Our finding that anxiety was associated with poorer receptive language among infants is one of a few published studies to observe differences in infants. It is possible that additional effects of these exposures might become apparent when the children are older.

This sample of infants was larger and more population-based than many previous clinical samples, which may have enabled us to detect subtle differences between groups. However, because the infants were generally developing typically, differences among them

due to maternal psychosocial characteristics may be slight and difficult to detect, and may partially account for null results. Also, we used a standard but lower threshold for the EPDS to enhance the sensitivity for a broader group of women who may have had minor depression in the postpartum period.[215] This inclusiveness may explain our inability to observe an association between depressive symptoms and infant development because maternal stress, depression, and anxiety may only adversely affect infant development when it is severe. We examined results using a cutpoint of 12/13 for the EPDS, and most parameter estimates were larger than our reported results but very imprecise. A larger sample of participants would enable detection of differences with greater precision.

The multiple imputation techniques assume that the missing data can be predicted from the available data; however, no statistical test can verify this.[236] The wide range of demographic and psychosocial variables in this dataset that were used to impute missing values provides some assurance that missingness was reasonably predicted, and imputation produced slightly more precise results than complete case analyses.

Several strengths distinguish this study. First, few studies have simultaneously assessed multiple aspects of infant development, not just global development, in relation to these exposures. The Mullen offers sub-scales corresponding to specific developmental areas, exhibits high test-retest reliability, and correlates strongly with the Bayley MDI.[217] The Mullen has not previously been used to examine the associations between maternal anxiety, stress and depressive symptoms and child development in a sample of typically developing infants. Some previous studies relied on maternal report for measures of infant outcomes which can inflate the association between poor maternal psychological health and infant behavior since depressed mothers may be more critical or less observant of their

child's development. This study employed trained interviewers who used standard assessment techniques with high reliability. This study also relied on well-established, validated instruments for measuring anxiety, stress, and depressive symptoms. The CES-D, EPDS, STAI, and Perceived Stress scales are commonly used instruments in psychosocial research and have been applied in many studies of pregnancy or postpartum health.[37, 40, 187, 226, 255] Finally, the longitudinal design of this study allowed assessment of maternal psychological health in the antepartum and postpartum periods before the developmental outcomes were assessed at 12 months. This avoided assessing the exposures based on recall or confusing the temporal sequence of the exposures and outcomes.

Conclusion

Infants of mothers with elevated levels of trait anxiety exhibited slightly lower receptive language and overall cognitive ability compared to infants of mothers with low levels of trait anxiety, after adjusting for preterm birth, maternal education, and self-esteem. No similar associations were observed for infants exposed to maternal minor or major depressive symptoms or to perceived stress. The effects of maternal psychological disorders might be more likely to produce subtle shifts in development rather than developmental delays. Continued efforts to follow this cohort for future language and general cognitive development would be valuable to see if the associations with anxiety persist or if associations with maternal depressive symptoms or stress become apparent. Clinicians should be aware of the potential detrimental effect of maternal anxiety on very early child development.

Table 6.1. Characteristics of mothers and infants in the Pregnancy, Infection, and Nutrition (PIN), PIN Postpartum and PIN Babies Studies and associations with maternal depressive symptoms, trait anxiety, and perceived stress, 2002-2006

	n (%)	*State-Trait Anxiety Inventory (trait anxiety score)*				*Antepartum and postpartum depressive symptoms*			*Cohen's Perceived Stress Scale score*			*Mullen Scales of Early Learning Composite*
						Few symptoms postpartum	Minor or major depressive symptoms postpartum	Minor or major symptoms postpartum and antepartum				
		Score = 0-<31 (n, %)	31-<40 (n, %)	40+ (n, %)	Missing (n, %)	EPDS score=0-9 (n, %)	EPDS score 10+ and both CES-D scores=0-16 (n, %)	EPDS score 10+ and at least 1 CES-D score= 17+ (n, %)	Score= 0-<11 (n, %)	11-<17 (n, %)	17+ (n, %)	Mean, SD
All women (n, %)	358 (100)	118 (33.0)	127 (35.5)	91 (25.4)	22 (6.2)	302 (84.4)	21 (5.9)	35 (9.8)	120 (33.5)	150 (41.9)	88 (24.6)	99.4 (13.6)
Maternal age (years)												
17-20	16 (4.5)	1 (6.3)	4 (25.0)	9 (56.3)	2 (12.5)	8 (50.0)	1 (6.3)	7 (43.8)	2 (12.5)	5 (31.3)	9 (56.3)	96.7 (17.2)
21-30	167 (46.6)	45 (27.0)	55 (32.9)	54 (32.3)	13 (7.8)	139 (83.2)	9 (5.4)	19 (11.4)	57 (34.1)	66 (39.5)	44 (26.4)	98.8 (13.5)
31-47	175 (48.9)	72 (41.1)	68 (38.9)	28 (16.0)	7 (4.0)	155 (88.6)	11 (6.3)	9 (5.1)	61 (34.9)	79 (45.1)	35 (20.0)	100.3 (13.4)
Self-esteem												
0-49	106 (29.6)	9 (8.5)	36 (34.0)	61 (57.6)	0 (0.0)	76 (71.7)	5 (4.7)	25 (23.6)	23 (21.7)	41 (38.7)	42 (39.6)	99.7 (13.6)
50-55	114 (31.8)	34 (29.8)	57 (50.0)	23 (20.2)	0 (0.0)	97 (85.1)	10 (8.8)	7 (6.1)	35 (30.7)	51 (44.7)	28 (24.6)	99.3 (13.3)
56-60	114 (31.8)	75 (65.8)	32 (28.1)	7 (6.1)	0 (0.0)	109 (95.6)	3 (2.6)	2 (1.8)	57 (50.0)	47 (41.2)	10 (8.8)	100.1 (13.7)

missing	24 (6.7)	0 (0.0)	2 (8.3)	0 (0.0)	22 (91.7)	20 (83.3)	3 (12.5)	1 (4.2)	5 (20.8)	11 (45.8)	8 (33.3)	96.4 (15.4)
Poverty												
<185% of federal level	49 (13.7)	3 (6.1)	14 (28.6)	23 (46.9)	9 (18.4)	32 (65.3)	5 (10.2)	12 (24.5)	10 (20.4)	18 (36.7)	21 (42.9)	96.7 (15.0)
185-350%	73 (20.4)	22 (30.1)	27 (37.0)	20 (27.4)	4 (5.5)	62 (84.9)	5 (6.9)	6 (8.2)	19 (26.0)	30 (41.1)	24 (32.9)	98.1 (14.5)
>350%	225 (62.8)	91 (40.4)	82 (36.4)	43 (19.1)	9 (4.0)	200 (88.9)	11 (4.9)	14 (6.2)	90 (40.0)	97 (43.1)	38 (16.9)	100.4 (13.0)
missing	11 (3.1)	2 (18.2)	4 (36.4)	5 (45.5)	0 (0.0)	8 (72.7)	0 (0.0)	3 (27.3)	1 (9.1)	5 (45.5)	5 (45.5)	101.3 (12.3)
Maternal education												
0-12 years	50 (14.0)	6 (12.0)	9 (18.0)	26 (52.0)	9 (18.0)	33 (66.0)	3 (6.0)	14 (28.0)	8 (16.0)	18 (36.0)	24 (48.0)	95.7 (14.7)
13-16	177 (49.4)	54 (30.5)	67 (38.9)	47 (26.6)	9 (5.1)	150 (84.8)	10 (5.7)	17 (9.6)	61 (34.5)	79 (44.6)	37 (20.9)	99.0 (13.8)
>16	131 (36.6)	58 (44.3)	51 (38.9)	18 (13.7)	4 (3.1)	119 (90.8)	8 (6.1)	4 (3.1)	51 (38.9)	53 (40.5)	27 (20.6)	101.5 (12.7)
Preterm birth												
Term	313 (87.4)	108 (34.5)	108 (34.5)	79 (25.2)	18 (5.8)	266 (85.0)	16 (5.1)	31 (9.9)	109 (34.8)	127 (40.6)	77 (24.6)	100.2 (13.3)
Preterm	45 (12.6)	10 (22.2)	19 (42.2)	12 (26.7)	4 (8.9)	36 (80.0)	5 (11.1)	4 (8.9)	11 (24.4)	23 (51.1)	11 (24.4)	94.1 (14.8)

Table 6.2. Results of linear regression models for the association between maternal trait anxiety score and scores on the Mullen Scales of Early Learning: Pregnancy, Infection, and Nutrition (PIN), PIN Postpartum and PIN Babies Studies, 2002-2006

	State-Trait Anxiety Inventory (trait anxiety score)			
	Score=0-<31 (n=118, 35.1%)	31-<40 (n=127, 37.8%)	40+ (n=91, 27.1%)	P for trend[a]
Mullen Scales of Early Learning				
Gross motor				0.60
Mean (SD)	50.3 (12.4)	48.7 (12.3)	50.9 (10.8)	
β (95% CI) (Unadjusted model)	-	-1.5 (-4.5, 1.5)	0.6 (-2.6, 3.8)	
β (95% CI) (Adjusted model)[b]	-	-1.8 (-5.0, 1.4)	1.1 (-2.9, 5.1)	
Visual reception				0.10
Mean (SD)	51.8 (11.1)	48.7 (11.4)	47.8 (10.0)	
β (95% CI) (Unadjusted model)	-	-3.0 (-5.8, -0.2)	-4.0 (-6.9, 1.0)	
β (95% CI) (Adjusted model)[b]	-	-2.6 (-5.6, 0.3)	-3.0 (-6.7, 0.6)	
Fine motor				0.50
Mean (SD)	51.3 (11.3)	48.6 (11.0)	50.0 (10.9)	
β (95% CI) (Unadjusted model)	-	-2.7 (-5.5, 0.0)	-1.3 (-4.4, 1.7)	
β (95% CI) (Adjusted model)[b]	-	-2.9 (-5.8, 0.1)	-1.4 (-5.0, 2.2)	
Receptive Language				0.02
Mean (SD)	46.8 (7.0)	45.4 (8.3)	44.8 (8.5)	
β (95% CI) (Unadjusted model)	-	-1.4 (-3.4, 0.6)	-2.0 (-4.1, 0.1)	
β (95% CI) (Adjusted model)[b]	-	-1.8 (-4.0, 0.3)	-2.9 (-5.6, -0.3)	
Expressive Language				0.53
Mean (SD)	53.2 (9.0)	53.2 (9.0)	52.8 (8.9)	
β (95% CI) (Unadjusted model)	-	0.0 (-2.2, 2.3)	-0.4 (-2.8, 2.0)	
β (95% CI) (Adjusted model)[b]	-	-0.2 (-2.6, 2.2)	-1.0 (-4.0, 2.1)	
Composite				0.05
Mean (SD)	101.9 (13.5)	98.3 (13.6)	97.9 (13.3)	
β (95% CI) (Unadjusted model)	-	-3.5 (-6.9, -0.2)	-4.0 (-7.6, -0.4)	
β (95% CI) (Adjusted model)[b]	-	-3.9 (-7.5, -0.3)	-4.5 (-8.9, 0.0)	

[a]Trend tests are reported for the adjusted models

[b]Covariates include preterm birth, maternal education, self-esteem

Table 6.3. Results of linear regression models for the association between maternal depressive symptoms and scores on the Mullen Scales of Early Learning: Pregnancy, Infection, and Nutrition (PIN), PIN Postpartum and PIN Babies Studies, 2002-2006

	Antepartum and postpartum depressive symptoms			
	Few symptoms postpartum	Minor or major depressive symptoms postpartum only	Minor or major symptoms postpartum and antepartum	
Mullen Scales of Early Learning	EPDS score=0-9 (n=302, 84.4%)	EPDS score 10+ & both CES-D scores=0-16 (n=21, 5.9%)	EPDS score 10+ & at least one CES-D score=17+ (n=35, 9.8%)	P for trend[a]
Gross motor				0.50
Mean (SD)	49.4 (11.9)	52.1 (15.1)	52.1 (11.3)	
β (95% CI) (Unadjusted model)	-	2.6 (-4.0, 9.2)	2.7 (-1.5, 6.8)	
β (95% CI) (Adjusted model)[b]	-	1.5 (-5.4, 8.5)	1.7 (-3.5, 6.9)	
Visual reception				0.61
Mean (SD)	50.0 (11.2)	45.7 (10.3)	47.3 (9.9)	
β (95% CI) (Unadjusted model)	-	-4.3 (-9.9, 1.4)	-2.7 (-6.3, 1.0)	
β (95% CI) (Adjusted model)[b]	-	-2.8 (-8.9, 3.3)	-0.9 (-5.5, 3.7)	
Fine motor				0.77
Mean (SD)	50.0 (11.1)	47.5 (11.6)	50.1 (11.1)	
β (95% CI) (Unadjusted model)	-	-2.5 (-8.3, 3.4)	0.1 (-3.8, 3.9)	
β (95% CI) (Adjusted model)[b]	-	-3.6 (-9.7, 2.6)	-0.9 (-5.6, 3.9)	
Receptive Language				0.79
Mean (SD)	45.6 (7.9)	45.9 (7.1)	46.2 (8.8)	
β (95% CI) (Unadjusted model)	-	0.3 (-4.1, 4.6)	0.5 (-2.1, 3.2)	
β (95% CI) (Adjusted model)[b]	-	-0.8 (-5.4, 3.8)	-0.9 (-4.2, 2.5)	
Expressive Language				0.44
Mean (SD)	53.2 (9.0)	50.5 (8.7)	53.2 (8.3)	
β (95% CI) (Unadjusted model)	-	-2.7 (-7.5, 2.2)	0.0 (-3.0, 3.1)	
β (95% CI) (Adjusted model)[b]	-	-4.4 (-9.8, 0.9)	-1.5 (-5.4, 2.4)	
Composite				0.64
Mean (SD)	99.7 (13.6)	95.2 (12.8)	98.6 (13.7)	
β (95% CI) (Unadjusted model)	-	-4.5 (-11.8, 2.8)	-1.2 (-5.7, 3.4)	
β (95% CI) (Adjusted model)[b]	-	-5.6 (-13.3, 2.0)	-2.0 (-7.6, 3.7)	

[a]Trend tests are reported for the adjusted models

[b]Covariates include preterm birth, maternal education, self-esteem, trait anxiety, postpartum perceived stress

Table 6.4. Results of linear regression models for the association between maternal perceived stress score in the fourth postpartum month and scores on the Mullen Scales of Early Learning: Pregnancy, Infection, and Nutrition (PIN), PIN Postpartum and PIN Babies Studies, 2002-2006

	Cohen's Perceived Stress Scale score			
	Score=0-<11 (n=120, 33.5%)	11-<17 (n=150, 41.9%)	17+ (n=88, 24.6%)	P for trend[a]
Mullen Scales of Early Learning				
Gross motor				0.19
Mean (SD)	49.6 (13.7)	49.0 (10.6)	51.8 (11.6)	
β (95% CI) (Unadjusted model)	-	-0.6 (-3.5, 2.3)	2.2 (-1.1, 5.5)	
β (95% CI) (Adjusted model)[b]	-	-0.9 (-3.9, 2.1)	3.0 (-0.8, 6.7)	
Visual reception				0.77
Mean (SD)	50.4 (11.2)	49.9 (11.6)	47.6 (9.6)	
β (95% CI) (Unadjusted model)	-	-0.5 (-3.2, 2.1)	-2.8 (-5.9, 0.3)	
β (95% CI) (Adjusted model)[b]	-	0.5 (-2.3, 3.2)	-0.8 (-4.2, 2.6)	
Fine motor				0.13
Mean (SD)	49.5 (11.5)	49.8 (11.1)	50.6 (10.8)	
β (95% CI) (Unadjusted model)	-	0.3 (-2.4, 3.0)	1.0 (-2.1, 4.1)	
β (95% CI) (Adjusted model)[b]	-	1.2 (-1.6, 4.0)	2.4 (-1.0, 5.8)	
Receptive Language				0.05
Mean (SD)	45.5 (7.5)	45.2 (8.4)	46.8 (7.8)	
β (95% CI) (Unadjusted model)	-	-0.3 (-2.2, 1.6)	1.3 (-0.9, 3.5)	
β (95% CI) (Adjusted model)[b]	-	0.2 (-1.8, 2.2)	2.8 (0.3, 5.3)	
Expressive Language				0.06
Mean (SD)	52.4 (9.7)	52.9 (8.8)	54.3 (8.1)	
β (95% CI) (Unadjusted model)	-	0.4 (-1.7, 2.6)	1.9 (-0.6, 4.3)	
β (95% CI) (Adjusted model)[b]	-	1.0 (-1.3, 3.2)	2.8 (-0.1, 5.6)	
Composite				0.09
Mean (SD)	99.4 (14.1)	99.3 (13.8)	99.8 (12.7)	
β (95% CI) (Unadjusted model)	-	-0.1 (-3.4, 3.2)	0.4 (-3.4, 4.2)	
β (95% CI) (Adjusted model)[b]	-	1.3 (-2.1, 4.6)	3.3 (-0.9, 7.4)	

[a]Trend tests are reported for the adjusted models

[b]Covariates include preterm birth, maternal education, self-esteem, trait anxiety

CHAPTER 7

RESULTS

Specific Aim 3: Perinatal depressive symptoms and the concentration of docosahexaenoic acid in breast milk

Introduction

Pregnancy and new motherhood are particularly stressful periods for many women, with depression during pregnancy affecting an estimated 12 to 18% of women and postpartum depression affecting up to 19% of new mothers.[23, 24] Several studies have shown that depressed women tend to have impaired interaction with their infant, and negative effects on child development have been observed into the school-age years.[26, 27, 29-31]

Low levels of long-chain polyunsaturated fatty acids (LCPUFAs), particularly docosahexaenoic acid (DHA), have been noted in the red blood cell and plasma cell membranes and brain orbitofrontal cortex tissue of clinically depressed adults.[43, 44, 195] Pregnancy may exacerbate low DHA status because maternal stores of DHA are mobilized to support the rapid development of the fetal brain.[49] A handful of studies have noted lower postpartum plasma DHA levels among women with postpartum depression, but the results have been inconsistent and none have examined DHA levels in the breast milk.[196, 204-206] A few clinical trials have evaluated whether n-3 fatty acid supplements might prevent perinatal depression or reduce symptoms, with mostly negative findings.[207-211]

In the postpartum period, maternal DHA stores are a major source of DHA for the infant via breast milk. Approximately 73% of U.S. infants are breastfed for at least some time, and 12% are exclusively breastfed for the first 6 months.[203] Some studies have observed a positive association between infant DHA intake and developmental outcomes such as visual acuity, although studies have often been conflicting.[116, 239, 256, 257]

If women with elevated perinatal depressive symptoms also have reduced DHA content of their breast milk, then it would appear that perinatal depression could have both psychosocial and nutritional influences on infant development. The aim of the current study was to examine whether women with depressive symptoms at various time points during pregnancy and postpartum have lower breast milk DHA content.

Methods

Study population

Data were collected as part of the Pregnancy, Infection, and Nutrition Study (PIN) and its postnatal follow-up component, PIN Postpartum. The goal of the PIN Study was to identify factors associated with preterm birth. The goal of PIN Postpartum was to identify modifiable behaviors associated with high gestational weight gain and postpartum weight retention.[45] All protocols were approved by the University of North Carolina (UNC) Biomedical Institutional Review Board; all participants provided written informed consent.

Women (n=1,169) were eligible for PIN Postpartum if they completed the third wave of the PIN Study, agreed to be contacted after delivery and lived in the study area. Medical constraints (n=24), inability to recontact (n=207) or schedule

(n=62), and refusal (n=187) resulted in 689 women who participated in a visit scheduled in the fourth postpartum month. Women were eligible to provide a breast milk sample if they delivered their child between February 2004 and May 2007 (n=519) and were still breastfeeding and living in the area, but 165 women never initiated or had discontinued breastfeeding by the time of the visit. Some women were unable (n=26) or declined (n=23) to provide a sample; 305 provided a sample (304 were analyzable).

Breast milk collection and fatty acid analysis

Women were asked to use a breast pump at around 10 AM on the day of the postpartum visit to provide three 1.5 ml tubes of milk for storage at -80 degrees Celsius at the study office. Samples were analyzed for fatty acid content by two laboratories. Samples collected before April 1, 2005 were analyzed by the Collaborative Studies Clinical Laboratory at the University of Minnesota Medical Center, Fairview (Minneapolis, Minnesota). This lab was unable to complete the analysis for the second batch of samples, so those collected after April 1, 2005 were analyzed by the Clinical Nutrition Research Center, UNC (Chapel Hill, North Carolina). Fatty acid extraction was performed on 0.5 ml samples of breast milk mixed with 0.5 ml 0.9% saline using the method of Bligh and Dyer.[241] The chloroform phase was transferred to a clean tube and evaporated to dryness under a nitrogen flow. Residual lipids were saponified and fatty acids were transmethylated by sequentially adding 1ml 4.25% NaOH in $CHCl_3$:MeOH (2:1, v/v) and 1N HCl in saline.[242] After vigorous mixing, the samples were centrifuged at 1500g for 5

minutes. The fatty acid methyl esters in the lower phase were evaporated to dryness under nitrogen and then resuspended in 50 μl undecane and analyzed by capillary gas chromatography (injector 240°, detector 280°). To check the efficacy of the extraction, 0.1 mg 17:0 standard (diheptadecanoyl phosphatidylcholine) was added. The individual fatty acids were identified by comparison with authentic standards (Nu Chek Prep, Elysian, MN). Data were analyzed using Perkin Elmer Totalchrom Chromatography Software, version 6.2 (Somerset, NJ).

Depressive symptoms

During pregnancy, information about depressive symptoms was collected via self-administered questionnaires before 20 weeks gestation and at 24-29 weeks gestation using the Centers for Epidemiologic Studies Depression (CES-D) scale.[212] The CES-D is intended to measure depressive symptoms in general population samples. Women who scored above 16 on the CES-D were classified as having minor or major depressive symptoms. The 16/17 cutpoint for the CES-D was chosen because several studies have reported reduced specificity of the CES-D using the 15/16 cutpoint for pregnant women because some symptoms of pregnancy (e.g., fatigue) are similar to CES-D components.[225-227]

In the fourth postpartum month, women were interviewed in the home and screened for depressive symptoms using the Edinburgh Postnatal Depression Scale (EPDS).[215] Women who scored above 9 on the EPDS were classified as having minor or major depressive symptoms. The 9/10 cutpoint for the EPDS has been

validated in previous studies and has been shown to increase the sensitivity of the instrument to minor depression.[228-230]

Covariate measurement

Age, education, and other demographic and lifestyle covariates were obtained via telephone at 17-22 weeks gestation. At the clinic visit at 24-29 weeks gestation, women were asked to complete a modified version of the Block Food Frequency Questionnaire (FFQ) to assess diet during the previous three months, from which estimated daily dietary intake of n-3 fatty acids for the three previous months was calculated.[258, 259] At the postpartum visit, information was collected about current smoking and the presence of a spouse or partner in the woman's life.

Statistical analysis

We used t-tests and one-way analysis of variance to examine the difference in mean breast milk DHA concentration between groups with elevated depressive symptoms (i.e., above the cutpoint for the particular depressive symptoms screener) versus few depressive symptoms (i.e., below the cutpoint for the screener) at each time point depressive symptoms were measured. To consider whether the DHA status of women who scored above the cutpoint multiple times differed from the DHA status of women who scored above the cutpoint zero or one times, we formed a variable for the count of the number of times women scored above a cutpoint. DHA concentration was approximately log-normally distributed, so it was log-transformed for use in regression

models. The association between depressive symptoms and logDHA was examined using linear regression.

Potential covariates were identified *a priori* based on previous studies and constructed Directed Acyclic Graphs.[234, 235] Multiple partial F-tests checked for the presence of effect measure modification. Covariates included in the regression models included maternal age (tertiles), education (0-12, 13-16, >16 years), estimated daily intake of n-3 fatty acids (quartiles), presence of a spouse or partner (yes/no), smoking (ever during pregnancy or postpartum versus none), and a variable for laboratory.

Results

All women except participated in at least one assessment of depressive symptoms, leaving 303 total women eligible for inclusion in this analysis. The proportion of women scoring above the cutpoint on the first or second prenatal CES-D or the postpartum EPDS was 11.9%, 15.8%, and 11.2%, respectively; and 8.6% scored above the relevant cutpoint at 2 or 3 of the time points these instruments were administered (Table 7.1). Elevated depressive symptoms were associated with fewer years of maternal education, absence of a spouse or partner, smoking, and younger maternal age; these associations were strongest among women who scored above the cutpoint 2-3 times.

The mean breast milk DHA concentration was 0.28% (SD=0.22) and ranged 0.07%-1.49%. Women with more years of education had higher breast milk DHA levels on average than women with less education. Mean DHA concentration also differed by laboratory ($t=-7.2$, $p<0.01$). DHA levels were 25% lower among women with elevated depressive symptoms at less than 20 weeks gestation compared to women with few

depressive symptoms at that time (adjusted β for logDHA= -0.22, 95% CI: -0.42, -0.03) (Table 7.2). There was a suggestion of a similar association with the number of times women scored above a depressive symptoms screener cutpoint. DHA levels were 19% lower for women who scored above a cutpoint 2-3 times compared to women who never scored above a cutpoint, although this estimate was imprecise (adjusted β for logDHA=-0.17, 95% CI: -0.40, 0.06). All women with elevated depressive symptoms at less than 20 weeks gestation had DHA levels below 0.45%, and all women with elevated symptoms at 2-3 time points had DHA levels below 0.35%. Women with elevated depressive symptoms at 24-29 weeks gestation or postpartum did not have lower breast milk DHA levels than women with few depressive symptoms at those time points. We re-examined models with dietary n-3 intake excluded and observed no difference in the results.

Discussion

The goal of this study was to examine whether women who experienced elevated depressive symptoms during pregnancy or postpartum also had lower DHA levels in their breast milk. A few previous studies have noted lower DHA levels as measured in plasma, but DHA levels in breast milk are a better reflection of infant dietary exposure.[196, 204, 206] Our interest lies in infant dietary exposure since infant exposure to LCPUFAs is a prominent component of this dissertation.

Depressive symptoms early in pregnancy could reflect maternal affect over a long period of time pre-conception through early pregnancy. Likewise, breast milk DHA levels are more reflective of long-term stores of fatty acids and lifetime diet than short

term influences.[260] If women who are chronically depressed have low DHA stores, then it might be reflected in the breast milk. In contrast, depression later in pregnancy or postpartum might be related to perinatal events, and women who have a brief depressive episode might not exhibit lower DHA levels in breast milk.

While there has been growing interest in the relation between depression and DHA, studies have been unable to verify the temporal direction of the association. It is possible that depressed individuals have lower dietary n-3 intake, but this appears to be an incomplete explanation as some studies have observed no association between dietary intake and depressive symptoms and the results of trials to evaluate DHA supplements to treat depression have often been negative.[206, 211, 261-263] Other indications are that depressed individuals may have impaired DHA synthesis or accelerated breakdown of DHA.[197] This study cannot verify the temporal direction; however, our intention was not to uncover the mechanisms involved but to understand the relationship between depressive symptoms and DHA status in the context of fatty acids in the diets of breastfed infants.

Few studies in this area have focused on maternal affect in the perinatal period (most are unrelated to pregnancy). Of the perinatal studies, one observed no relationship between postpartum depression and DHA status, while three others observed that decreasing DHA levels were accompanied by increasing depressive symptoms, based on plasma DHA levels.[196, 204-206]

The proportion of women who experienced significant depressive symptoms in this study is similar to other studies, and the mean DHA concentration in the breast milk

samples in this study was in the range of other U.S. studies. For instance, Birch et al. found mean DHA levels of 0.29%.[63]

Some weaknesses of our study deserve mention. First, a clinical diagnosis of depression might be preferred over the use of depressive symptoms screeners; however, if an effect is observed at sub-clinical levels of depressive symptoms, it indicates a larger proportion of individuals might be affected, not only those who are clinically depressed. Second, we cannot rule out reduced dietary intake of n-3 fatty acids among individuals with elevated depressive symptoms as the reason for low DHA levels. However, we included n-3 intake in our regression models, and our results did not change when it was removed from the models. Several issues limit our ability to extend this analysis further at this time. For instance, incorporating information about red blood cell DHA concentrations during pregnancy would provide more information about antepartum DHA status, and red blood cell levels are a better indicator of maternal DHA status. Also, the PIN Babies Study has too few infants to examine the interaction between maternal perinatal depressive symptoms and infant exposure to LCPUFAs, so we cannot examine whether infants who are exposed to both maternal psychological distress and low levels of LCPUFAs demonstrate poorer developmental outcomes than infants exposed to only one or none of these.

One strength of this study is that it included three depressive symptoms assessments instead of just one, and two of the three assessments occurred during pregnancy which no previous studies have included. Second, we chose to measure DHA in breast milk, which may be a better marker of infant DHA exposure than DHA measured in plasma and red blood cells even though plasma and red blood cells may be

better markers of maternal DHA status. Third, most previous studies of perinatal depression and DHA status involved patients with clinical depression. We were able to examine associations in a sample of women unselected for pre-existing mental health conditions who experienced from very few to many depressive symptoms.

Conclusion

Women who experienced elevated depressive symptom early in pregnancy had 25% less DHA in their breast milk than women who experienced few symptoms. This difference may or may not be meaningful in terms of infant DHA exposure. The difference in DHA content of the treatment versus control formulas in infant feeding trials is greater than this, and the results of these trials (usually focused on neurodevelopment) are mixed. It is possible that any benefits might be more apparent at high doses.[116, 239, 256, 257] Regardless, perinatal depression remains an exposure of concern for clinicians and families due to its psychosocial influence on child development. A possible negative effect on infant nutrition is another reason to seek ways to reduce the prevalence of perinatal depression. If DHA supplementation can prevent or lessen depressive symptoms, it could have the added benefit of increasing the amount of DHA available to infants, if DHA indeed benefits child development.

Table 7.1. Characteristics of Women in the PIN Postpartum Study and Associations with Depressive Symptoms and Breast Milk DHA Concentration, 2002-2006

	CES-D, <20 weeks gestation (n, %)			*CES-D, 24-29 weeks gestation (n, %)*			*EPDS, 4th month postpartum (n, %)*		*Mean DHA concentration of breast milk (% of total fatty acids) (SD)*
	0-16	17+	missing	0-16	17+	missing	0-9	10+	
Overall	253 (83.5)	36 (11.9)	14 (4.6)	225 (74.3)	48 (15.8)	30 (9.9)	269 (88.8)	34 (11.2)	0.28 (0.22)
Maternal education									
0-12 years	11 (64.7)	4 (23.5)	2 (11.8)	8 (47.1)	7 (41.2)	2 (11.8)	13 (76.5)	4 (23.5)	0.31 (0.24)
13-16	112 (78.3)	24 (16.8)	7 (4.9)	103 (72.0)	25 (17.5)	15 (10.5)	124 (86.7)	19 (13.3)	0.25 (0.19)
>16	130 (90.9)	8 (5.6)	5 (3.5)	114 (79.7)	16 (11.2)	13 (9.1)	132 (92.3)	11 (7.7)	0.17 (0.09)
Smoking									
Yes	9 (56.3)	7 (43.8)	0	8 (50.0)	7 (43.8)	1 (6.3)	12 (75.0)	4 (25.0)	0.30 (0.24)
No	244 (85.0)	29 (10.1)	14 (4.9)	217 (75.6)	41 (14.3)	29 (10.1)	257 (89.6)	30 (10.5)	0.28 (0.22)
Maternal age (years)									
17-20	3 (50.0)	3 (50.0)	0	2 (33.3)	3 (50.0)	1 (16.7)	5 (83.3)	1 (16.7)	0.16 (0.05)
21-30	113 (79.0)	20 (14.0)	10 (7.0)	101 (70.6)	27 (18.9)	15 (10.5)	124 (86.7)	19 (13.3)	0.28 (0.23)
31-47	137 (89.0)	13 (8.4)	4 (2.6)	122 (79.2)	18 (11.7)	14 (9.1)	140 (90.9)	14 (9.1)	0.28 (0.21)
Daily dietary n-3 intake									
>=75th percentile	55 (80.9)	11 (16.2)	2 (2.9)	53 (77.9)	12 (17.7)	3 (4.4)	61 (89.7)	7 (10.3)	0.32 (0.29)
50-<75th percentile	63 (91.3)	6 (8.7)	0	57 (82.6)	12 (17.4)	0	65 (94.2)	4 (5.8)	0.24 (0.13)
25-<50th percentile	56 (87.5)	6 (9.4)	2 (3.1)	52 (81.3)	10 (15.6)	2 (3.1)	56 (87.5)	8 (12.5)	0.28 (0.25)
0-<25th percentile	64 (83.1)	11 (14.3)	2 (2.6)	60 (77.9)	14 (18.2)	3 (3.9)	69 (89.6)	8 (10.4)	0.28 (0.19)
missing	15 (60.0)	2 (8.0)	8 (32.0)	3 (12.0)	0	22 (88.0)	18 (72.0)	7 (28.0)	0.24 (0.12)
Presence of spouse or partner									
Yes	250 (84.8)	32 (10.9)	13 (4.4)	222 (75.3)	46 (15.6)	27 (9.2)	264 (89.5)	31 (10.5)	0.28 (0.22)
No	3 (37.5)	4 (50.0)	1 (12.5)	3 (37.5)	2 (25.0)	3 (37.5)	5 (62.5)	3 (37.5)	0.24 (0.11)
Laboratory									
Minnesota	149 (85.1)	19 (10.9)	7 (4.0)	132 (75.4)	27 (15.4)	16 (9.1)	153 (87.4)	22 (12.6)	0.20 (0.14)
North Carolina	104 (81.3)	17 (13.3)	7 (5.5)	93 (72.7)	21 (16.4)	14 (10.9)	116 (90.6)	12 (9.4)	0.38 (0.26)

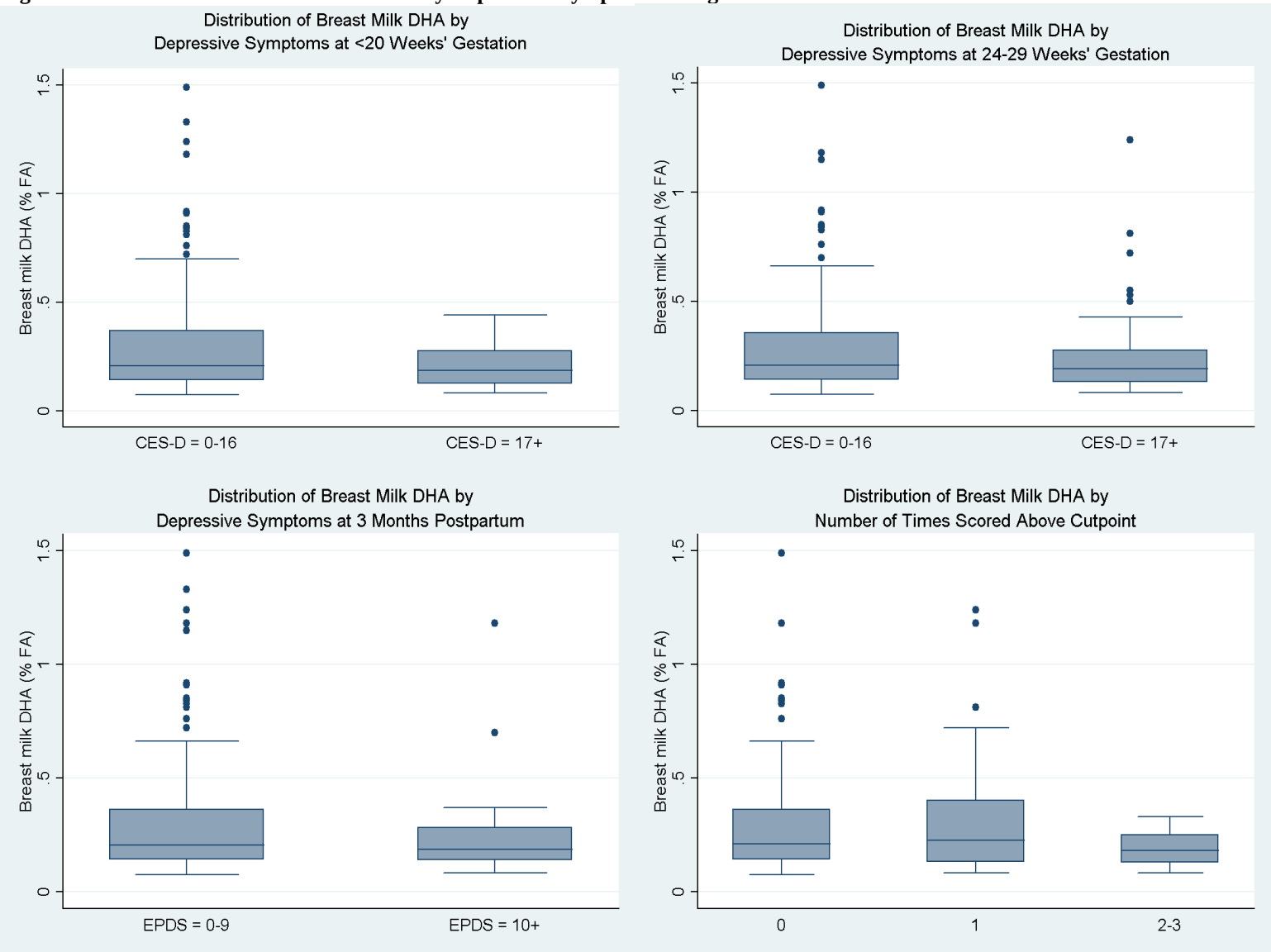
Table 7.2. Breast milk fatty acid concentration (logDHA) by depressive symptoms categories: Pregnancy, Infection, and Nutrition (PIN) and PIN Postpartum studies, 2002-2006

LogDHA	*CES-D (<20 weeks gestation) (0-16=ref)*	*CES-D (24-29 weeks gestation) (0-16=ref)*	*EPDS (4th month postpartum) (0-9=ref)*	*Number of times scored above a depressive symptoms screener cutpoint[a] (0=ref)*	
	17+	17+	10+	1	2-3
β (95% CI) Unadjusted model	-0.20 (-0.42, 0.02)	-0.07 (-0.26, 0.12)	-0.11 (-0.33, 0.12)	0.03 (-0.17, 0.23)	-0.23 (-0.48, 0.02)
β (95% CI) Adjusted model[b]	-0.22 (-0.42, -0.03)	-0.05 (-0.22, 0.12)	-0.02 (-0.21, 0.17)	0.00 (-0.17, 0.18)	-0.17 (-0.40, 0.06)

[a] Count of the number of times scored above 16 on the CES-D or above 9 on the EPDS

[b] Adjusted models included: maternal age, estimate of daily dietary n-3 intake during pregnancy, education, smoking, presence of a spouse or partner, and laboratory

Figure 7.1. Distribution of Breast Milk DHA by Depressive Symptoms Categories



CHAPTER 8

CONCLUSIONS

Overview

The goal of the present study was to fill gaps in the understanding of the roles of LCPUFA intake and maternal perinatal depression in infant neurodevelopment. Impaired development early in life can translate into lifelong cognitive deficits and behavioral disorders. Improved understanding of the role of LCPUFA intake in cognitive development can help optimize early life nutrition for development. Additionally, refined understanding of the role of perinatal depression, particularly when considered in relation to infant nutrition and other psychosocial influences, can potentially help direct mental health services or nutritional interventions.

This study built on an existing large pregnancy cohort study with an infant follow-up component and, thus, had a large database of prospective psychosocial, nutritional, and developmental data from which to draw. We developed a Conceptual Model that integrated the exposures and outcomes under study within the context of the perinatal period and infancy.

The results of our analysis suggest that differences in visual reception, language, and motor skills, and overall cognitive development among infants at 12 months may be explained by factors other than DHA and AA exposure, breastfeeding duration and exclusivity, and the socio-demographic factors that underlie choices about feeding method. We also examined the role of maternal psychological health in cognitive development and

found that maternal trait anxiety, but not perinatal depressive symptoms or perceived stress, was associated with reduced receptive language ability and overall cognition, but the differences were small. However, women who experienced elevated depressive symptoms early in pregnancy or at several points during the perinatal period had lower levels of DHA available to their infants via their breast milk. It is possible that the effects of LCPUFA exposure and maternal psychological health become more apparent after infancy or that our sample size limited the ability to detect small differences.

Strengths and limitations

LCPUFA exposure measurement

Despite the large number of studies that have examined the association between the LCPUFA content of infant formulas and various neurodevelopmental outcomes, there is no consensus about whether LCPUFAs enhance development. One reason for this may be the variety of exposure measures used in these studies and their ability to reflect infants' complex feeding patterns. Most studies do not take into account the wide variation in the extent to which infants are breastfed or formula fed and the differences in LCPUFA content of breast milk. Infant feeding trials generally use intent-to-treat analysis which can limit the generalizability of findings. This study had the benefit of detailed information about breastfeeding and formula use over time, and we used this information to develop a novel exposure measure that combined both sources. It was clear that feeding practices changed fairly frequently in this cohort: shifts in the number of feedings that were breastfed to formula fed and changes in the formula products used over time. Our exposure measurement

approach was able to capture this complexity and so is more reflective of actual infant feeding patterns compared to the intent-to-treat driven approaches used in infant feeding trials. Also, we were able to include some infants fed formulas with no DHA or AA added. This increased the variability of exposure compared to what might be observed in a sample of infants born more recently because almost all formula products today contain DHA and AA. In fact, a cohort of infants enrolled just a couple of years after this one might have zero infants fed only formulas without DHA and AA, making it impossible to have an unexposed comparison group.

Despite these strengths, this study was subject to some limitations pertaining to the ability to measure infant exposure to LCPUFAs. First, we had very few infants fed only formulas without supplemental DHA and AA for all months to allow direct comparison with infants fed only formulas with DHA and AA. This is because formulas with DHA and AA have increasingly displaced formulas without DHA and AA. Also, the formulas tend to have similar amounts of DHA and AA added, and this somewhat limits the variability in exposure. Second, despite the detailed nature of our novel exposure measure, it cannot capture some of the inter-individual and intra-individual variability in LCPUFA exposure across time. For instance, the study relied on a single breast milk measurement as the basis for estimating LCPUFA exposure from breast milk, thereby not capturing any possible variability in LCPUFA content of breast milk across lactation. Also, the study did not have information available on the amount of formula or breast milk consumed per feeding (or how this changed over time) so some variability in LCPUFA exposure was not accounted for. In addition, because some women stopped breastfeeding before the study visit or refused to provide a breast milk sample, we were unable to directly measure their infants' exposure via

breast milk. Finally, two depression symptoms instruments were used in this study; consistent use of a single instrument might have been preferred. Because of these limitations, the results of this study should be interpreted with caution. It is possible that this study underestimated the potential benefits of breastfeeding or LCPUFA exposure on infant development, particularly in light of the null results for the breastfeeding models which one might have expected to suggest a benefit. The null results for LCPUFA exposure are less surprising given that the majority of studies in this area have shown no benefit to cognitive development.

Outcome measurement – infant development

Assessing infant development is difficult and somewhat subjective because infant behavior in testing situations is variable and to some degree relies on input from mothers, who may vary in their ability to provide objective information. Assessments of young children are sensitive to rapport with the examiner as well. In addition, developmental assessments of infants tend to have somewhat limited predictive value largely because of the rapid developmental changes occurring in infancy. Few studies of LCPUFA exposure assess infant development beyond a measure of global development, like the Bayley. A major strength of this study was the use of the Mullen which simultaneously assesses multiple domains of infant development using five sub-scales and an overall composite. As a result, this study was able to assess whether the exposures varied in their associations by domain.

Study design and study population

Several features of the PIN Study design served as strengths and limitations to the analyses presented here. First, the prospective design of this study was a strength in that it avoided assessing the exposures based on recall or confusing the temporal sequence of the exposures and outcomes. The length of the follow-up period allowed for study of exposures during pregnancy with developmental outcomes more than one year later. Some previous studies were not able to study prenatal exposures or to follow infants for as many months. Second, while the women were recruited into this observational study via prenatal care clinics, they were not selected based on any pre-existing condition or screened for mental health characteristics. Also, infants were breastfed or formula fed according to what the family chose, not assigned to one particular method or product. These are strengths in that this study population might resemble the general population of women and infants more closely than a sample selected for prior depression history, for instance. Consequently, the results of this study might be more applicable to the general population than the clinical trials in this area. However, because the exposure prevalence (e.g., for maternal perinatal depressive symptoms) was fairly low in this sample and the sample was limited in size, we might have been unable to detect small differences between groups in this study that might have been apparent in a larger sample. This might explain some of our null results. Also because of our limited sample size we were unable to examine the effect of changes in depressive symptoms across pregnancy and the postpartum period. Third, with any prospective cohort study, selection bias is a limitation. The infants who participated in the developmental assessments at 12 months of age were a select group—those whose mothers completed the PIN Study and elected to continue into the PIN Postpartum component to 12

months. Women who experienced significant depressive symptoms or anxiety during pregnancy or high perceived stress postpartum were somewhat less likely to participate postpartum. If these children would also have scored lower on the Mullen scales than participating children, our results may underestimate the true association, but the data to evaluate this were unavailable. Finally, a prospective cohort study is normally considered a weaker design than a randomized controlled trial due to the trial's ability to handle confounding factors by randomization. However, when infant feeding trials include a non-randomized breastfed comparison group, this advantage no longer applies to comparisons involving this group, especially if key confounders are not addressed in the analysis. As such, the observational design of the present study is not necessarily a disadvantage, especially since we were able to incorporate key confounders into our analyses pertaining to feeding method.

Implications

Our findings suggest that maternal trait anxiety interferes to a slight extent with optimal infant cognitive development, and this is apparent by 12 months of age. Because trait anxiety is a stable characteristic of the individual, it is not easily modifiable but techniques for stress management and reliance on supportive family and friends might help buffer the potential effects of anxiety. Pharmaceutical interventions to control anxiety may be unwise during pregnancy and lactation. Our findings about depressive symptoms and breast milk DHA status support existing proposals that improving DHA status during pregnancy might help relieve depressive symptoms. Since DHA is naturally available in certain foods like fatty fish and also in over-the-counter supplements, recommendations for

pregnant women at risk of depression to consume more DHA could be implemented. To date, clinical trials in this area have failed to show relief from postpartum depression from DHA supplementation, but there have only been a few small studies. Also, there is uncertainty about the effects of large doses of DHA and concern about exposure to high levels of mercury and polychlorinated biphenyls in seafood. Whether consuming more DHA can help manage anxiety as well remains to be seen, but at least one study suggests it is a possibility.[264] Aside from its potential to reduce depressive symptoms in adults, the benefits of DHA to infant development remain uncertain as well. Our results indicated no benefit from LCPUFA intake, whether from breast milk or formula. Many previous feeding trials have shown similar results, and results overall have been equivocal.[17, 103, 110, 114] Most clinical trials that randomized women to LCPUFA supplements to examine effects on infant development have been unable to show any benefit to development from this method of supplementation.[143-148] As a result, if it is found that maternal supplementation helps with depressive symptoms, it cannot be expected that maternal supplementation will benefit infant development simultaneously. Given the conflicting results among previous studies and the strengths and limitations of this study, no actions are warranted to change infant feeding practices at this time except to provide a note of caution that the effects of LCPUFA supplementation of infants remain unclear and deserve further study.

Directions for Future Research

Future studies in five key areas are recommended to advance this field of research. First, studies of infant development should follow children longer into the school age years and examine specific developmental domains in relation to early life exposures to maternal

psychosocial influences, LCPUFAs, and the interaction between them. Second, larger studies should be designed to better pinpoint the optimal intake of n-3 fatty acids for women and LCPUFAs for infants. Feeding or supplementation dose-ranging trials covering a wider range of doses would be useful. The increasing acceptance and ubiquity of formula products and baby foods with LCPUFAs added might make forming an unexposed comparison group of infants for these studies more difficult, however. Third, methods development studies should be conducted to develop better ways of assessing infant exposure to LCPUFAs in ways that are applicable to the general population of infants and their complex feeding patterns. Fourth, more basic research into the neurophysiologic mechanisms involved in the relationship between n-3 fatty acids and mental health would help clarify the direction of the association between them and better direct efforts to improve mental health and optimize n-3 fatty acid status. Finally, because infant cognition is a product of complex interactions between multiple influences, future studies of infant development in this area should avoid examining one exposure alone and instead focus on the interactions between genetics (e.g., fatty acid desaturase genes), diet, and the psychosocial environment.

APPENDIX 1.

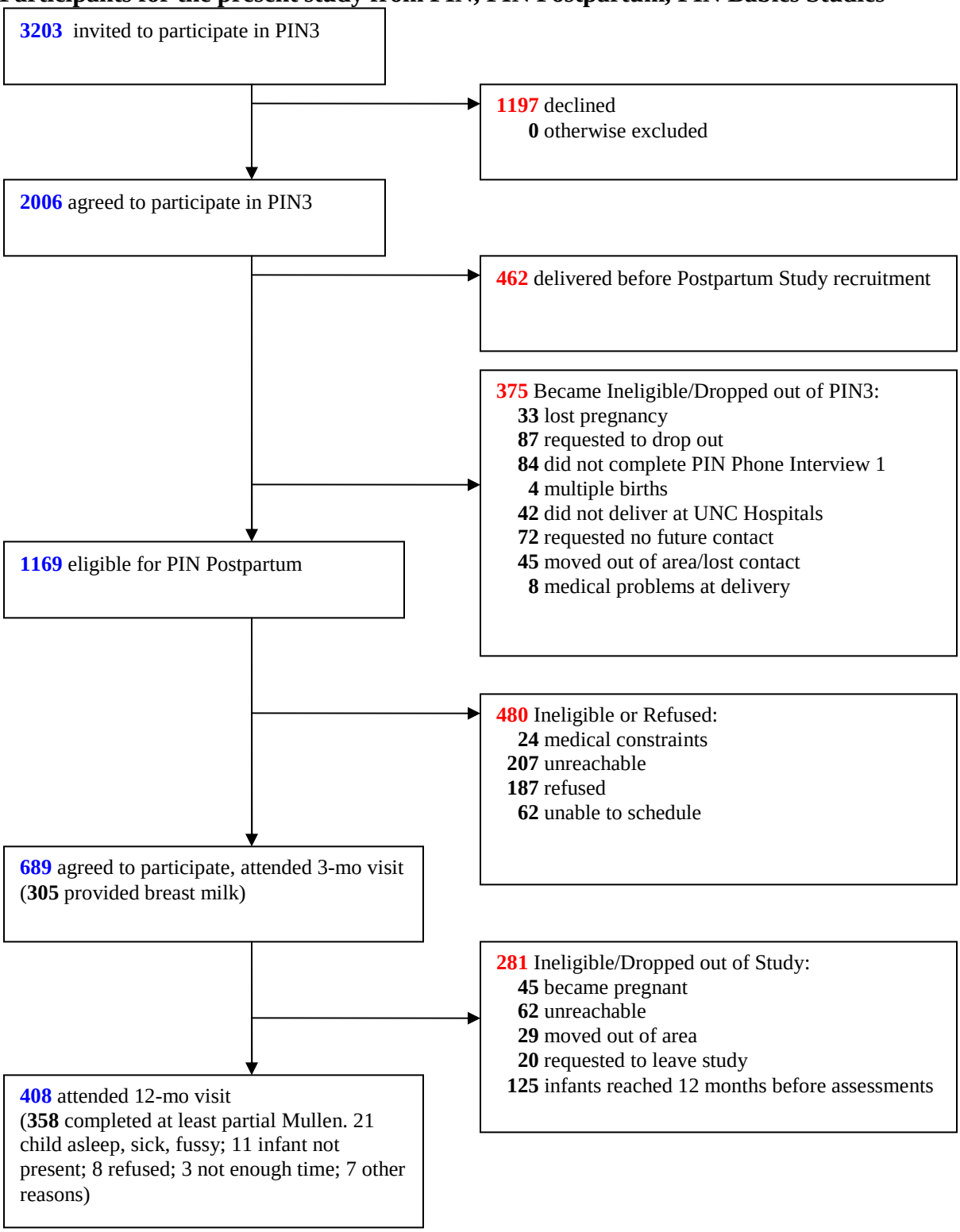
DHA and AA Content of Common Infant Formulas in Powder Form, 2002-2006[a]

Manufacturer	*Formula Product*	*DHA (g/100g edible portion)*	*AA (g/100g edible portion)*
Mead Johnson	Enfamil AR LIPIL	0.087	0.164
	Enfamil EnfaCare	0	0
	Enfamil EnfaCare LIPIL	0.080	0.170
	Enfamil Lactose Free	0	0
	Enfamil Lactose Free LIPIL	0.090	0.180
	Enfamil LIPIL	0.090	0.175
	Enfamil LIPIL with iron	0.089	0.168
	Enfamil low iron	0	0
	Enfamil with iron	0	0
	Nutramigen	0	0
	Nutramigen LIPIL	0.080	0.170
	ProSobee	0	0
	ProSobee LIPIL	0.085	0.170
Ross	Alimentum	0	0
	Alimentum Advance	0.011	0.018
	Isomil	0	0
	Isomil Advance	0.096	0.187
	Neosure	0	0
	Similac Advance	0.096	0.187
	Similac Lactose Free	0	0
	Similac low iron	0	0
	Similac with iron	0	0
Carnation	Good Start	0	0
	Good Start with iron, DHA, ARA	0.084	0.167
Other	Kirkland DHA, iron	0.103	0.181

[a] Source: U.S. Department of Agriculture Nutrient Database for Standard Reference[221] (except for Carnation products, source: Nestle Infant Nutrition Center website[222]).

APPENDIX 2

Participants for the present study from PIN, PIN Postpartum, PIN Babies Studies



REFERENCES

1. Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med.* May 1998;14(4):245-258.

2. Brooks-Gunn J, McCarton CM, Casey PH, et al. Early intervention in low-birth-weight premature infants. Results through age 5 years from the Infant Health and Development Program. *JAMA.* Oct 26 1994;272(16):1257-1262.

3. Brown AS, van Os J, Driessens C, Hoek HW, Susser ES. Further evidence of relation between prenatal famine and major affective disorder. *Am J Psychiatry.* Feb 2000;157(2):190-195.

4. Marlow N, Hennessy EM, Bracewell MA, Wolke D. Motor and executive function at 6 years of age after extremely preterm birth. *Pediatrics.* Oct 2007;120(4):793-804.

5. Schnaas L, Rothenberg SJ, Flores MF, et al. Reduced intellectual development in children with prenatal lead exposure. *Environ Health Perspect.* May 2006;114(5):791-797.

6. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. *Lancet.* Nov 3 1973;2(7836):999-1001.

7. van Kuijk FJ, Buck P. Fatty acid composition of the human macula and peripheral retina. *Invest Ophthalmol Vis Sci.* Dec 1992;33(13):3493-3496.

8. Kishimoto Y, Agranoff BW, Radin NS, Burton RM. Comparison of the fatty acids of lipids of subcellular brain fractions. *J Neurochem.* Mar 1969;16(3):397-404.

9. Anderson RE. Lipids of ocular tissues. IV. A comparison of the phospholipids from the retina of six mammalian species. *Exp Eye Res.* Oct 1970;10(2):339-344.

10. Sun GY, Sun Y. Phospholipids and acyl groups of synaptosomal and myelin membranes isolated from the cerebral cortex of squirrel monkey (Saimiri sciureus). *Biochim Biophys Acta.* Oct 5 1972;280(2):306-315.

11. Birch EE, Birch DG, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. *Invest Ophthalmol Vis Sci.* Oct 1992;33(11):3242-3253.

12. Carlson SE, Werkman SH, Rhodes PG, Tolley EA. Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am J Clin Nutr.* Jul 1993;58(1):35-42.

13. Carlson SE, Werkman SH, Tolley EA. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr.* May 1996;63(5):687-697.

14. Innis SM, Adamkin DH, Hall RT, et al. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. *J Pediatr.* May 2002;140(5):547-554.

15. O'Connor DL, Hall R, Adamkin D, et al. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics.* Aug 2001;108(2):359-371.

16. Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M. Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. *Pediatr Res.* Aug 1995;38(2):262-266.

17. Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. *Dev Med Child Neurol.* Mar 2000;42(3):174-181.

18. Lucas A, Stafford M, Morley R, et al. Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant-formula milk: a randomised trial. *Lancet.* Dec 4 1999;354(9194):1948-1954.

19. Makrides M, Gibson RA. Long-chain polyunsaturated fatty acid requirements during pregnancy and lactation. *Am J Clin Nutr.* Jan 2000;71(1 Suppl):307S-311S.

20. Nolen-Hoeksema S. Sex differences in unipolar depression: evidence and theory. *Psychol Bull.* Mar 1987;101(2):259-282.

21. Weissman MM, Bland RC, Canino GJ, et al. Cross-national epidemiology of major depression and bipolar disorder. *JAMA.* Jul 24-31 1996;276(4):293-299.

22. Andrade L, Caraveo-Anduaga JJ, Berglund P, et al. The epidemiology of major depressive episodes: results from the International Consortium of Psychiatric Epidemiology (ICPE) Surveys. *Int J Methods Psychiatr Res.* 2003;12(1):3-21.

23. Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal depression: a systematic review of prevalence and incidence. *Obstet Gynecol.* Nov 2005;106(5 Pt 1):1071-1083.

24. Bennett HA, Einarson A, Taddio A, Koren G, Einarson TR. Prevalence of depression during pregnancy: systematic review. *Obstet Gynecol.* Apr 2004;103(4):698-709.

25. Friedman E, Clark D, Gershon S. Stress, anxiety, and depression: Review of biological, diagnostic, and nosologic issues. *Journal of Anxiety Disorders.* 1992;6(4):337-363.

26. Brennan PA, Hammen C, Andersen MJ, Bor W, Najman JM, Williams GM. Chronicity, severity, and timing of maternal depressive symptoms: relationships with child outcomes at age 5. *Dev Psychol.* Nov 2000;36(6):759-766.

27. Sharp D, Hay DF, Pawlby S, Schmucker G, Allen H, Kumar R. The impact of postnatal depression on boys' intellectual development. *J Child Psychol Psychiatry.* Nov 1995;36(8):1315-1336.

28. Hay DF, Pawlby S, Angold A, Harold GT, Sharp D. Pathways to violence in the children of mothers who were depressed postpartum. *Dev Psychol.* Nov 2003;39(6):1083-1094.

29. Murray L, Kempton C, Woolgar M, Hooper R. Depressed mothers' speech to their infants and its relation to infant gender and cognitive development. *J Child Psychol Psychiatry.* Oct 1993;34(7):1083-1101.

30. Murray L, Sinclair D, Cooper P, Ducournau P, Turner P, Stein A. The socioemotional development of 5-year-old children of postnatally depressed mothers. *J Child Psychol Psychiatry.* Nov 1999;40(8):1259-1271.

31. Murray L, Fiori-Cowley A, Hooper R, Cooper P. The impact of postnatal depression and associated adversity on early mother-infant interactions and later infant outcome. *Child Dev.* Oct 1996;67(5):2512-2526.

32. Hay DF, Kumar R. Interpreting the effects of mothers' postnatal depression on children's intelligence: a critique and re-analysis. *Child Psychiatry Hum Dev.* Spring 1995;25(3):165-181.

33. Sinclair D, Murray L. Effects of postnatal depression on children's adjustment to school. Teacher's reports. *Br J Psychiatry.* Jan 1998;172:58-63.

34. Beck CT. Maternal depression and child behaviour problems: a meta-analysis. *J Adv Nurs.* Mar 1999;29(3):623-629.

35. Silverstein M, Augustyn M, Cabral H, Zuckerman B. Maternal depression and violence exposure: double jeopardy for child school functioning. *Pediatrics.* Sep 2006;118(3):e792-800.

36. McMahon C, Barnett B, Kowalenko N, Tennant C, Don N. Postnatal depression, anxiety and unsettled infant behaviour. *Aust N Z J Psychiatry.* Oct 2001;35(5):581-588.

37. Zelkowitz P, Papageorgiou A, Bardin C, Wang T. Persistent maternal anxiety affects the interaction between mothers and their very low birthweight children at 24 months. *Early Hum Dev.* Jul 14 2008.

38. Galler JR, Harrison RH, Ramsey F, Forde V, Butler SC. Maternal depressive symptoms affect infant cognitive development in Barbados. *J Child Psychol Psychiatry.* Sep 2000;41(6):747-757.

39. Weinberg MK, Tronick EZ. Emotional characteristics of infants associated with maternal depression and anxiety. *Pediatrics.* Nov 1998;102(5 Suppl E):1298-1304.

40. Murray L. The impact of postnatal depression on infant development. *J Child Psychol Psychiatry.* Mar 1992;33(3):543-561.

41. Ghodsian M, Zajicek E, Wolkind S. A longitudinal study of maternal depression and child behaviour problems. *J Child Psychol Psychiatry.* Jan 1984;25(1):91-109.

42. Hibbeln JR. Seafood consumption, the DHA content of mothers' milk and prevalence rates of postpartum depression: a cross-national, ecological analysis. *J Affect Disord.* May 2002;69(1-3):15-29.

43. Peet M, Murphy B, Shay J, Horrobin D. Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry.* Mar 1 1998;43(5):315-319.

44. Edwards R, Peet M, Shay J, Horrobin D. Omega-3 polyunsaturated fatty acid levels in the diet and in red blood cell membranes of depressed patients. *J Affect Disord.* Mar 1998;48(2-3):149-155.

45. Savitz DA, Dole N, Williams J, et al. Determinants of participation in an epidemiological study of preterm delivery. *Paediatr Perinat Epidemiol.* 1999 Jan 1999;13(1):114-125.

46. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev.* Mar 1979;3(1):79-83.

47. Dobbing J, Sands J. Quantitative growth and development of human brain. *Arch Dis Child.* Oct 1973;48(10):757-767.

48. Rice D, Barone S, Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect.* Jun 2000;108 Suppl 3:511-533.

49. Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog Lipid Res.* Jan-Mar 2001;40(1-2):1-94.

50. Gleason C. Fetal alcohol exposure: effects on the developing brain. *NeoReviews.* 2001;2:231-237.

51. Pope SK, Whiteside L, Brooks-Gunn J, et al. Low-birth-weight infants born to adolescent mothers. Effects of coresidency with grandmother on child development. *JAMA.* Mar 17 1993;269(11):1396-1400.

52. Siega-Riz AM, Adair LS, Hobel CJ. Maternal underweight status and inadequate rate of weight gain during the third trimester of pregnancy increases the risk of preterm delivery. *J Nutr.* Jan 1996;126(1):146-153.

53. Agaoglu L, Torun O, Unuvar E, Sefil Y, Demir D. Effects of iron deficiency anemia on cognitive function in children. *Arzneimittelforschung.* 2007;57(6A):426-430.

54. Lozoff B, Georgieff MK. Iron deficiency and brain development. *Semin Pediatr Neurol.* Sep 2006;13(3):158-165.

55. McCann JC, Ames BN. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. *Am J Clin Nutr.* Apr 2007;85(4):931-945.

56. Zeisel SH. Choline: critical role during fetal development and dietary requirements in adults. *Annu Rev Nutr.* 2006;26:229-250.

57. Smithells RW, Sheppard S, Schorah CJ. Vitamin dificiencies and neural tube defects. *Arch Dis Child.* Dec 1976;51(12):944-950.

58. Zimmermann MB. The adverse effects of mild-to-moderate iodine deficiency during pregnancy and childhood: a review. *Thyroid.* Sep 2007;17(9):829-835.

59. Hamosh M, Salem N, Jr. Long-chain polyunsaturated fatty acids. *Biol Neonate.* 1998;74(2):106-120.

60. Simopoulos AP. Omega-3 Polyunsaturated. In: Caballero B, Allen L, Prentice A, eds. *Encyclopedia of Human Nutrition*. 2nd ed: Elsevier; 2005:205-219.

61. *Health Effects of Polyunsaturated Fatty Acids in Seafoods*. Orlando, FL: Academic Press; 1986.

62. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. *Am J Clin Nutr.* Apr 1985;41(4):787-800.

63. Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C. Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr Res.* Aug 1998;44(2):201-209.

64. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr.* Jun 2007;85(6):1457-1464.

65. Muskiet FA, Kuipers RS, Smit EN, Joordens JC. The basis of recommendations for docosahexaenoic and arachidonic acids in infant formula: absolute or relative standards? *Am J Clin Nutr.* Dec 2007;86(6):1802-1803.

66. Oliw E, Gramstrom E, Anggard E. The prostaglandins and essential fatty acids. In: Pace-Asciak E, Gramstrom E, eds. *Prostaglandins and related substances / editors, C. Pace-Asciak and E. Granstrom*. Amsterdam: Elsevier; 1983:1-19.

67. Innis SM. Essential fatty acids in growth and development. *Prog Lipid Res.* 1991;30(1):39-103.

68. Bazan NG, Scott BL. Dietary omega-3 fatty acids and accumulation of docosahexaenoic acid in rod photoreceptor cells of the retina and at synapses. *Ups J Med Sci Suppl.* 1990;48:97-107.

69. Martinez M. Polyunsaturated fatty acids in the developing human brain, red cells and plasma: influence of nutrition and peroxisomal disease. *World Rev Nutr Diet.* 1994;75:70-78.

70. Clandinin MT, Chappell JE, Heim T, Swyer PR, Chance GW. Fatty acid utilization in perinatal de novo synthesis of tissues. *Early Hum Dev.* Sep 1981;5(4):355-366.

71. Martinez M. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. *Brain Res.* Jun 26 1992;583(1-2):171-182.

72. Carnielli VP, Wattimena DJ, Luijendijk IH, Boerlage A, Degenhart HJ, Sauer PJ. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. *Pediatr Res.* Jul 1996;40(1):169-174.

73. Heird WC, Lapillonne A. The role of essential fatty acids in development. *Annu Rev Nutr.* 2005;25:549-571.

74. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA. Arachidonic acid status correlates with first year growth in preterm infants. *Proc Natl Acad Sci U S A.* Feb 1 1993;90(3):1073-1077.

75. Al MD, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. *Eur J Clin Nutr.* Aug 1997;51(8):548-553.

76. Jensen RG. Lipids in human milk. *Lipids.* Dec 1999;34(12):1243-1271.

77. Jorgensen MH, Hernell O, Lund P, Holmer G, Michaelsen KF. Visual acuity and erythrocyte docosahexaenoic acid status in breast-fed and formula-fed term infants during the first four months of life. *Lipids.* Jan 1996;31(1):99-105.

78. Sanders TA, Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. *J Pediatr.* Apr 1992;120(4 Pt 2):S71-77.

79. Jorgensen MH, Hernell O, Hughes E, Michaelsen KF. Is there a relation between docosahexaenoic acid concentration in mothers' milk and visual development in term infants? *J Pediatr Gastroenterol Nutr.* Mar 2001;32(3):293-296.

80. Ribeiro M, Balcao V, Guimaraes H, et al. Fatty acid profile of human milk of Portuguese lactating women: prospective study from the 1st to the 16th week of lactation. *Ann Nutr Metab.* 2008;53(1):50-56.

81. Mitoulas LR, Gurrin LC, Doherty DA, Sherriff JL, Hartmann PE. Infant intake of fatty acids from human milk over the first year of lactation. *Br J Nutr.* Nov 2003;90(5):979-986.

82. Boersma ER, Offringa PJ, Muskiet FA, Chase WM, Simmons IJ. Vitamin E, lipid fractions, and fatty acid composition of colostrum, transitional milk, and mature milk: an international comparative study. *Am J Clin Nutr.* May 1991;53(5):1197-1204.

83. Makrides M, Simmer K, Neumann M, Gibson R. Changes in the polyunsaturated fatty acids of breast milk from mothers of full-term infants over 30 wk of lactation. *Am J Clin Nutr.* Jun 1995;61(6):1231-1233.

84. Agostoni C, Marangoni F, Giovannini M, Riva E, Galli C. Long-chain polyunsaturated fatty acids, infant formula, and breastfeeding. *Lancet.* Nov 21 1998;352(9141):1703-1704.

85. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. *Eur J Clin Nutr.* Jun 1996;50(6):352-357.

86. Michaelsen KF, Larsen PS, Thomsen BL, Samuelson G. The Copenhagen Cohort Study on Infant Nutrition and Growth: breast-milk intake, human milk macronutrient content, and influencing factors. *Am J Clin Nutr.* Mar 1994;59(3):600-611.

87. Motil KJ. Infant feeding: a critical look at infant formulas. *Curr Opin Pediatr.* Oct 2000;12(5):469-476.

88. Administration FaD. Nutrient requirements for infant formulas. Vol 50: Federal Register; 1985:45106-45108.

89. Raitan D, Talbot J, Waters J. LSRO report: Assessment of nutrient requirements for infant formulas. *Journal of Nutrition.* 1998;128:2059-2093S.

90. Aggett PJ, Haschke F, Heine W, et al. Comment on the content and composition of lipids in infant formulas. ESPGAN Committee on Nutrition. *Acta Paediatr Scand.* Aug-Sep 1991;80(8-9):887-896.

91. Koletzko B, Agostoni C, Carlson SE, et al. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. *Acta Paediatr.* Apr 2001;90(4):460-464.

92. Jensen CL, Heird WC. Lipids with an emphasis on long-chain polyunsaturated fatty acids. *Clin Perinatol.* Jun 2002;29(2):261-281, vi.

93. Koletzko B, Sinclair A. Long-chain polyunsaturated fatty acids in diets for infants: choices for recommending and regulating bodies and for manufacturers of dietary products. *Lipids.* Feb 1999;34(2):215-220.

94. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr.* Aug 1994;60(2):189-194.

95. Farquharson J, Cockburn F, Patrick WA, Jamieson EC, Logan RW. Effect of diet on infant subcutaneous tissue triglyceride fatty acids. *Arch Dis Child.* Nov 1993;69(5):589-593.

96. Farquharson J, Jamieson EC, Logan RW, Patrick WJ, Howatson AG, Cockburn F. Age- and dietary-related distributions of hepatic arachidonic and docosahexaenoic acid in early infancy. *Pediatr Res.* Sep 1995;38(3):361-365.

97. Hoefer C, MC. H. Later development of breast fed and artificially fed infants. *JAMA.* 1929;92:615-620.

98. Faldella G, Govoni M, Alessandroni R, et al. Visual evoked potentials and dietary long chain polyunsaturated fatty acids in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* Sep 1996;75(2):F108-112.

99. Uauy RD, Birch DG, Birch EE, Tyson JE, Hoffman DR. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatr Res.* Nov 1990;28(5):485-492.

100. Carlson SE, Cooke RJ, Rhodes PG, Peeples JM, Werkman SH, Tolley EA. Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids. *Pediatr Res.* Nov 1991;30(5):404-412.

101. Simmer K. Longchain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev.* 2001(4):CD000376.

102. Agostoni C, Riva E, Scaglioni S, Marangoni F, Radaelli G, Giovannini M. Dietary fats and cholesterol in italian infants and children. *Am J Clin Nutr.* Nov 2000;72(5 Suppl):1384S-1391S.

103. Agostoni C, Zuccotti GV, Radaelli G, et al. Docosahexaenoic acid supplementation and time at achievement of gross motor milestones in healthy infants: a randomized, prospective, double-blind, placebo-controlled trial. *Am J Clin Nutr.* Jan 2009;89(1):64-70.

104. Auestad N, Halter R, Hall RT, et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics.* Aug 2001;108(2):372-381.

105. Ben XM, Zhou XY, Zhao WH, et al. Growth and development of term infants fed with milk with long-chain polyunsaturated fatty acid supplementation. *Chin Med J (Engl).* Aug 2004;117(8):1268-1270.

106. Bouwstra H, Boersma ER, Boehm G, Dijck-Brouwer DA, Muskiet FA, Hadders-Algra M. Exclusive breastfeeding of healthy term infants for at least 6 weeks improves neurological condition. *J Nutr.* Dec 2003;133(12):4243-4245.

107. Bouwstra H, Dijck-Brouwer DA, Boehm G, Boersma ER, Muskiet FA, Hadders-Algra M. Long-chain polyunsaturated fatty acids and neurological developmental outcome at 18 months in healthy term infants. *Acta Paediatr.* Jan 2005;94(1):26-32.

108. Bouwstra H, Dijck-Brouwer DA, Wildeman JA, et al. Long-chain polyunsaturated fatty acids have a positive effect on the quality of general movements of healthy term infants. *Am J Clin Nutr.* Aug 2003;78(2):313-318.

109. Carlson SE. Lipid requirements of very-low-birth-weight infants for optimal growth and development. Paper presented at: Lipids, Learning, and the Brain: Fats in Infant Formulas, 1993; Columbus, OH.

110. Clandinin MT, Van Aerde JE, Merkel KL, et al. Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. *J Pediatr.* Apr 2005;146(4):461-468.

111. Fang PC, Kuo HK, Huang CB, Ko TY, Chen CC, Chung MY. The effect of supplementation of docosahexaenoic acid and arachidonic acid on visual acuity and neurodevelopment in larger preterm infants. *Chang Gung Med J.* Oct 2005;28(10):708-715.

112. Fewtrell MS, Morley R, Abbott RA, et al. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. *Pediatrics.* Jul 2002;110(1 Pt 1):73-82.

113. Fewtrell MS, Abbott RA, Kennedy K, et al. Randomized, double-blind trial of long-chain polyunsaturated fatty acid supplementation with fish oil and borage oil in preterm infants. *J Pediatr.* Apr 2004;144(4):471-479.

114. Makrides M, Gibson RA, McPhee AJ, et al. Neurodevelopmental outcomes of preterm infants fed high-dose docosahexaenoic acid: a randomized controlled trial. *JAMA.* Jan 14 2009;301(2):175-182.

115. O'Connor DL, Jacobs J, Hall R, et al. Growth and development of premature infants fed predominantly human milk, predominantly premature infant formula, or a combination of human milk and premature formula. *J Pediatr Gastroenterol Nutr.* Oct 2003;37(4):437-446.

116. Scott DT, Janowsky JS, Carroll RE, Taylor JA, Auestad N, Montalto MB. Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? *Pediatrics.* Nov 1998;102(5):E59.

117. van Wezel-Meijler G, van der Knaap MS, Huisman J, Jonkman EJ, Valk J, Lafeber HN. Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. *Acta Paediatr.* 2002;91(9):942-950.

118. Werkman SH, Carlson SE. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months. *Lipids.* Jan 1996;31(1):91-97.

119. Carlson SE, Werkman SH. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. *Lipids.* Jan 1996;31(1):85-90.

120. Willatts P, Forsyth JS, DiModugno MK, Varma S, Colvin M. Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet.* Aug 29 1998;352(9129):688-691.

121. Sacker A, Quigley MA, Kelly YJ. Breastfeeding and developmental delay: findings from the millennium cohort study. *Pediatrics.* Sep 2006;118(3):e682-689.

122. Agostoni C, Marangoni F, Giovannini M, Galli C, Riva E. Prolonged breast-feeding (six months or more) and milk fat content at six months are associated with higher developmental scores at one year of age within a breast-fed population. *Adv Exp Med Biol.* 2001;501:137-141.

123. Angelsen NK, Vik T, Jacobsen G, Bakketeig LS. Breast feeding and cognitive development at age 1 and 5 years. *Arch Dis Child.* Sep 2001;85(3):183-188.

124. Dee DL, Li R, Lee LC, Grummer-Strawn LM. Associations between breastfeeding practices and young children's language and motor skill development. *Pediatrics.* Feb 2007;119 Suppl 1:S92-98.

125. Bier JA, Oliver T, Ferguson AE, Vohr BR. Human milk improves cognitive and motor development of premature infants during infancy. *J Hum Lact.* Nov 2002;18(4):361-367.

126. Florey CD, Leech AM, Blackhall A. Infant feeding and mental and motor development at 18 months of age in first born singletons. *Int J Epidemiol.* 1995;24 Suppl 1:S21-26.

127. Gomez-Sanchiz M, Canete R, Rodero I, Baeza JE, Avila O. Influence of breast-feeding on mental and psychomotor development. *Clin Pediatr (Phila).* Jan-Feb 2003;42(1):35-42.

128. Innis SM, Nelson CM, Lwanga D, Rioux FM, Waslen P. Feeding formula without arachidonic acid and docosahexaenoic acid has no effect on preferential looking acuity or recognition memory in healthy full-term infants at 9 mo of age. *Am J Clin Nutr.* Jul 1996;64(1):40-46.

129. Feldman R, Eidelman AI. Direct and indirect effects of breast milk on the neurobehavioral and cognitive development of premature infants. *Dev Psychobiol.* Sep 2003;43(2):109-119.

130. Eidelman AI, Feldman R. Positive effect of human milk on neurobehavioral and cognitive development of premature infants. *Adv Exp Med Biol.* 2004;554:359-364.

131. Jacobson JL, Jacobson SW, Muckle G, Kaplan-Estrin M, Ayotte P, Dewailly E. Beneficial effects of a polyunsaturated fatty acid on infant development: evidence from the inuit of arctic Quebec. *J Pediatr.* Mar 2008;152(3):356-364.

132. Lucas A, Morley R, Cole TJ, Gore SM. A randomised multicentre study of human milk versus formula and later development in preterm infants. *Arch Dis Child Fetal Neonatal Ed.* Mar 1994;70(2):F141-146.

133. Lucas A, Morley R, Cole TJ, et al. Early diet in preterm babies and developmental status in infancy. *Arch Dis Child.* Nov 1989;64(11):1570-1578.

134. Morley R, Cole TJ, Powell R, Lucas A. Mother's choice to provide breast milk and developmental outcome. *Arch Dis Child.* Nov 1988;63(11):1382-1385.

135. Pinelli J, Saigal S, Atkinson SA. Effect of breastmilk consumption on neurodevelopmental outcomes at 6 and 12 months of age in VLBW infants. *Adv Neonatal Care.* Apr 2003;3(2):76-87.

136. Rogan WJ, Gladen BC. Breast-feeding and cognitive development. *Early Hum Dev.* Jan 1993;31(3):181-193.

137. Strain JJ, Davidson PW, Bonham MP, et al. Associations of maternal long-chain polyunsaturated fatty acids, methyl mercury, and infant development in the Seychelles Child Development Nutrition Study. *Neurotoxicology.* Sep 2008;29(5):776-782.

138. Vohr BR, Poindexter BB, Dusick AM, et al. Beneficial effects of breast milk in the neonatal intensive care unit on the developmental outcome of extremely low birth weight infants at 18 months of age. *Pediatrics.* Jul 2006;118(1):e115-123.

139. Morrow-Tlucak M, Haude RH, Ernhart CB. Breastfeeding and cognitive development in the first 2 years of life. *Soc Sci Med.* 1988;26(6):635-639.

140. Ounsted M, Moar VA, Scott A. Neurological development of small-for-gestational age babies during the first year of life. *Early Hum Dev.* Mar 1988;16(2-3):163-172.

141. Temboury MC, Otero A, Polanco I, Arribas E. Influence of breast-feeding on the infant's intellectual development. *J Pediatr Gastroenterol Nutr.* Jan 1994;18(1):32-36.

142. Vestergaard M, Obel C, Henriksen TB, Sorensen HT, Skajaa E, Ostergaard J. Duration of breastfeeding and developmental milestones during the latter half of infancy. *Acta Paediatr.* Dec 1999;88(12):1327-1332.

143. Helland IB, Saugstad OD, Smith L, et al. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics.* Nov 2001;108(5):E82.

144. Gibson RA, Neumann MA, Makrides M. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. *Eur J Clin Nutr.* Sep 1997;51(9):578-584.

145. Lauritzen L, Jorgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. *Reprod Nutr Dev.* Sep-Oct 2005;45(5):535-547.

146. Jensen CL, Voigt RG, Prager TC, et al. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am J Clin Nutr.* Jul 2005;82(1):125-132.

147. Judge MP, Harel O, Lammi-Keefe CJ. Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. *Am J Clin Nutr.* Jun 2007;85(6):1572-1577.

148. Tofail F, Kabir I, Hamadani JD, et al. Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants. *J Health Popul Nutr.* Mar 2006;24(1):48-56.

149. Jain A, Concato J, Leventhal JM. How good is the evidence linking breastfeeding and intelligence? *Pediatrics.* Jun 2002;109(6):1044-1053.

150. Garcia PT, Holman RT. Competitive inhibitions in the metabolism of polyunsaturated fatty acids studied via the composition of phospholipids, triglycerides and cholesteryl esters of rat tissues. *J Am Oil Chem Soc.* Dec 1965;42(12):1137-1141.

151. Mooney KL. The Child Behavior Checklist. In: Keyser DJ, Sweetland RD, eds. *Test critiques*. Kansas City, MO: Westport; 1984:181-182.

152. Lewis M, McGurk H. Evaluation of Infant Intelligence: Infant intelligence scores--true or false? *Science.* Dec 15 1972;178(4066):1174-1177.

153. McCall RB, Carriger MS. A meta-analysis of infant habituation and recognition memory performance as predictors of later IQ. *Child Dev.* Feb 1993;64(1):57-79.

154. Bornstein MH, Sigman MD. Continuity in mental development from infancy. *Child Dev.* Apr 1986;57(2):251-274.

155. Bayley N. *The Bayley Scales of Infant Development*. San Antonio: Pyschological Corporation; 1993.

156. Lutter C. Breastfeeding. In: Caballero B, Allen L, Prentice A, eds. *Encyclopedia of Human Nutrition*. 2nd ed: Elsevier; 2005.

157. McCarter-Spaulding D, Horowitz JA. How does postpartum depression affect breastfeeding? *MCN Am J Matern Child Nurs.* Jan-Feb 2007;32(1):10-17.

158. Dennis CL. Breastfeeding initiation and duration: a 1990-2000 literature review. *J Obstet Gynecol Neonatal Nurs.* Jan-Feb 2002;31(1):12-32.

159. Lanting CI, Patandin S, Weisglas-Kuperus N, Touwen BC, Boersma ER. Breastfeeding and neurological outcome at 42 months. *Acta Paediatr.* Dec 1998;87(12):1224-1229.

160. Lu MC, Lange L, Slusser W, Hamilton J, Halfon N. Provider encouragement of breast-feeding: evidence from a national survey. *Obstet Gynecol.* Feb 2001;97(2):290-295.

161. Chatterji P, Bonuck K, Dhawan S, Deb N. *WIC participation and the initiation and duration of breastfeeding.* Madison, WI: Institute for Research on Poverty; 2002.

162. Wright AL. The rise of breastfeeding in the United States. *Pediatr Clin North Am.* Feb 2001;48(1):1-12.

163. Loveland Cook CA, Flick LH, Homan SM, Campbell C, McSweeney M, Gallagher ME. Posttraumatic stress disorder in pregnancy: prevalence, risk factors, and treatment. *Obstet Gynecol.* Apr 2004;103(4):710-717.

164. Andersson L, Sundstrom-Poromaa I, Bixo M, Wulff M, Bondestam K, aStrom M. Point prevalence of psychiatric disorders during the second trimester of pregnancy: a population-based study. *Am J Obstet Gynecol.* Jul 2003;189(1):148-154.

165. Halbreich U. Postpartum disorders: multiple interacting underlying mechanisms and risk factors. *J Affect Disord.* Sep 2005;88(1):1-7.

166. Halbreich U, Karkun S. Cross-cultural and social diversity of prevalence of postpartum depression and depressive symptoms. *J Affect Disord.* Apr 2006;91(2-3):97-111.

167. Beck CT. Predictors of postpartum depression: an update. *Nurs Res.* Sep-Oct 2001;50(5):275-285.

168. Hernandez-Martinez C, Arija V, Balaguer A, Cavalle P, Canals J. Do the emotional states of pregnant women affect neonatal behaviour? *Early Hum Dev.* Jun 18 2008.

169. Mulder EJ, Robles de Medina PG, Huizink AC, Van den Bergh BR, Buitelaar JK, Visser GH. Prenatal maternal stress: effects on pregnancy and the (unborn) child. *Early Hum Dev.* Dec 2002;70(1-2):3-14.

170. Alder J, Fink N, Bitzer J, Hosli I, Holzgreve W. Depression and anxiety during pregnancy: a risk factor for obstetric, fetal and neonatal outcome? A critical review of the literature. *J Matern Fetal Neonatal Med.* Mar 2007;20(3):189-209.

171. Halbreich U. The association between pregnancy processes, preterm delivery, low birth weight, and postpartum depressions--the need for interdisciplinary integration. *Am J Obstet Gynecol.* Oct 2005;193(4):1312-1322.

172. Talge NM, Neal C, Glover V. Antenatal maternal stress and long-term effects on child neurodevelopment: how and why? *J Child Psychol Psychiatry.* Mar-Apr 2007;48(3-4):245-261.

173. Kato T. Molecular genetics of bipolar disorder and depression. *Psychiatry Clin Neurosci.* Feb 2007;61(1):3-19.

174. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* Jul 18 2003;301(5631):386-389.

175. Zhang X, Gainetdinov RR, Beaulieu JM, et al. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron.* Jan 6 2005;45(1):11-16.

176. Schumacher J, Jamra RA, Becker T, et al. Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. *Biol Psychiatry.* Aug 15 2005;58(4):307-314.

177. Burr W, Leigh G, Day R, Constantine J. Symbolic interaction and the family. In: Burr W, Hill R, Nye F, IR. R, eds. *Contemporary theories about the family.* Vol 2. New York: New York Press; 1979:42-111.

178. Mercer RT. Predictors of maternal role attainment at one year postbirth. *West J Nurs Res.* Feb 1986;8(1):9-32.

179. Harwood K, McLean N, Durkin K. First-time mothers' expectations of parenthood: What happens when optimistic expectations are not matched by later experiences? *Dev Psychol.* Jan 2007;43(1):1-12.

180. Gross D, Conrad B, Fogg L, Wothke W. A longitudinal model of maternal self-efficacy, depression, and difficult temperament during toddlerhood. *Res Nurs Health.* Jun 1994;17(3):207-215.

181. Silver EJ, Heneghan AM, Bauman LJ, Stein RE. The relationship of depressive symptoms to parenting competence and social support in inner-city mothers of young children. *Matern Child Health J.* Jan 2006;10(1):105-112.

182. Beck CT. A meta-analysis of predictors of postpartum depression. *Nurs Res.* Sep-Oct 1996;45(5):297-303.

183. Cutrona CE, Troutman BR. Social support, infant temperament, and parenting self-efficacy: a mediational model of postpartum depression. *Child Dev.* Dec 1986;57(6):1507-1518.

184. Lovejoy MC, Graczyk PA, O'Hare E, Neuman G. Maternal depression and parenting behavior: a meta-analytic review. *Clin Psychol Rev.* Aug 2000;20(5):561-592.

185. Field T, Healy B, Goldstein S, et al. Infants of depressed mothers show "depressed" behavior even with nondepressed adults. *Child Dev.* Dec 1988;59(6):1569-1579.

186. Bornstein MH. *Handbook of Parenting*. Mahwah, NJ: Lawrence Erlbaum Associates; 1995.

187. Brouwers E, van Baar A, Pop V. Maternal anxiety during pregnancy and subsequent infant development. *Infant Behavior and Development.* 2001;24:95-106.

188. Carvalho AE, Martinez FE, Linhares MB. Maternal anxiety and depression and development of prematurely born infants in the first year of life. *Span J Psychol.* Nov 2008;11(2):600-608.

189. Cornish AM, McMahon CA, Ungerer JA, Barnett B, Kowalenko N, Tennant C. Postnatal depression and infant cognitive and motor development in the second postnatal year: The impact of depression chronicity and infant gender. *Infant Behavior and Development.* 2005;28:407-417.

190. DiPietro JA, Novak MF, Costigan KA, Atella LD, Reusing SP. Maternal psychological distress during pregnancy in relation to child development at age two. *Child Dev.* May-Jun 2006;77(3):573-587.

191. Kurstjens S, Wolke D. Effects of maternal depression on cognitive development of children over the first 7 years of life. *J Child Psychol Psychiatry.* Jul 2001;42(5):623-636.

192. Lyons-Ruth K, Zoll D, Connell D, Grunebaum HU. The depressed mother and her one-year-old infant: environment, interaction, attachment, and infant development. *New Dir Child Dev.* Winter 1986(34):61-82.

193. Spielberger C. *Manual for the State-Trait Anxiety Inventory*. Palo Alto: Consulting Psychologists Press; 1983.

194. Cicchetti D, Rogosch FA, Toth SL. Maternal depressive disorder and contextual risk: contributions to the development of attachment insecurity and behavior problems in toddlerhood. *Dev Psychopathol.* Spring 1998;10(2):283-300.

195. McNamara RK, Hahn CG, Jandacek R, et al. Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biol Psychiatry.* Jul 1 2007;62(1):17-24.

196. Otto SJ, de Groot RH, Hornstra G. Increased risk of postpartum depressive symptoms is associated with slower normalization after pregnancy of the functional docosahexaenoic acid status. *Prostaglandins Leukot Essent Fatty Acids.* Oct 2003;69(4):237-243.

197. Young G, Conquer J. Omega-3 fatty acids and neuropsychiatric disorders. *Reprod Nutr Dev.* Jan-Feb 2005;45(1):1-28.

198. Hibbeln JR, Salem N, Jr. Dietary polyunsaturated fatty acids and depression: when cholesterol does not satisfy. *Am J Clin Nutr.* Jul 1995;62(1):1-9.

199. Hibbeln JR. Fish consumption and major depression. *Lancet.* Apr 18 1998;351(9110):1213.

200. Tanskanen A, Hibbeln JR, Tuomilehto J, et al. Fish consumption and depressive symptoms in the general population in Finland. *Psychiatr Serv.* Apr 2001;52(4):529-531.

201. Timonen M, Horrobin D, Jokelainen J, Laitinen J, Herva A, Rasanen P. Fish consumption and depression: the Northern Finland 1966 birth cohort study. *J Affect Disord.* Nov 1 2004;82(3):447-452.

202. Vuori E, Kiuru K, Makinen SM, Vayrynen P, Kara R, Kuitunen P. Maternal diet and fatty acid pattern of breast milk. *Acta Paediatr Scand.* Nov 1982;71(6):959-963.

203. Breastfeeding outcome indicators – Ever Breastfed, Breastfeeding at 6 months, Breastfeeding at 12 months, Exclusive breastfeeding at 3 months, Exclusive breastfeeding at 6 months, 2004 births. National Immunization Survey. Hyattsville, MD: Centers for Disease Control and Prevention.

204. De Vriese SR, Christophe AB, Maes M. Lowered serum n-3 polyunsaturated fatty acid (PUFA) levels predict the occurrence of postpartum depression: further evidence that lowered n-PUFAs are related to major depression. *Life Sci.* Nov 7 2003;73(25):3181-3187.

205. Makrides M, Crowther CA, Gibson RA, Gibson RS, Skeaff CM. Docosahexaenoic acid and post-partum depression - is there a link? *Asia Pac J Clin Nutr.* 2003;12 Suppl:S37.

206. Browne JC, Scott KM, Silvers KM. Fish consumption in pregnancy and omega-3 status after birth are not associated with postnatal depression. *J Affect Disord.* Feb 2006;90(2-3):131-139.

207. Freeman MP, Davis M, Sinha P, Wisner KL, Hibbeln JR, Gelenberg AJ. Omega-3 fatty acids and supportive psychotherapy for perinatal depression: a randomized placebo-controlled study. *J Affect Disord.* Sep 2008;110(1-2):142-148.

208. Freeman MP, Hibbeln JR, Wisner KL, Watchman M, Gelenberg AJ. An open trial of Omega-3 fatty acids for depression in pregnancy*. *Acta Neuropsychiatrica.* 2006;18(1):21-24.

209. Freeman MP, Hibbeln JR, Wisner KL, Brumbach BH, Watchman M, Gelenberg AJ. Randomized dose-ranging pilot trial of omega-3 fatty acids for postpartum depression. *Acta Psychiatr Scand.* Jan 2006;113(1):31-35.

210. Llorente AM, Jensen CL, Voigt RG, Fraley JK, Berretta MC, Heird WC. Effect of maternal docosahexaenoic acid supplementation on postpartum depression and information processing. *Am J Obstet Gynecol.* May 2003;188(5):1348-1353.

211. Marangell LB, Martinez JM, Zboyan HA, Chong H, Puryear LJ. Omega-3 fatty acids for the prevention of postpartum depression: negative data from a preliminary, open-label pilot study. *Depress Anxiety.* 2004;19(1):20-23.

212. Radloff LS. The CES-D Scale: A self-report depression scale for research in the general population. *Applied Psychological Measurement.* 1977;1(3):385-401.

213. Rosenberg M. *Society and the adolescent self-image*. Princeton, NJ: Princeton University Press; 1965.

214. Sherbourne CD, Stewart AL. The MOS social support survey. *Soc Sci Med.* 1991;32(6):705-714.

215. Cox J, Holden J. *Perinatal mental health: A guide to the Edinburgh Postnatal Depression Scale*. London: Gaskell; 2003.

216. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav.* Dec 1983;24(4):385-396.

217. Mullen E. *Mullen Scales of Early Learning*. Circle Pines, MN: American Guidance Service, Inc.; 1995.

218. Eickmann SH, de Lira PI, Lima Mde C, Coutinho SB, Teixeira Mde L, Ashworth A. Breast feeding and mental and motor development at 12 months in a low-income population in northeast Brazil. *Paediatr Perinat Epidemiol.* Mar 2007;21(2):129-137.

219. Labbok M, Krasovec K. Toward consistency in breastfeeding definitions. *Stud Fam Plann.* Jul-Aug 1990;21(4):226-230.

220. Organization WH. Indicators for Assessing Breastfeeding Practices. Geneva; 1991.

221. USDA Nutrient Database for Standard Reference. 2008. http://www.ars.usda.gov/nutrientdata. Updated Last Updated Date. Accessed February 13, 2009.

222. Nestle. www.nestle-infantnutrition.com. Accessed February 13, 2009.

223. Spacapan S, Oskamp S, eds. *The Social Psychology of Health*. Newbury Park: Sage; 1988.

224. Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ. Assessing depressive symptoms in five psychiatric populations: a validation study. *Am J Epidemiol.* Sep 1977;106(3):203-214.

225. Orr ST, Miller CA. Maternal depressive symptoms and the risk of poor pregnancy outcome. Review of the literature and preliminary findings. *Epidemiol Rev.* 1995;17(1):165-171.

226. Zuckerman B, Amaro H, Bauchner H, Cabral H. Depressive symptoms during pregnancy: relationship to poor health behaviors. *Am J Obstet Gynecol.* May 1989;160(5 Pt 1):1107-1111.

227. Hoffman S, Hatch MC. Depressive symptomatology during pregnancy: evidence for an association with decreased fetal growth in pregnancies of lower social class women. *Health Psychol.* Nov 2000;19(6):535-543.

228. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry.* Jun 1987;150:782-786.

229. Harris B, Huckle P, Thomas R, Johns S, Fung H. The use of rating scales to identify post-natal depression. *Br J Psychiatry.* Jun 1989;154:813-817.

230. Murray L, Carothers AD. The validation of the Edinburgh Post-natal Depression Scale on a community sample. *Br J Psychiatry.* Aug 1990;157:288-290.

231. Matthey S, Henshaw C, Elliott S, Barnett B. Variability in use of cut-off scores and formats on the Edinburgh Postnatal Depression Scale: implications for clinical and research practice. *Arch Womens Ment Health.* Nov 2006;9(6):309-315.

232. Akshoomoff N. Use of the Mullen Scales of Early Learning for the assessment of young children with Autism Spectrum Disorders. *Child Neuropsychol.* Aug 2006;12(4-5):269-277.

233. Schraeder BD. Assessment of measures to detect preschool academic risk in very-low-birth-weight children. *Nurs Res.* Jan-Feb 1993;42(1):17-21.

234. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology.* 1999;10:37-48.

235. Robins JM. Data, design, and background knowledge in etiologic inference. *Epidemiology.* 2001;11:313-320.

236. Rubin D. *Multiple Imputation for Nonresponse in Surveys*. New York: John Wiley & Sons; 1987.

237. Schafer D. Covariate measurement error in generalized linear models. *Biometrika.* 1987;74(2):385-391.

238. *SAS software, v9.1* [computer program]. Version. Cary, NC: SAS Institute Inc.; 2002-2003.

239. Makrides M, Neumann MA, Simmer K, Gibson RA. A critical appraisal of the role of dietary long-chain polyunsaturated fatty acids on neural indices of term infants: a randomized, controlled trial. *Pediatrics.* Jan 2000;105(1 Pt 1):32-38.

240. Carlson SE, Cooke RJ, Werkman SH, Tolley EA. First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. *Lipids.* Nov 1992;27(11):901-907.

241. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol.* Aug 1959;37(8):911-917.

242. Tacconi M, Wurtman RJ. Rat brain phosphatidyl-N,N-dimethylethanolamine is rich in polyunsaturated fatty acids. *J Neurochem.* Sep 1985;45(3):805-809.

243. Keim SA, Daniels J, Dole N, Herring AH, Siega-Riz AM, Scheidt PC. A prospective study of maternal anxiety, perceived stress, and depressive symptoms in relation to infant cognitive development. *In review.*

244. White H. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. *Econometrica.* 1980;48(4):817-838.

245. Beck CT. The effects of postpartum depression on maternal-infant interaction: a meta-analysis. *Nurs Res.* Sep-Oct 1995;44(5):298-304.

246. Assel MA, Landry SH, Swank PR, Steelman L, Miller-Loncar C, Smith KE. How do mothers' childrearing histories, stress and parenting affect children's behavioural outcomes? *Child Care Health Dev.* Sep 2002;28(5):359-368.

247. Atashili J, Ta M. Paper 032-2007 A SAS macro for automating the 'change-in-estimate' strategy for assessing confounding. *SAS Global Forum 2007.* Orlando; 2007.

248. Fenson L, Dale P, Reznick J, et al. *MacArthur Communicative Development Inventories. User's Guide and Technical Manual.* San Diego, London: Singular Publishing Group, Inc.; 1993.

249. Feeley N, Gottlieb L, Zelkowitz P. Infant, mother, and contextual predictors of mother-very low birth weight infant interaction at 9 months of age. *J Dev Behav Pediatr.* Feb 2005;26(1):24-33.

250. Zelkowitz P, Papageorgiou A, Bardin C, Wang T. Persistent maternal anxiety affects the interaction between mothers and their very low birthweight children at 24 months. *Early Hum Dev.* Jan 2009;85(1):51-58.

251. Irwin JR, Carter AS, Briggs-Gowan MJ. The social-emotional development of "late-talking" toddlers. *J Am Acad Child Adolesc Psychiatry.* Nov 2002;41(11):1324-1332.

252. Needleman HL, Leviton A, Bellinger D. Lead-associated intellectual deficit. *N Engl J Med.* Feb 11 1982;306(6):367.

253. Feldman R, Eidelman AI, Rotenberg N. Parenting stress, infant emotion regulation, maternal sensitivity, and the cognitive development of triplets: a model for parent and child influences in a unique ecology. *Child Dev.* Nov-Dec 2004;75(6):1774-1791.

254. Singer LT, Salvator A, Guo S, Collin M, Lilien L, Baley J. Maternal psychological distress and parenting stress after the birth of a very low-birth-weight infant. *JAMA.* Mar 3 1999;281(9):799-805.

255. Stancil TR, Hertz-Picciotto I, Schramm M, Watt-Morse M. Stress and pregnancy among African-American women. *Paediatr Perinat Epidemiol.* Apr 2000;14(2):127-135.

256. Birch EE, Castaneda YS, Wheaton DH, Birch DG, Uauy RD, Hoffman DR. Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. *Am J Clin Nutr.* Apr 2005;81(4):871-879.

257. Carlson SE, Ford AJ, Werkman SH, Peeples JM, Koo WW. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediatr Res.* May 1996;39(5):882-888.

258. Block G, Coyle LM, Hartman AM, Scoppa SM. Revision of dietary analysis software for the Health Habits and History Questionnaire. *Am J Epidemiol.* Jun 15 1994;139(12):1190-1196.

259. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol.* Sep 1986;124(3):453-469.

260. van Goor SA, Smit EN, Schaafsma A, Dijck-Brouwer DA, Muskiet FA. Milk of women with lifetime consumption of the recommended daily intake of fish fatty acids should constitute the basis for the DHA contents of infant formula. *J Perinat Med.* 2008;36(6):548-549.

261. Rogers PJ, Appleton KM, Kessler D, et al. No effect of n-3 long-chain polyunsaturated fatty acid (EPA and DHA) supplementation on depressed mood and cognitive function: a randomised controlled trial. *Br J Nutr.* Feb 2008;99(2):421-431.

262. Su KP, Huang SY, Chiu CC, Shen WW. Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur Neuropsychopharmacol.* Aug 2003;13(4):267-271.

263. Marangell LB, Martinez JM, Zboyan HA, Kertz B, Kim HF, Puryear LJ. A double-blind, placebo-controlled study of the omega-3 fatty acid docosahexaenoic acid in the treatment of major depression. *Am J Psychiatry.* May 2003;160(5):996-998.

264. Buydens-Branchey L, Branchey M, Hibbeln JR. Associations between increases in plasma n-3 polyunsaturated fatty acids following supplementation and decreases in anger and anxiety in substance abusers. *Prog Neuropsychopharmacol Biol Psychiatry.* Feb 15 2008;32(2):568-575.