POLYUNSATURATED FATTY ACID INTAKE AND INFECTION IN THE PRENATAL PERIOD AND THE RISK OF CEREBRAL PALSY: AN EPIDEMIOLOGIC ANALYSIS USING THE MOBAND-CP DATASET

By

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ABSTRACT

POLYUNSATURATED FATTY ACID INTAKE AND INFECTION IN THE PRENATAL PERIOD AND THE RISK OF CEREBRAL PALSY: AN EPIDEMIOLOGIC ANALYSIS USING THE MOBAND-CP DATASET

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Cerebral palsy (CP) is the most common childhood motor disability worldwide. Two large, prospective pregnancy cohorts, the Danish National Birth Cohort (DNBC) and the Norwegian Mother and Child Cohort (MoBa), have been harmonized to create The MOthers and BAbies of Norway and Denmark- Cerebral Palsy Study (MOBAND-CP), which has facilitated novel research on prenatal risk factors for CP. Nevertheless, prenatal nutrition is an area of CP research that is still largely untouched and is also suited for analysis using MOBAND-CP data.

Using a sample of singleton children born at 35 weeks gestation or later (*i.e.,* term or near-term), the primary aim of this research is to evaluate the association between prenatal consumption of two poly-unsaturated fatty acids, docosahexaenoic acid (DHA) and arachidonic acid (ARA), and the risk of CP in term- or near-term children. Secondary aims are to: (a) calculate the ratio of DHA to ARA, (b) evaluate the association between the DHA to ARA ratio and CP, (c) estimate the risk of CP for children whose mothers reported an infection during pregnancy and (d) explore the effect modification of the association between infection and CP by docosahexaenoic quartile.

We calculated the prevalence of CP for children born at or near-term by exposure to assess the suitability of combining the source cohorts' data for models. To estimate risk of CP, we used multiple imputation to retain observations missing covariate and infection data. We generated log-binomial models to estimate the risk of CP by exposure.

Term- and near-term children with DHA exposure in the second quartile had a 1.85-fold increased risk of CP compared to children whose DHA exposure was in the first quartile. The risk of CP for children in the third and fourth quartile of DHA exposure was similar to the risk for children exposed in the first quartile. Risk of CP by ARA quartile differed between the two cohorts that comprise MOBAND-CP. For term- and near-term children from the Danish National Birth Cohort (DNBC), the risk of CP decreased as ARA quartile increased. In the Norwegian Mother and Child Cohort (MoBa), the risk of CP increased as ARA quartile increased. The ratio of DHA to ARA also differed by cohort. In the DNBC, the risk of CP increased as the ratio quartile increased. In the MoBa, the risk of CP decreased as ratio quartile increased.

Risk of CP was similar for term- and near-term children whose mothers did and did not report an infection during pregnancy. The results of the effect modification analysis demonstrated that within children with prenatal DHA exposure in the third quartile, the risk of CP was 1.92 times higher for those whose mothers reported a prenatal infection compared to children whose mothers did not.

Exposure to DHA *in utero* affects a child's risk of CP, and it may modify the relationship between infection and CP. The association between ARA and CP remains unclear, as does the association between the DHA to ARA ratio and CP. DHA intake in the second quartile was associated with the greatest risk of CP, though further research is needed to explain the underlying mechanisms. Further research is needed to clarify the association between ARA and CP, and the reasons that children in the third quartile of DHA exposure have significantly higher risk of CP if their mothers reported a prenatal infection compared to the children whose mothers did not.

This dissertation is dedicated to Peter V. Without you, I would not be who I am.
In memory of Draper

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KEY TO ABBREVIATIONS

ADDM Autism and Developmental Disabilities Monitoring Network

ARA arachidonic acid

BMI body mass index

BPDI Bayley Psychomotor Development Index

CI confidence interval

CP cerebral palsy

DHA docosahexaenoic acid

DNBC Danish National Birth Cohort

DPA docosapenaenoic acid

EDEN Study of Pre and Early Postnatal Determinants of Child Health and Development

EPA Eicosapentaenoic acid

FFQ food frequency questionnaire

HR hazrd ratio

KO knock-out

LOWESS Locally Weighted Scatterplot Smoothing

MDI Bayley Scale of Mental Development

MMT Maastricht Motor Test

MoBa Norwegian Mother and Child Cohort

MOBAND-CP MOthers and BAbies of Norway and Denmark- Cerebral Palsy Study

n-3 omega-3

n-6 omega-6

OR	odds ratio
PFAS	perfluoroalkyl substance
PMT-5	Peg Moving Test-5
PS	phosphatidylserine
PUFA	poly-unsaturated fatty acid
RGSC	Registrar General's Social Class
SCPE	Surveillance of Cerebral Palsy in Europe
UTI	urinary tract infection

Chapter 1: Background and Literature Review

Definition and Prevalence of Cerebral Palsy

Cerebral palsy (CP) is a group of disorders related to the development of movement and posture. The disorders are permanent and are frequently the result of injuries to developing fetal and infant brains.(1) CP can be accompanied by other disorders of neurodevelopment such as autism, epilepsy, and intellectual disability.(1) According to the Surveillance of Cerebral Palsy in Europe (SCPE) Network standards, there are four subtypes of CP: spastic, dyskinetic, ataxic, and unclassifiable.(2) Classification is primarily based on the type and consistency of the muscle tone a child exhibits.(2) In the SCPE standards, spastic cerebral palsy is further broken down into unilateral if only one side of the body is affected, and bilateral if both sides are affected.(2)

CP is the most common childhood motor disability world-wide.(3) Table 1.1 reviews prevalence estimates of CP and their data sources. A meta-analysis of 49 studies estimated worldwide prevalence of CP was 2.11 cases per 1,000 live births (95% confidence interval [95% CI]: 1.98, 2.25).(4) While the prevalence of CP was highest for children born at less than 28 weeks gestation (prevalence: 82.25 cases per 1,000 live births [95% CI: 54.49, 124.17]), the prevalence estimate for children born 32 to 36 weeks gestation was 6.75 cases (95% CI: 4.59, 9.94), and the prevalence for children born at 37 weeks gestation or later was 1.35 per 1,000 live births at term (95% CI: 1.15, 1.59).(4) The fact that the overall prevalence estimate and the term-birth estimate were closer than the overall prevalence and the preterm prevalence indicates that children born at term contribute a large proportion of the cases of CP.

The lack of national CP registry in the United States makes it difficult to estimate the prevalence of CP for the entire country, though the Autism and Developmental Disabilities Monitoring Network (ADDM) makes efforts to identify and follow children with CP in four metropolitan areas (see https://www.cdc.gov/NCBDDD/cp/research.html). The birth prevalence of CP for children who survived to one year in the Metropolitan Atlanta Developmental Disabilities Surveillance Program was 2.2 cases per 1,000 live births in 2003.(5) Birth prevalence is calculated as the number of cases of CP in a defined population divided by the number of live births that contributed to that population. This study also noted that the proportion of children with CP born at term was 40% from 1998-2002, and the proportion of children with CP born between 32 and 36 weeks gestation was about 20%, demonstrating that a large proportion of children diagnosed with CP arose from the term and late-preterm population.(5) The ADDM Network estimated the across-network prevalence of CP to be 3.3 cases per 1,000 eight-year olds in 2008,(6) and the metropolitan Atlanta cohort estimated there were about 3.4 cases of CP per 1,000 eight-year old children in 2010.(7) Additionally, this study noted there was little change in the prevalence of CP from 1991 to 2010.(7) A recent study that used Medicaid data from 15 states estimated the prevalence of CP for children and adolescents was 1.78 cases per 1,000 Medicaid patients through age 20.(8) Medicaid data may underestimate the prevalence of CP because it relies on administrative data to identify cases, and administrative data may not be sensitive to mild cases of CP. A study that used the National Health Interview Survey estimated the 2009-2016 prevalence of CP to be 3.2 cases per 1,000 children ages 3-17 years old (95% CI: 2.7, 3.7), which is similar to the ADDM estimates.(9) Prevalence rates differ by the populations and time frames for which they are calculated. For

example, the prevalence rate within eight-year old children is higher than the birth prevalence in part because the denominator includes children who survive to eight years old, which is a smaller number than live births. The denominator of birth prevalence includes live births, and not all children will survive to eight years old. Additional differences may stem from different inclusion criteria in the CP case definition. The ADDM Network's case definition of CP includes children with both congenital and acquired CP.

Estimating the prevalence of CP in Europe is facilitated by the availability of population-based CP registries that subscribe to the SCPE standards. The estimated CP prevalence across Europe in 2003 was 1.77 cases per 1,000 births.(10) Estimates from Scandinavia are similar. A study from the Norwegian Cerebral Palsy Registry found the prevalence of CP for children born between 1996 and 1998 was 2.1 cases per 1,000 live births(11), while a study from the Norwegian Patient Register found the 2008-2010 percentage of children age five or older in Norway with cerebral palsy was about 3 per 1,000.(12) A study from Norway that used the CP Registry of Norway and covered the years 1999 to 2010 indicated that the prevalence of CP was decreasing; it declined from 2.35 per 1,000 live births in the 1999 birth cohort to 1.89 cases per 1,000 live births in the 2010 birth cohort.(13) The differences in prevalence estimates stem from the different denominators, dates of inclusion, and different sources of data. The CP Registry of Norway includes children born from 1996 to present,(11) and cases of CP that were not reported to the CP Registry of Norway can be identified through the Norwegian Patient Registry.(14)

A study from the Danish CP Registry demonstrated the 2005-2007 prevalence of CP was 1.8 cases per 1,000 live births, and almost 60% of the cases were born between 37 and 41

weeks gestation.(15) An additional 17.2% of CP cases in this study were born between 32 and 36 weeks gestation.(15) From a study that used the Medical Birth Registry of Norway and the National Insurance Scheme, the prevalence of CP for children born at term or post-term was 1.15 per thousand live births (95% CI:1.10, 1.20).(16) The prevalence estimates of CP demonstrate the importance of understanding CP risk factors in for children born at term or near-term (*i.e.,* 35-36 weeks gestation).

Relevance of CP to Public Health

Though CP is a rare, there are significant economic and socio-emotional costs. In 2003, the estimated combined direct and indirect lifetime costs of CP for people born in 2000 was USD $11.5 billion, and the estimated lifetime disorder-related cost for an individual living with CP in the United States was USD $921,000.(17) In Denmark the lifetime cost of CP for an individual was estimated to be about €800,000 (i.e. U.S. $905,000) for people alive in the year 2000.(18) The Danish study found that the care-taking costs of CP and the losses of productivity contributed the most to the total estimated lifetime costs of CP.(18) Economic costs alone demonstrate that CP is an important public health challenge.

Along with the financial costs of living with CP, there are health-costs and quality of life costs. Adults with CP have shorter lifespans than those who are unaffected, though the association between CP and mortality risk is modified by the severity of CP.(19) As severity of CP increases, the risk of mortality also increases.(19, 20) From 1983 to 2010, annual change in mortality for children under the age of 15 was noted to be on the decline, for people age 15-59, annual change was stable, and for those who were 60 and older, annual mortality was increasing.(21) Adults with CP are more likely than the general population to die from

cardiovascular and respiratory diseases.(22) Adults with CP also have a higher prevalence of chronic diseases compared to adults without CP, including hypertension, myocardial infarction, dyslipidemia, asthma, and osteoporosis, and multi-morbidity.(23)

Pain from CP and changes in motor function impact the quality of life of people with CP at all ages. CP associated pain was found to negatively impact the quality of life in children in multiple domains.(24) Parent-reported physical, psychological, and self-perception quality of life scores had higher odds of being in the lowest quartile if the child had moderate or severe pain.(24) Predicted gross motor function curves show that for children with severe gross motor function deficits, predicted gross motor function plateaus earlier than children with less severe motor deficits.(25) Children were followed to track motor deficit decline, and it was found that as children aged into young adults, motor function declined by four to eight points on the scale used to measure gross motor function.(25, 26) Adults under the age of 50 with CP often have multiple musculoskeletal problems brought on by overuse, physical strain, and early joint deterioration.(27)

There is also substantial burden on care givers of people with CP. Care-givers of individuals with CP report lower quality of life than others.(28, 29) In a study that compared quality of life measures in a sample of mothers of children with CP to a sample of women in the general population, mothers of children with CP reported lower quality of life scores across all domains, including physical functioning, body pain, social functioning, and mental health. Differences in mean scores ranged from 20-30 points.(28) Even after spinal fusion intervention for CP, which has been shown to improve quality of life for children with Gross Motor Function Classification System levels of IV and V,(30) burden of care for care givers was not reduced.(31)

A 2019 meta-analysis demonstrated parents of children with CP had a higher prevalence of depression compared to parents of non-disabled children. The weighted effect size of the mean difference in depression scores between parents of children with CP and parental controls was 0.6, which the author considered a moderately strong association.(32) The health and quality of life costs for people living with CP and their care-takers are important challenges that demonstrate that CP is a public health concern that deserves more attention.

Risk Factors for CP

While the risk of CP is higher for pre-term infants, a large proportion of CP cases arise in infants born at term or near- term (i.e:35-36 weeks gestation).(5, 33) The following section focuses primarily on risk factors for CP for children born at or near-term. It is reasonable to include births in gestational weeks 35 and 36 when studying CP in term children because the risk of complications resulting from preterm birth decline after 34 weeks gestation. The risk of death or severe neurological conditions for children born after 35 weeks gestation is half that of children born at 34 weeks.(34) A 2008 meta-analysis demonstrated that the prevalence of CP declined as gestational age increased, with 14.6% of children born between 22 and 27 weeks having a CP diagnosis, 6.2% of children born 28 to 31 weeks were diagnosed with CP, 0.7% of children born 32 to 36 weeks had a diagnosis of CP, and 0.1% of children born at 37 weeks or later were diagnosed with CP.(35) A similar pattern was noted using linked Norwegian patient records.(36) Risk of CP also changes within children born at term. A study that used data from the Norwegian National Birth Registry and Norwegian National Insurance Scheme found that for term and post-term births, children born at 37 weeks and 42 weeks had the highest prevalence and the greatest risk of CP compared to children born at 40 weeks.(16) For children

born at 37 weeks, the prevalence of CP was 1.9 per 1,000 live births, and the relative risk of CP compared to children born at 40 weeks was 1.9 (95% CI: 1.6, 2.4), while for children born at 42 weeks the prevalence was 1.36 per 1000 live births and risk of CP was increased by 1.4-fold compared to children born at 40 weeks (95% CI: 1.2, 1.6). Children born after 42 weeks had a prevalence of CP of 1.4 per 1,000 live births, and a relative risk of CP compared to children born at 40 weeks of 1.4 (95% CI: 1.1, 1.8).(16) The prevalence of CP for children born at 40 weeks was 1 case per 1,000 live births. These differences persisted after adjustment for child sex, socioeconomic factors, and maternal age.(16)

Birth asphyxia and birth defects are consistently shown to be important risk factors for CP. A cohort study that used linked records found that mild to severe birth asphyxia was a common birth complication for children with CP compared to the general population.(37) Placental abruption and cord prolapse were also more prevalent for the cases of CP compared to the general population. Placental abruption was most common for children born between 28 and 36 weeks gestation, and cord prolapse was most common for children born prior to 28 weeks gestation.(37) The evidence of an association between cord prolapse and CP is mixed. A study from the Collaborative Perinatal Project found no association between cord prolapse and CP.(38) Evidence from a study from the Western Australia Register of Developmental Anomalies- Cerebral Palsy demonstrated that asphyxia was found in 8.5% of cases of CP for children born after 34 weeks gestation (odds ratio:1.6 [95% CI: 1.1, 3.2]). Evidence of asphyxia during birth was determined by the presence of uterine rupture, amniotic embolism, tight nuchal cord, cord prolapse, placental abruption, severe intrapartum hemorrhage, maternal cardiac arrest, and severe shoulder dystocia.(39) This study also noted birth defects diagnosed

by age six were found in 42.3% of cases of CP. The odds of birth defects for CP cases were 12.6 (95% CI: 8.3, 19.0) fold higher than the odds for of birth defects for controls.(39) A study using the SCPE data calculated that congenital malformations were present in 11.9% of the CP cases identified by the registry.(40) The difference between the Australian and the European birth defect estimates may result from the difference in the length of time allowed for diagnosis of a birth defect. A case-control study from California found the odds of congenital abnormalities were 5.2 times higher for children with CP compared to the control children (95% CI: 2.8, 9.7).(41)

Prenatal exposure to infection and inflammation, and treatment of these are important risk factors for CP. Infection during the hospitalization for delivery was found to increase the odds of CP for children born at term (odds ratio: 1.8 [95% CI: 1.1, 2.8]).(42) In the study from the Western Australia Register of Developmental Anomalies- Cerebral Palsy, inflammation was found in 4.8% of cases of CP for children born after 34 weeks gestation. The odds ratio for an inflammatory event for CP cases compared to controls was 2.1 (95% CI: 1.0, 4.2). Inflammatory events were defined as maternal pyrexia of 37.5 degrees Celsius or higher, uterine tenderness, malodorous amniotic fluid, high leukocyte count, maternal or fetal tachycardia, and inflammatory placental histology.(39) In a case-control study from Washington state, the odds of CP for children born at term were increased by 80% if the child was prenatally exposed to infection (odds ratio: 1.8 [95% CI: 1.1, 2.8]).(43) Use of paracetamol during pregnancy, a possible indicator of presence of fever, was found to increase the risk of CP by 30% (relative risk: 1.3 [95% CI: 1.0, 1.7), and risk of bilateral spastic CP increased 2.4 fold for children who

were prenatally exposed to aspirin.(44) Risk of CP related to prenatal infection is discussed in a later section of this review.

Intrauterine growth restriction, defined as birthweight at least two standard deviations below optimal for gestational age, gender, maternal height, and parity or a diagnosis of growth restriction, was present in 16.5% of CP cases in the study from the Western Australia Register of Developmental Anomalies- Cerebral Palsy, and had an odds ratio of 3.5 (95% CI: 2.2, 5.5) for CP compared to children who were not growth restricted.(39) The importance of fetal growth restriction has been replicated in other case-control studies of CP risk factors for children born at term or near-term. Odds of CP were four-fold higher for children who were born with marked fetal growth restriction compared to children who were born at an appropriate weight for gestational age.(45) The SCPE studied fetal growth as a risk factor for CP. Using a fetal standard for growth, they found that regardless of gestational age, children whose growth was assessed as 3 standard deviations below the mean had the highest prevalence of CP.(46) The prevalence of CP was highest in the children who were born between 28 and 31 weeks gestation and whose fetal growth placed them three standard deviations below the mean.(46)

Other important risk factors for CP include maternal body mass index greater than 23 kg/m^2(BMI), maternal age, socio-demographic characteristics, prenatal smoking, and prenatal alcohol use. An analysis from the MOthers and BAbies of Norway and Denmark-CP (MOBAND-CP) study investigated maternal BMI as a risk factor for CP. The prevalence of CP increased as maternal BMI increased, and the risk of CP was higher for the children whose mothers' pre-pregnancy BMI fell in the high-normal range (relative risk: 1.4 [95% CI: 1.0, 1.8]), overweight range (relative risk: 1.6 [95% CI:1.2, 2.0]), and obese range (relative risk: 1.6 [95% CI: 1.1,2.2 for

obese women]).(47) A retrospective cohort study that used Swedish registries also found early pregnancy maternal BMI was associated with risk of CP in children.(48) This study found that a BMI classification of overweight early in pregnancy increased risk of CP in children by 20%, a BMI classification of obesity 1 increased risk of CP by 28%, a BMI classification of obesity 2 increased risk of CP by 54%, and a BMI classification of obesity 3 increased risk two-fold.(48)

Heavy alcohol use during pregnancy, defined as having an alcohol use disorder diagnosis during pregnancy, was shown to increase the odds of CP by 2.5 to 3.3 fold when compared to pregnancies not complicated by alcohol use.(49) However, a case-control study demonstrated no association between maternal alcohol use and CP when alcohol was measured via self-report as drinks per week.(50) This same study found that maternal smoking during pregnancy was associated with a 40% increase in odds of CP compared to mothers who did not smoke during pregnancy.(50) A study from the Danish National Birth Cohort (DNBC) also found maternal smoking during pregnancy was associated with an increased risk of CP.(51) Women who smoked ten or more cigarettes a day had offspring with 1.8 times the risk of CP compared to women who did not smoke during pregnancy (95% CI: 1.1, 2.9).(51)

Maternal age greater than 35 was found to be associated with a two-fold increase in risk of CP for children born at 36 weeks gestation or later,(52) though another study did not find an association between maternal age and risk of CP.(48) A systematic review found that maternal age greater than 40 years was consistently associated with increased CP risk for all children and children born at term. This systematic review noted that maternal age less than 20 was associated with increased risk of CP, but the individual studies' estimates were inconsistent.(53) Parity has also been associated with CP. In one study, the occurrence of CP was increased by

nine-fold for the children of primiparous mothers compared to the children of multiparous mothers.(54) Villamor *et al* demonstrated that as parity increased, the rate of CP decreased.(48) A case-control study found that primiparity was more common for the mothers of children who had perinatal arterial ischemic stroke compared to the control children (74% versus 44%, p-value: 0.002), though the association was attenuated in multivariate analysis.(55) Perinatal arterial stroke has been associated with the occurrence of CP.(56) Parental socioeconomic status impacts the risk of CP. A study conducted using the Medical Birth Registries of Norway and Denmark found that the children of women with partners had 21% lower risk of CP compared to the children of single women.(57) This study also found that risk of CP decreased as both maternal and paternal education increased.(57) A study that used the Registrar General's Social Class (RGSC) based on occupation found no link between paternal RGSC and CP.(58)

From a public health perspective, it may be impossible to eliminate many of these risk factors for CP. This raises the question of how we might interrupt the pathways from these exposures to the outcome of CP in order to reduce the occurrence and the severity of CP. Polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA), and arachidonic acid (ARA) taken prenatally are potential disruptors of inflammatory insults that lead to CP.

The Role of Fatty Acids in Prenatal Brain Development

Fatty acids DHA and ARA are two of the most important constituents of the fetal brain and belong to the omega-3 (n-3) and omega-6 (n-6) fatty acid families, respectively.(59) Early in pregnancy, accretion of PUFAs in the fetal body is minimal, but accretion begins to accelerate at 25 weeks gestation and rises to almost 7 grams per day throughout the body of a baby born at

term.(60) The potential for PUFAs to influence pregnancy outcomes has been explored in both observational research and randomized control trials, and the role of PUFAs for optimal pregnancy and development outcomes has been reviewed.(60-70) The precursors to DHA and ARA, the n-3 alpha-linolenic acid and n-6 linoleic acid, respectively, are "essential" in that they cannot be produced by the human body but must be acquired through diet.(61) DHA and ARA can either be acquired through diet or synthesized from their precursors.(61) Alpha- linolenic acid and linoleic acid compete for the same resources when they are metabolized, and there are fewer steps required to convert linoleic acid into ARA than required to convert alpha-linolenic acid in DHA. (Figure 1.1).(70, 71) The next sections review the biological importance of DHA and ARA in brain development, describes the food sources for DHA, ARA, and their precursor fatty acids, and gives evidence that DHA and ARA intake during pregnancy may impact risk of CP in children.

DHA

DHA is an important fatty acid during fetal brain development because it supports many processes necessary for optimal brain function. DHA supports both neuronal development and structural membrane integrity.(60) DHA is an important constituent in the phospholipid membranes; it impacts fluidity, and when DHA is unavailable, docosapentanoic (DPA) is accreted into neuron membranes.(72) Membrane fluidity is the capacity of a membrane to flex and change as needed to facilitate movement of molecules and actions of enzymes.(72, 73) Membrane fluidity influences protein functions, protein trafficking, and vesicle budding and fusion, all of which are important functions for cell survival and maintenance.(72) DHA's

structure has been shown to facilitate conformational changes in membranes more effectively than DPA.(74)

DHA is a precursor to many chemicals that affect fetal brain development, many of which are anti-inflammatory or inhibit vasoconstriction. (72) For example, DHA is the preferred precursor to the aminophospholipid phosphatidylserine (PS), which acts as a flag in apoptotic cells, allowing them to be more quickly identified and phagocytized.(75) It has been shown that the time to resolution of inflammation is enhanced in tissues with high levels of PS.(75) PS also plays a role in cell signaling and neurotransmitter release, acting as a part of protein docking sites for proteins involved in synaptogenesis, neuronal survival, and neurite growth.(76) Cells with more DHA than DPA have higher levels of PS, which enhances cell survival.(72)

Table 1.2 describes primary sources of the n-3 fatty acid DHA, which include fatty fish oils, including salmon oil, cod liver oil, and sardine oil (see USDA Food Composition Databases at http://ndb.nal.usda.gov). Fatty fish are also a primary source of dietary DHA, including caviar, mackerel, and salmon.

DHA's precursor is the n-3 fatty acid alpha-linolenic acid, which must be acquired through diet.(61) Conversion of alpha-linolenic acid into DHA is inefficient in women, and only about 9% of alpha-linolenic acid is converted into DHA.(77) Table 1.2 describes primary sources of alpha-linolenic acid, which include seeds and seed- based oils, including flax seed oil, chia seeds, and canola oil (see USDA Food Composition Databases at http://ndb.nal.usda.gov). Alpha-linolenic acid is not efficiently processed into DHA in the fetus so the fetus is dependent on the mother for DHA.(60)

Throughout the decade of the 2000s and into the 2010s, the recommended dietary intake of DHA during pregnancy in the United States and Europe was 200 mg per day, and the recommendations focus on achieving this level through intake of one to two servings of fatty fish per week.(78, 79) Supplementation is only recommended if the recommended daily intake cannot be met through food.(79)

ARA

ARA in an n-6 fatty acid that acts as a precursor to many chemicals that can affect fetal brain development, many of which are pro-inflammatory, some of which can be pro-inflammatory or anti-inflammatory and promote vasoconstriction.(72) ARA is a precursor to adrenic acid, which is an important component of myelin,(70) and multiple endocannabinoids, which are important neurotransmitters involved in many functions, including the resolution of inflammation.(80) For example, in animal models, anandamide, an important endocannabinoid, was protective against white matter damage, increased survival of preoligodendrocytes, and preserved myelination when rats were exposed to excitotoxic brain injury.(81) Arachidonic acid has also been found to act as an antioxidant, protecting the hippocampus from oxidative stressors,(82) and it supports the growth of neurite processes.(83)

ARA can be obtained through conversion of linoleic acid by delta-6 desaturase, though fetuses still rely on maternal intake of linoleic acid and ARA.(60) Conversion of linoleic acid to ARA may be inefficient in humans.(84) Table 1.2 describes the primary sources of ARA, which include beef and lamb (see USDA Food Composition Databases at http://ndb.nal.usda.gov). ARA's precursor, the n-6 fatty acid linoleic acid, is more prevalent than ARA in the western diet, and the primary sources include soy oil, corn oil, and seeds roasted in oil like sunflower seeds

(see USDA Food Composition Databases at http://ndb.nal.usda.gov). There is no recommended daily intake of ARA during pregnancy, though combined intake of n-6 and n-3 PUFAs should total about 10% of total energy intake.(79)

CP and PUFAs

There is little published literature on the topic of CP and exposure to PUFAs during pregnancy. Searches in Pubmed yielded few studies that assessed the relationship between CP and exposure to PUFAs during pregnancy (see Table 1.3). A case-control study of CP did find that the odds of CP were reduced by 30% for each additional serving of fish eaten per week during pregnancy.(85) The study did not account for gestational age at birth and relied on recall of diet during pregnancy three to eight years after birth.(85) Trials have demonstrated that PUFA supplementation during pregnancy may affect some of the most important risk factors for CP: preterm birth and IUGR. A recent trial of fish-oil supplementation demonstrated that the mean duration of gestation of women in the intervention arm of the trial was two days longer compared to the control group, and the mean birthweight of the children in the intervention arm was 97 grams higher than the mean birthweight in the control group.(66) As discussed previously, preterm birth and impaired fetal growth are two important risk factors for CP, and these findings support consumption of n-3 fatty acids during pregnancy. However, in this study there were differences in the trial arm and the control arm of baseline characteristics such as maternal smoking status, with the trial arm having fewer smokers than the control arm, which raises concerns about confounding.(66)

Animal models of motor development can help shed some light on the mechanisms through which prenatal intake of DHA and ARA may affect risk of CP in children. For example, in

a mouse study, mice without genes for the delta-six desaturase, the enzyme that processes alpha linolenic acid into DHA and linoleic acid into ARA, were fed diets containing only alpha-linolenic acid and linoleic acid (control), or alpha-linolenic acid, linoleic acid, and ARA.(86) There was an additional control group of wild-type mice. The knock-out (KO) control mice and the KO plus ARA mice demonstrated significantly less motor coordination compared to the wild-type control mice. The KO plus ARA mice showed increased spontaneous movement compared to the KO control mice.(86) An update to this research showed that KO mice who were fed a combination of precursors plus DHA and ARA exhibited more movement than KO control mice, and similar amounts of spontaneous movements as the wild-type mice.(87) The study also found the KO ARA plus DHA mice had the highest level of motor control of all the groups, and that the KO plus ARA only mice were less coordinated in their movement than the KO plus DHA and ARA mice. KO plus DHA only mice did not show more movement than the KO control mice, but they did have more motor control.(87) The findings from this series of studies suggests that both DHA and ARA are essential for optimal motor development.

There are many studies that examine the relationship between exposure to fatty acids during pregnancy and neurodevelopment, though results are mixed. The Maastricht Motor Test (MMT) is a motor development test that is validated in five to six year old children.(88) The MMT gives both qualitative and quantitative ratings of movements across four scales: static balance, dynamic balance, ball skills, and manual dexterity.(88) A study of umbilical plasma DHA concentration and motor development in seven-year old school children found a positive association between umbilical DHA concentration and MMT total and quality scores.(89) The Peg Moving Test-5 (PMT-5) measures motor development by timing the length of time it takes

a child to move pegs, with increasing time indicating decreasing motor skills development. The PMT-5 was used in the Study of Pre and Early Postnatal Determinants of Child Health and Development (EDEN) cohort and was found to have a positive relationship with the omega-6/omega-3 fatty acid ratio that was marginally statistically significant.(90) An analysis of data from a small, randomized controlled trial of fish oil supplementation for women with allergies demonstrated higher mean hand-eye coordination sub-scores of the Griffiths Mental Development Scale for the children whose mothers were supplemented with 1:2 ratio of EPA to DHA during pregnancy; the mean score for children whose mothers took fish oil was 114.0, compared to a mean score of 108.0 for children whose mothers received olive oil (p-value: 0.02).(91) The baseline characterisitcs of the women in this analysis were different between the treatment and control arm on important variables such as maternal age and maternal education.(91) The importance of a a low ratio of n-6 to n-3 fatty acids was demonstrated again in a community-based pregnancy cohort study that used 24-hour dietary recall to assess fatty acid intake and subsequent neurodevelopment in children.(69) This study divided mothers' estimated linoleic acid to alpha-linolenic acid ratio into quartiles and conducted the Bayley Psychomotor Development Index (BPDI) in children at six months of age. Results indicated that children whose mother's whose ratio fell into a quartile higher than the first had double the odds of a BPDI index that fell below 85, which indicates delayed psychomotor performance.(69) In a trial that supplemented DHA and ARA during pregnancy and lactation, the authors found a marginally significant difference in the BPDI for children at 18 months of age. Children whose mothers were supplemented with DHA had psychomotor development scores that were on average 4 points higher than the children in the control group (p-value: 0.08).(92) This study

terminated participation if a pregnancy was delivered prior to 37 weeks gestation, so the analyses only included children born at term. Exclusion of participants after randomization is problematic in randomized control trials because it increases the risk that confounding will arise in the baseline characteristics of the mothers. However, key baseline characteristics remained balanced between the intervention and control groups after exclusion of preterm births.(92) Another study of psychomotor development using the BPDI in 18 month-olds born at term found no association between umbilical blood concentrations of DHA or ARA and BPDI score.(93) A study that used a measure of general movement in three month old children whose essential fatty acid status at birth was determined from cord blood found that infants with abnormal general movements had lower ARA concentrations and higher total omega-9 fatty acid concentrations compared to their counterparts with normal general movements.(94) Finally, a study of fish oil supplementation during pregnancy did not find a relationship between fish oil supplementation and cognition, language, or fine motor skills in 12 year old children.(95)

CP and Infection

Infections during pregnancy are well-established risk factors for CP. In 1986, Nelson and Ellenberg found that chorionitis was present in 10% of cases of CP in the Collaborative Perinatal Project sample.(96) A 1997 case-control study of children in California found the odds of CP of unknown etiology increased for children born at a normal weight whose mothers spiked a fever during labor or were diagnosed with clinical chorioamnionitis (OR: 9.3, CI:2.7, 31.0). (97) Around the same time, a case control study in Ohio investigated the association between maternal urinary tract infection in pregnancy and CP outcomes. In unadjusted analyses, the authors found an increase in the odds of CP for the mothers who experienced UTI during

pregnancy (OR: 3.9, CI 1.7-8.9), and in adjusted analyses, the odds increased to 4.8 (p-value: less than 0.05).(98) A nested case-control study of preterm children found that in univariate analysis, odds ratios for CP were higher for children whose mothers experienced fever prior to onset of labor compared to children whose mothers did not experience fever prior to the onset of labor, but the effect was marginally significant (OR: 2.3 [95% CI:1.0, 5.2]). This study also found fever prior to onset of labor had a significant odds ratio for children with spastic diplegia (OR: 3.1 [95% CI:1.1, 8.4]).(99) A population based case-control study in Washington State found children whose mothers had any infection during the hospitalization for delivery had increased odds of CP compared to children whose mothers did not have an infection (OR:3.1 [95% CI 2.3-4.2]), though the odds were not as high for infants born at term compared to preterm (OR term: 1.8 [95% CI: 1.1, 2.8] OR preterm: 2.3 [95% CI: 1.3, 4.2]).(43) A study from the DNBC found higher hazard ratios for CP for children whose mothers experienced vaginal infections, pyelonephritis, and fever during pregnancy compared to children whose mothers did not have these exposures. In sensitivity analysis of children born at term, vaginal infections (HR: 1.7 [95% CI 1.1, 2.7]) and fever (HR: 1.6 [95% CI 1.1, 2.4]) during pregnancy were both associated with CP.(51) These findings point to the importance of stratifying by gestational age to better understand the factors that increase risk of CP.

Infections during pregnancy are important risk factors for CP for children born at term. A case-control study from western Sweden demonstrated that infectious events during pregnancy and labor in near-term and term infants were associated with increased odds of spastic cerebral palsy. Infectious events included bacterial growth in urine during pregnancy (OR: 2.1 [95% CI: 1.1, 4.1]) any infectious disease during pregnancy (OR: 1.7 [95% CI: 1.2,2.3]),

and severe infection (OR: 2.0 [95% CI: 1.0,3.8]).(100) In this study, spastic hemiplegia was more strongly associated with infection during pregnancy than other subtypes of CP. Bacterial growth in urine (OR: 4.7 [95% CI: 1.5, 15.2]), any infection (OR:2.9 [95% CI: 1.7-4.8]), and severe infection (OR: 15.4 [95% CI: 3.0,78.1]) were all associated with CP in children with spastic hemiplegia.(100) A retrospective cohort study of the Danish National Birth Registry explored infection as a risk factor for CP for children who were born at term. The hazard ratio of CP for term infants exposed to genitourinary tract infection during pregnancy was 1.9 (95% CI: 1.4,3.3)(101). This study used time to diagnosis with cerebral palsy as recorded in the Danish National Cerebral Palsy registry, but there have been noted issues with this source of timing of diagnosis, including a large proportion of children diagnosed with CP at six months of age (see mobawiki.fhi.no). In a case-control study, urinary tract infection during hospital admission for delivery increased the odds of CP by two-fold compared to children whose mothers did not have a urinary tract infection (OR: 2.2 [95% CI: 0.9, 5.1]), though maternal fever did not increase odds of CP (OR: 1.2 [95% CI: 0.5, 2.8]).(43)

CP, PUFAs, and Infection

CP that develops in the antenatal period is likely to have multiple contributing events. Several “multi-hit” theories of CP have been developed.(102-104) An example of a multi-hit theory developed in children born at extremely low gestational age is from Leviton *et al*. Leviton *et al* describe a sequential order of exposures leading to brain damage in very low gestational age newborns that starts with being born small for gestational age and then experiencing systemic inflammation in the two-week post-natal period.(102) Across multiple measures of systemic inflammation, pre-term newborns who were born SGA and expressed

markers of systemic inflammation had higher odds of a low score on the Bayley Scale of Mental Development (MDI). Infants who did not express high levels of markers of systemic inflammation but were SGA had elevated odds of an MDI score of less than 55, as did infants who were not SGA but exhibited high levels of markers for systemic inflammation, but the estimates were lower than the estimates for the infants who experienced both SGA and high levels of systemic inflammation.(102) In the same extremely low gestational age cohort, Korzeniewski *et al* found an increased odds of ventriculomegaly for preterm infants who had been exposed to different combinations of histologically confirmed chorioamnionitis, acute fetal inflammatory response, and an inflammation-inducing illness during the neonatal period. Korzeniewski *et al* included the antenatal exposure of chorioamnionitis in this multi-hit model, and found infants who experienced multiple conditions, such as chronic chorioamnionitis and a neonatal inflammation-inducing illness had higher odds of ventriculomegaly than infants who experienced none of the conditions (OR: 4.8 [95% CI: 1.3, 17.1]).(103) Mor *et al* demonstrated the multi-hit hypothesis of CP by studying infants whose gestations were complicated by pre-eclampsia.(104) The prevalence of CP for the children of women who had pre-eclampsia was 0.2%, compared to 0.1% in the normotensive group. Mor *et al* found that for offspring whose gestation was complicated by pre-eclampsia, those who experienced infections had 8.1 times the odds of developing CP compared to offspring who only experienced pre-eclampsia (95% CI: 1.8, 36.9).(104) These models serve as examples for understanding how conditions might work together to cause CP. Given the role that DHA and ARA play in fetal brain development and their roles in resolution of inflammation, it is possible that DHA and ARA modify the association between infection and CP.

Conclusions

World-wide, CP is the most common childhood motor disability.(3) There is evidence that the prevalence of CP is decreasing in Norway (13), but the causes of CP and effective prevention strategies remain elusive, particularly for children born near or at term. Exposures during pregnancy like infection increase the risk of CP in children, but we do not know the reason some children who are exposed to infection develop CP and why others do not. Fetal brain development is a sensitive process and maternal nutritional exposures constitute an area of research that has been studied very little in relation to CP. DHA and ARA are the two most prevalent fatty acids in the brain, and maternal intake of the two fatty acids may be important factors in the biological pathway of CP development. There is evidence that maternal intake of PUFAs during pregnancy affects the risk of other neurodevelopmental conditions. Multi-hit theories of CP have been developed and suggest that inflammatory exposures compound the effects of other negative exposures like pre-eclampsia.(104) DHA and ARA both have important roles in resolving inflammation in the brain, and maternal intake of DHA and ARA may impact the availability of the two fatty acids in the fetal brain for response to inflammatory insults. DHA and ARA intake during pregnancy may affect risk of CP independently, and may also modify the association between infection during pregnancy and CP.

Chapter 1a. A Short Review of CP Research in the MOBAND-CP Cohorts

CP research has been undertaken in the Danish National Birth Cohort (DNBC) and the Norwegian Mother and Child Cohort (MoBa), but the majority of CP research has been initiated through the combined cohort study MOthers and BAbies of Norway and Denmark- Cerebral Palsy (MOBAND-CP) study.

The Danish National Birth Cohort

The DNBC has been used to study a wide range of exposures and their associations with CP. Parental sub-fertility, maternal infections, fever, smoking, and environmental toxins are some of the exposures that have been studied in the DNBC. One study from the DNBC explored underlying sub-fertility as a risk factor for CP.(105) Parental sub-fertility measured as time to pregnancy was found to not be associated with CP, but children born through *in vitro* fertilization or intracytoplasmic sperm injection had an increased risk of CP (hazard ratio: 2.30 (95% CI: 1.12, 4.73). Self-reported prenatal fever, vaginal infections, and smoking were associated with CP in another study from the DNBC.(51) Vaginal infections increased risk of CP 1.52 times (95% CI: 1.04, 2.24), fever increased risk of CP 1.5 times (95% CI: 1.1, 2.2), and smoking 10 or more cigarettes per day increased risk of CP by 1.8-fold (95% CI: 1.1, 2.9).(51) Perfluoroalkyl substance (PFAS) exposure was studied in a nested case-control study that used CP cases and a sample of typically developing children that arose from the DNBC. Prenatal PFAS exposure, measured in maternal plasma, was found to increase the risk of CP for boys in the study.(106) For each increase in the natural log of perfluorooctane sulfonate the risk of CP increased by 1.7 fold (95 % CI: 1.0, 2.8) and for each increase in the natural log of

perfluorooctanoic acid the risk of CP increased two-fold (adjusted odds ratio: 2.1 [95% CI: 1.2, 3.6]). The effect sizes were larger in boys born at term.(106)

The Norwegian Mother and Child Cohort

One study from the MoBa cohort studied the association between CP and prenatal caffeine intake. The study found that high intake of caffeinated soft-drinks prior to pregnancy was associated with an increased risk of CP (hazard ratio 1.9 [95% CI: 1.2, 3.6]), and consumption of three to five servings of caffeinated soft-drinks during gestation weeks 13-30 increased the hazard of CP by 1.7-fold (95% CI: 1.1, 2.8). In the first half of pregnancy, consuming 51 to 100 mg of caffeine from soft drinks daily was associated with 1.9-fold increase in risk of CP (95% CI: 1.1, 3.6).(107)

MOthers and BAbies of Norway and Denmark- Cerebral Palsy

Studies from the MOBAND-CP study have covered a variety of exposures such as pre-pregnancy body mass index (BMI), maternal thyroid disorders, and the use of anti-inflammatory medications like paracetamol. Maternal pre-pregnancy BMI in the upper-normal range (23.0 to 24.9 kg/m^2) the overweight range (25 to 29.9 kg/m^2), and the obese range (30 kg/m^2 or higher) were associated with an increased risk of CP compared to the lower-normal range (18.5 to 22.9 kg/m^2) For children whose mothers were in the upper-normal range of pre-pregnancy BMI, risk of CP increased by 1.35 fold (95 %CI: 1.03, 1.78), and for the children of mothers whose pre-pregnancy BMI fell into the overweight and obese categories, the risk of CP in each group was elevated about 60% (overweight relative risk:1.56 [95% CI:1.21, 2.01], obese relative risk: 1.55 [95% CI: 1.11, 2.18]) .(47) Self-reported thyroid disorders were not associated with CP in the MOBAND population, (108) but paracetamol use during pregnancy increased risk of CP by 1.3

fold (95% CI: 1.0, 1.7) exposed for children compared to the unexposed children. This study also found risk of CP changed by trimester in which paracetamol was used, with children who were exposed in the second trimester having a 1.6-fold higher risk of unilateral spastic CP compared to unexposed children. Use of aspirin at any point in pregnancy was also associated with a 2.4 fold increase in risk of bilateral spastic CP as well (95% CI: 1.1, 5.3).(44)

Chapter 2: Aims and Hypotheses

Aims

Within children born at 35 weeks gestation or later, this study will

I. Describe cerebral palsy (CP) and CP risk factors in order to identify prenatal factors important for the proposed analyses of the relationships between maternal intake of PUFAs during pregnancy, infection during pregnancy, and CP

II. Elucidate the association between maternal prenatal intake of docosahexaenoic acid (DHA) and the risk of CP

III. Elucidate the association between maternal prenatal intake of arachidonic acid (ARA) and the risk of CP

IV. Estimate the risk of CP in relation to the ratio of maternal DHA to ARA intake during pregnancy

V. Elucidate the association between infection during pregnancy and the risk of CP

VI. Explore effect modification of PUFAs on the relationship between infection during pregnancy and the risk of CP

VII. Assess the effects of potential confounding variables on the associations identified in Aims 2-6

Hypotheses

The following hypotheses will be tested within a sample of children born at 35 weeks of gestation or later.

Hypothesis 1

- Hypothesis Ia: The risk of CP will be highest for children whose mothers DHA consumption during pregnancy was in the lowest and highest quartiles compared to the children of women whose DHA consumption was in the middle two quartiles.
- Hypothesis Ib: Women who reported urinary tract infection or fever during pregnancy will have children with a higher risk of CP compared to the children of women who did not report urinary tract infection or fever during pregnancy

If one of the above hypotheses is not refuted, we will explore effect modification of DHA on infection during pregnancy

- Hypothesis Ic: The association between urinary tract infection or fever during pregnancy and CP will be modified by maternal DHA consumption during pregnancy. Within each quartile of DHA exposure, children of women who reported urinary tract infection or fever during pregnancy will have higher risk of CP than the offspring of women who reported urinary tract infection or fever during pregnancy, but the relative risk will narrow as DHA quartile increases
- Hypothesis Id: These associations will be robust to adjustment for confounding variables

Hypothesis 2

- Hypothesis IIa: The risk of CP will be highest for the children of women who had ARA intake in the highest quartile compared to the children of women whose ARA intake was in lower quartiles
- Hypothesis IIb: The risk of CP will decrease as the ratio of DHA to ARA increases. The risk of CP will be greatest for the offspring of women who reported consuming a diet in pregnancy in which the ratio of DHA to ARA was in the lowest quartile will have offspring with a higher risk of CP than women who consumed a diet with a ratio DHA to ARA in the highest quartile

If hypothesis IIb is not refuted, we will explore effect modification of DHA/ARA ratios on infection during pregnancy

- Hypothesis IIc: The association between infection during pregnancy and CP will be modified by the ratio of DHA to ARA consumed by the mother during pregnancy. Within each quartile of the DHA/ARA ratio exposure, the offspring of women who reported urinary tract infection or fever during pregnancy will have higher risk of CP than the offspring of women who reported no urinary tract infection or fever, however the relative risk will increase as quartile increases
- Hypothesis IId: These associations will be robust to adjustment for confounding variables

Chapter 3: Methods

Data Source

The data source for this study is the MOthers and Babies of Norway and Denmark-Cerebral Palsy (MOBAND-CP) study. MOBAND-CP combines data from the Danish National Birth Cohort (DNBC) and the Norwegian Mother and Child Cohort (MoBa). The unit of analysis in the MOBAND-CP data is the child, and a woman could contribute multiple pregnancies and children to the cohorts. MOBAND-CP includes over 200,000 children and 400 cases of CP. For aim one, we included all children who survived to age one in our analyses. For aims two through seven, we used a subset of the MOBAND-CP children who survived to age one and included children delivered at 35 weeks gestation or later and women with data collected via food frequency questionnaires.

The MOBAND-CP study team harmonized data from the DNBC and MoBa. Variables selected form harmonization were chosen because they would be useful for CP research across many studies.(14) Variables included in the MOBAND-CP dataset are regarded as either completely harmonized or partially harmonized. Complete harmonization indicates that variables' method of collection was nearly identical for the two cohorts, and collected data share an interpretation. Partial harmonization indicates that variables' method of collection differed and the questions that generated the data could be interpreted differently, but the resulting data overlapped enough to create a meaningful variable. Several other variables were considered for inclusion, but differences in collection were too great to be able to harmonize the data between the two cohorts.(14)

Outcome

CP data were derived from the Danish and Norwegian CP registries, both of which are member of the SCPE Network (see http://www.scpenetwork.eu/en/scpe-members/). The harmonization of CP in MOBAND-CP is regarded as complete. The cases of CP included in MOBAND-CP were acquired prenatally. Children with CP acquired after birth due to known brain injury are included in the denominator, but are not included in the numerator due to differences in registry inclusion between the two countries (see https://mobawiki.fhi.no).

Source Cohorts

DNBC

The Danish National Birth Cohort (DNBC) recruited pregnant women from 1996-2002. Women were invited to the study by the general practitioner they visited for their first prenatal care appointment.(109) Women who consented to the study were interviewed via telephone at 12 weeks and 30 weeks gestation. Limited data about diet during pregnancy were collected during the first and second interview. A food frequency questionnaire (FFQ), which asked women to recall eating habits for the previous month, was mailed at 25 to 26 weeks of gestation. An interview that covered the last part of pregnancy, birth, and the neonatal period was administered at six months post-partum.(109) The third interview included questions about use of fish oil supplements and consumption of fish, but the diet data used in this study was derived from the FFQ.

MoBa

The Norwegian Mother and Child cohort study (MoBa) recruited pregnant women from 1999-2009.(110) Women who received an ultrasound scan at gestational week 17 were

recruited by letter, which included the informed consent and the initial questionnaire. A second questionnaire was mailed to women who consented to participate at gestational week 30. Data about use of dietary supplements were collected in the two questionnaires. An FFQ was mailed at 22 weeks of gestation, and women recalled their eating habits for the first four to five months of pregnancy. A questionnaire that covered the last part of pregnancy, birth, and the neonatal period was mailed at six months post-partum.(110) This questionnaire asked about use of fish oil supplements after the second questionnaire through six months post-partum, but the diet data from the FFQ were used in this study.

Inclusion and Exclusion Criteria

For a child to be included in the fatty acid analyses, the mother must have completed the first and second interviews (DNBC) or first and third questionnaires (MoBa). We included only singleton children for whom CP diagnosis was not missing. Mothers must have completed the FFQ for the pregnancy and have had an estimated daily caloric intake in a plausible range of 4,500 kilojoules to 20,000 kilojoules. Figure 3.1 shows the inclusion and exclusion of children.

Measures of CP

CP diagnosis was defined as binary variable which could take a value of yes or no. CP diagnosis in the MOBAND-CP data includes only perinatally acquired CP. Children with CP who acquired a brain injury leading to CP in the post-natal period were included in the denominator, but not counted in the numerator as a case of CP.

The Danish CP Registry

CP cases from Denmark were ascertained from the Danish Cerebral Palsy Registry.(111) CP cases were reported by hospital-based pediatric departments to the registry and reviewed by a pediatric neurologist. Pediatric neurologists used standardized definitions of CP from the SCPE Network. The Danish Cerebral Palsy Registry was established in 1967, and its maintenance was taken on by the National Institute of Public Health in Denmark in 1991.(111) In 1997, the Danish CP Registry joined the SCPE. The Danish CP Registry covered eastern Denmark until 2001 when it expanded to cover the entire country.(111) To ensure completeness of CP case ascertainment, the CP registry compares reported cases to the National Patient Registry. Children with hospital stays for CP but who have not been previously reported to the Danish CP Registry by pediatric departments are added to the registry.(111) A 1997 validation study found the Danish CP Registry 85% complete and used the National Patient Registry to identify 35 more cases.(112) CP acquired after birth is not recorded in the Danish CP Registry.

The CP Registry of Norway

In MOBAND-CP, 90% of the Norwegian CP cases were identified through the Cerebral Palsy Registry of Norway.(11, 14) The CP Registry of Norway was established in 2006 and it

includes CP cases from children born in 1996 and later (see: http://www.scpenetwork.eu/en/scpe-members/norway/). Cases were reported by public hospitals and habilitation centers. Cases reported to the registry were reviewed by pediatric neurologists given standardized definitions for diagnosis of CP and subtype based on the SCPE standards.(11) The remaining 10% of Norwegian CP cases included in MOBAND-CP dataset were identified through linkage to the Norwegian Patient Registry.(12, 14) CP patients were identified by CP related ICD-10 codes from hospitalizations. Potential cases were reviewed by two pediatric neurologists.(12)

Measures of Polyunsaturated Fatty Acids

Grams of DHA per day and grams of ARA per day were estimated from FFQs administered to women in the second trimester. The DNBC FFQ was mailed to participants at 25 to 26 weeks gestation and included 360 questions.(109, 113) The FFQ asked women to recall their dietary habits, vitamin use, and supplement use for the previous month.(109) A validation study was conducted later in pregnancy which compared the FFQ to nutritional biomarkers obtained from serum and a seven day weighed food diary ascertained in gestational weeks 32-38.(113) The FFQ estimated n-3 fatty acid eicosapentaenoic acid was compared to erythrocyte eicosapentaenoic acid, and the two were found to be moderately correlated ($r = 0.37$, p-value <0.0001).(113) A more recent validation study of the DNBC FFQ found a correlation coefficient of 0.18 for the total percentage of fatty acids of DHA estimated by the FFQ and in plasma collected at a similar time point.(114) The MoBa FFQ was mailed to participants 22 weeks gestation and included 340 questions.(115, 116) The MoBa FFQ asked women to recall their dietary habits and supplement use for the first four to five months of pregnancy. The FFQ was

validated against a 4 day weighed food diary,(117) and fish intake was validated against biomarkers.(118) Erythrocyte DHA concentrations were correlated with fatty fish intake and supplement use (r= 0.25, p-value 0.01).(118) Another study noted there were significant differences in concentrations of the ratio of n-6 to n-3 fatty acids between supplement and non-supplement users, and there was a moderate correlation between total dietary intake of the ratio of n-6 to n-3 fatty acids estimated by FFQ and proportion of fatty acids in erythrocyte phospholipids.(119) Both the DNBC and MoBa FFQs were analyzed with the FoodCalc software using the Danish and Norwegian Food Tables, respectively.(113, 116)

Ratio of DHA to ARA

We calculated the ratio of DHA to ARA by dividing the estimated value of DHA in grams per day by the estimated value of ARA in grams per day. We then categorized the ratio into quartiles within each cohort. Twelve pregnancies had a zero value of ARA consumption and the ratio was not calculated. These observations were added to the highest quartile.

Fatty Acid Categorization

We tried different methods of categorization for the fatty acid variables. Locally weighted scatterplot smoothing (LOWESS), a non-parametric method of exploring data patterns, was used to identify a pattern of association between CP and DHA, ARA, and the ratio of DHA to ARA (see methods supplement figures 3a, 3b, and 3c). LOWESS creates a continuous variable for CP by outputting the binary variable on the logit scale and plots the logit value of CP against the continuous fatty acid variables. This process is useful for determining if there are cut points other than percentiles for classification of fatty acid. We tested spline regressions using cut points derived from the LOWESS process, which indicate if the cut points reflect significant

changes in the slope of the smoothed line. Spline regression did not indicate there were significant changes in the slope of the smoothed line at the identified knots. We also tested quartile and tertile cut points and compared those to the smoothed plots. We found that quartile cut points aligned with the smoothed plots most closely, so we decided to use quartiles to categorize fatty acid exposures. We calculated quartiles by cohort, so a participant from the DNBC was assigned a quartile according to the values of the fatty acid measure within the DNBC, and a MoBa participant was assigned a quartile according to the MoBa fatty acid values.

Measures of Infection

MOBAND-CP has self-reported and clinical measures of infection during pregnancy. Urinary tract infection (UTI) and fever up to 28 weeks gestation were self-reported. Other measures such as respiratory infection during pregnancy, which were self-reported, were not harmonized because the data were collected differently in Norway and Denmark and the differences were unreconcilable. The measures of clinical chorioamnionitis and fever during labor were ascertained from medical records of the mother and the baby. Clinical chorioamnionitis was recorded as present in only 0.1% of pregnancies eligible for this study, while fever during labor was recorded as present only in 0.4% of eligible pregnancies. This was unexpectedly low, as clinical chorioamnionitis is reported in 1% to 13% of term pregnancies in the United States(120). Due to the small number of events available for analysis for clinical chorioamnionitis and fever during labor and the likelihood the data system did not capture all instances of the two conditions, we decided to limit the infection variables to the partially-harmonized self-reported fever and UTI variables. Details on harmonization of the infection

variables can be found in the MOBAND-CP wiki site (https://mobawiki.fhi.no/mobandwiki/doku).

Urinary Tract Infection

UTIs were reported by women in 9.9% of pregnancies in the sample. UTI was ascertained at three time-points: the first interview or questionnaire that covered early pregnancy, the second interview or questionnaire that covered pregnancy from the first interview or questionnaire to 30 weeks, and the third interview or questionnaire that was administered six months post-partum and covered late pregnancy, birth, and the neonatal period. There was substantial missingness (13.5%) in the UTI data in the third interview/questionnaire due to non-response, so we used only the data for the first and second interviews/questionnaires in the binary measure for UTI. Harmonization of the UTI variables is considered partially complete as there were significant differences in how the questions about UTI were asked between the two cohorts, but there was enough overlap in the collected data to generate a binary variable for each point of data collection that indicated presence or absence of UTI. The DNBC included cystitis and pyelitis in its definitions of UTI at interviews two, but MoBa asked only about cystitis in questionnaire two. As a result, the DNBC has a higher frequency of UTIs compared to MoBa in the second interview/questionnaire (8.0% and 5.0%, respectively). The frequency of UTI up to interview/questionnaire one was similar for the two cohorts (DNBC: 5.0%. MoBa: 6.3%).

We generated a binary variable that was coded as yes if a participant reported that she had experienced a UTI at any point in pregnancy through the second questionnaire and no if a participant reported never experiencing a UTI. Participants who reported they had not had a

UTI at one time point but were missing data for the other time point were coded as missing because we could not be certain they remained UTI-free. We also ran separate regressions for UTI at each data collection point as a sensitivity analysis.

Fever Up To 28 Weeks of Gestation

Fever up to 28 weeks of gestation was reported in 21.9% of pregnancies in the sample. Fever was ascertained at two time-points: the first interview/questionnaire and the second interview/questionnaire. Harmonization of the fever prior to 28 weeks of gestation was considered partial. In the total MOBAND-CP sample, 27.3% of participants in the DNBC sample reported a fever through the second interview, while only 15.8% of MoBa participants reported a fever through the second interview. In the DNBC, women were asked if they had experienced episodes of fever, while the questions in MoBa asked about fevers with specific conditions, such as a fever with rash or a fever greater than 38.5 C. If a participant was missing an observation for fever at interview/questionnaire 1 or 2 the value for fever up to 28 weeks gestation was coded as missing.

Infection

The infection variable is a composite of the binary UTI variable and the binary fever variable. If a participant reported either having a UTI or fever in the first 28 weeks of pregnancy, the infection variable was coded as yes. If there was no reported UTI or fever in interviews/questionnaires 1 and 2, the infection variable was coded as no. If an observation was missing data for either UTI or fever for one of the data collection points, the infection variable was coded as missing.

Power Calculations

Power was calculated using a two-sided test (Table 3.1). We used the software package G*Power3 (University of Dusseldorf) to estimate the power to detect an odds ratio of 1.5 for a logistic regression model.(121) We used the total number of mothers who contributed one or more pregnancies to our eligible sample as our sample size. For DHA, ARA, and the ratio of DHA to ARA we used the lowest quartile of the variable as the proportion of the population exposed. For fever up to 28 weeks of gestation, the prevalence in the unexposed was 21.9% and UTI prevalence was 9.9%. We used multivariable linear regressions to estimate the R-squared value of the potential confounders on the exposure variable. CP is a rare disorder with a prevalence in the unexposed population of 0.2%. Because CP is rare we expected the odds ratios used to calculate the power would be similar to estimates of relative risk. Due to CP being rare, the power to detect a difference for the interaction between the infection variables and the fatty acid variables is limited.

Analytic Methods

Aim One

Aim one sought to describe CP in sample of singleton children born at term or near term. For this aim, we included all singleton children who survived to age one. Analyses were conducted using Stata version 16 (Stata Corp, College Station, Texas), and SAS 9.4 (SAS Institute, Cary, NC). We divided the sample into two birth categories: singleton children born at 35 weeks gestation or later and singleton children born prior to 35 weeks. We calculated the prevalence of CP in each birth category. Within the children who had CP, we used frequency tables and relative risks to compare the CP subtypes, gross motor function, and CP with comorbidities

across birth categories. For all children, we compared maternal characteristics and birth outcomes by birth category using means, percentages and relative risks. We used means (standard errors), frequencies (percentages), and relative risks to describe the maternal sociodemographic characteristics and birth outcomes by CP status.

Aims Two through Seven

Aims two through seven sought to estimate the risk of CP in children born near or at term according to maternal exposure status for DHA, ARA, the ratio of DHA to ARA, UTI, fever, and infection. We determined that the DHA exposure could be analyzed in the combined cohorts, but differences in the prevalence of CP by ARA quartile necessitated conducting the analyses for ARA and the ratio of DHA to ARA in each cohort separately. Splitting the sample by cohort for the ARA and ratio analyses resulted in empty cells for the effect modification questions, and this approach prohibited testing effect modification. We hypothesized that the lowest and the highest quartiles of DHA would have the greatest risk for CP compared to the middle two quartiles, and the highest quartile of ARA would have the highest risk of CP. To aid in interpretation, we used the lowest quartile of DHA, ARA, and the ratio of DHA to ARA for reference groups. For infection variables, the reference categories were no infection, no UTI, and no fever. We calculated frequencies and percentages stratified by categorized exposure variables to describe categories of maternal age at birth, previous live births, previous still births, planned or unplanned pregnancy, maternal occupational status, marital status, alcohol use in the first part of pregnancy, smoking in the first part of pregnancy, diabetes, maternal exercise in the first part of pregnancy, and maternal pre-pregnancy body mass index.

Covariate Selection

Covariates were identified *a priori* through the literature review. We considered variables that impact CP risk prior to birth and variables that are associated with the health behaviors of pregnant women for inclusion as covariates. We lacked harmonized data for maternal education and household income (48, 57), but we did have data on maternal employment status which has been used as a socioeconomic variable in another MOBAND-CP study.(108) Maternal age has been identified as a risk factor for CP for children born at term, and so we included maternal age as a covariate.(52) Parity is an important risk factor for CP, and as parity increases, risk of CP decreases.(48, 54) We tested both previous number of live births and previous number of stillbirths for inclusion as covariates. There is little evidence to suggest that the risk of CP is different for children of planned and unplanned pregnancies,(105) but pregnancy planning may influence maternal health behaviors so we decided to include it in our list of potential covariates. Being in a relationship with a partner during pregnancy is associated with a decreased risk of CP.(48, 57) We included a binary variable for marital status, which was coded as yes if a participant was married or had a partner and coded as no if a participant did not a partner. Heavy alcohol use has been associated with increased odds of CP,(49) and maternal alcohol use has been included as a confounder in another MOBAND-CP study.(108) Use of cigarettes during pregnancy has been shown to increase odds of CP.(50) Exercise and body mass are important components of total energy intake. Total energy is related to metabolic efficiency, total physical activity, and body mass.(122) Pre-pregnancy body mass index has been associated with CP risk (47, 48), and exercise in the first part of pregnancy is a health behavior, so we tested them to use as covariates as well.

Variables that were theoretically associated with both the exposure and outcome were included in models that contained only the exposure variable and the covariate. We did not consider statistical significance for covariate inclusion, but instead used change in effect estimate of the primary exposure. If the covariate changed the unadjusted risk ratio by 2% or more, the covariate was retained in the model. If the covariate did not change the unadjusted risk ratio by more than 2%, it was removed from the final model. We used a low threshold of inclusion of covariates because there were few covariates that had a large impact on the unadjusted relative risks, and we did not want to exclude variable that previous research had shown to be important. Energy intake in kilojoules/day was divided into quartiles and included in models to control for differences in fatty acid estimates to account for differences in total energy consumed.

Models

We used log-binomial models for the binary outcome of CP. Models without interaction terms followed the equation:

$$\text{Log}(\pi_{mp}) = \beta_0 + \beta_1 X_{1mp} + \beta_2 X_{2mp} + \beta_3 X_{3mp\ldots} + \beta_k X_{kmp}$$

in which m is the clustering variable for the mother and p is the pregnancy, which is the unit of analysis. Models with interaction terms followed the equation

$$\text{Log}(\pi_{mp}) = \beta_0 + \beta_1 X_{1mp} + \beta_2 X_{2mp} + \beta_3 X_{3mp\ldots} + \beta_k X_{kmp} + \beta_{k+1} X_{1mp} * X_{2mp}$$

Log-binomial models are from the family of generalized linear regression models and they produce risk ratios when beta estimates are exponentiated.(123) To test the associations between exposure and outcome, we used the combined cohorts and ran separate models for the cohorts when the patterns of prevalence for the exposure diverged. In order to retain all

observations, we used multiple imputation. Multiple imputation is used to generate a distribution of plausible values for an observation that is missing data for a variable.(124) Multiple imputation uses a model to generate values for the missing observations over a set number of imputations, which creates a dataset that has a range of plausible values for each missing observation. We imputed our datasets 30 times, which generated a dataset that had thirty observations for each original observation. When an observation in the original dataset was missing data for a particular variable, the imputed dataset would have replaced the missing value with 30 different values based on the predicted values generated by the imputation model. We generated three imputed datasets using chained equations. For the imputation models, we used multinomial logistic regression and logistic regression models to impute categorical and binary variables, respectively, using Stata mi impute (Stata Corp, College Station, TX). Each imputation model included the three fatty acid exposures, and one infection variable. The imputation models also included the outcome, the variables included in the final model, and auxiliary variables that were correlated with the variables in the final model. The auxiliary variables were maternal age, marital status, and smoking early in pregnancy. The imputation models included the following variables: CP, DHA quartile, ARA quartile, ratio quartile, maternal age, an infection variable, maternal occupation, exercise in the first part of pregnancy, pre-pregnancy BMI, number of previous live births, marital status, alcohol use during the first part of pregnancy, and cigarette smoking during the first part of pregnancy. We used the mi passive (Stata Corp, College Station, Texas) command to generate the values of the interaction terms after running the imputation model. To account for differences in distributions of covariates between the two cohorts, we generated the imputed data by

cohort.(124) About 25% of the observations were missing data for a covariate. We used 30 imputations based on the suggestion made by White, Royston, and Wood, who recommend the number of imputations be equal to or greater than the proportion of observations with missing data multiplied by 100.(124) This suggestion is rooted in finding balance between precision of results and efficiency; the greater the number of imputations, the more precise and consistent the results of the models that make use of the imputed data will be. However, running models with hundreds of imputations can be inefficient for time with only small gains in precision. White, Royston, and Wood's analyses suggest that their suggestion provides a sufficient balance between precision and efficiency.(124)

Robustness and Sensitivity Analysis

To test the robustness of the models, we restricted the analyses to children born at 37 weeks and after. This excluded 3,795 children. We also conducted analyses to include preterm births that met inclusion criteria. This added 1,801 children. We incorporated complete case analysis to check the validity of the multiple imputation. We expected the results of complete case analyses to look similar to the results of the models generated from the imputed data.

To test the sensitivity of the models to unmeasured confounding, we calculated the e-value for each model, which is a measure of the minimum strength of association an unmeasured confounding variable would need with both the exposure and the outcome in order to explain away the effect.(125) The interpretation of the e-value depends on the extent to which model covariates effectively remove confounding. The e-value for relative risks above one is calculated using the following formula:

E-value = RR+√ [RRX(RR-1)]

In which RR is the estimated relative risk of the outcome given the exposure. For relative risk values below one, the following formula is applied:

E-value =(1/RR)+√ [(1/RR)X([1/RR]-1)]

Confidence limits for the e-value can be calculated with the confidence intervals for the effect estimate. If a confidence interval crosses the null, the confidence limit for the e-value is equal to one, the null value for relative risk. If the confidence interval of the effect estimate does not cross the null, the e-value confidence limit is calculated using the effect estimate's upper or lower confidence limit, depending on if the effect estimate is higher or lower than the null, respectively.(125)

Chapter 4: Descriptive Epidemiology of Cerebral Palsy in Term or Near-Term Children

Introduction

World-wide, CP is the most common childhood motor disability and many of the cases arise from children born at or near term (*i.e.,* 35 weeks gestation or later).(3, 4) The MOBAND-CP study offers the opportunity to describe the pre-, peri-, and post-natal conditions for children born at or near term, and to compare these conditions to preterm singleton children. Additionally, MOBAND-CP allows for the comparison of pre- peri- and post-natal conditions between singleton term or near-term children who were diagnosed with CP and those who were not.

Methods

We sought to describe CP in a sample of singleton children born at or near term. The unit of analysis was the child and mothers could contribute more than once pregnancy to the sample. We included children of singleton pregnancies that resulted in a live birth delivered at 35 weeks and later, for which the child survived to one year of age. Children included in the DNBC cohort were born between 1996 and 2003, while children in the MoBa cohort were born between 1999 and 2009.

We calculated the prevalence of CP per 1,000 singleton children who survived to age one for children born at 35 weeks gestation or later for the whole sample and by cohort. We also calculated the prevalence of CP by birth category (*i.e:* singleton children born at or near term and singleton children born before 35 weeks gestation). We calculated prevalence in the sample of children born at or near term in three gestational age categories: 35-36 weeks, 37-39 weeks, and 40 weeks or higher. We used Stata 16 (StataCorp LLC, College Station, TX) STRATE

procedure to calculate the rates of CP accounting for clustering of pregnancies by mother. Within the children with CP, we compared CP characteristics such as subtype, by birth category. Characteristics compared across the birth categories are listed in Table 4.1. In addition to frequencies, we calculated risk ratios for each level of characteristic by birth category. We used the singleton term or near-term children with CP category as the reference. For all children, we compared maternal characteristics and birth outcomes by birth category. We calculated the relative risk for categories of maternal characteristics for the singleton preterm children using the singleton term or near-term children as the reference group. For the categorical birth outcomes, we calculated the relative risk of falling into a birth outcome category for preterm singleton children compared to singleton children born at or near term. We used Stata 16 GLM procedures clustered by mother to estimate the relative risk for each category.

We then compared the characteristics of the children diagnosed with CP to the children without a CP diagnosis in the sample of singleton term or near-term children. We calculated means (standard errors) and frequencies (percentages) using SAS 9.4 (SAS Institute, Cary, NC) and included a category for missing observations (see Table 4.2) In addition, we calculated the risk ratios for CP by characteristic.

Results

Prevalence of CP

There were 209,716 children in the overall MOBAND-CP sample that survived to age one. Of these children, 197,765 (94.3%) were singleton children born at 35 weeks gestation or later. The estimated prevalence of CP in the combined cohort term or near-term children was 1.50 cases per 1,000 children (95% CI: 1.34, 1.69). The estimated prevalence of CP for singleton

children born prior to 35 weeks gestation was 22.59 per 1,000 (95%CI: 18.50, 27.89). The DNBC prevalence of CP for singleton, term or near-term children was lower than the MoBa prevalence. The prevalence of CP for DNBC children in the term or near-term group was 1.41 (95% CI: 1.19, 1.68) per thousand, while the prevalence of CP for MoBa children was 1.58 per thousand (95% CI: 1.36, 1.84). (Table 4.3)

The prevalence of children with CP in the near or at term sample varied by gestational age. Within children born 35-36 weeks gestation, the prevalence was 3.42 cases per 1,000 children who survived to age one (95% CI: 2.28, 5.59). The prevalence of CP for children born 37-39 weeks was 1.60 per 1,000 children (95% CI: 1.35, 1.92), and it dropped for children born at 40 weeks or later to 1.34 cases per 1,000 children (95% CI: 1.15, 1.57). (Table 4.3)

Comparison of Term or Near-term Children to Preterm Children

CP characteristics by birth category

Within children who were diagnosed with CP, the most common subtype of CP within singleton children born at 35 weeks or later was spastic unilateral (42.4%), while spastic bilateral CP was the most common subtype for the singleton children born prior to 35 weeks gestation (see Table 4.4). Singleton preterm children were less likely to have spastic unilateral CP compared to the term- or near-term singleton children (relative risk: 0.63 [95%CI: 0.44, 0.91]), while the singleton, preterm children had a 90% increase risk of being diagnosed with spastic bilateral CP compared to the term- or near-term singleton children. There were also more children in the term and near- term group who were diagnosed with dyskinetic CP compared to the preterm children. Most children were classified as having a GMFCS score of one, regardless of group. However, there were more singleton children born at or near term

with a GMFCS of one compared to the preterm children. The relative risk for having a GMFCS of one was 0.73 for the preterm singleton children compared to the term singleton children (95% CI: 0.54, 0.97). Singleton children born at term or near term had a lower prevalence of GMFCS scores of 3 compared to the singleton preterm children (4.7% versus 14.4%). The singleton children in the preterm group had a higher risk of having a GMFCS score of three compared to the singleton term- or near-term children (relative risk 3.06 [95% CI: 6.28]). The complete table of relative risk estimates is available in supplement Table 4a.

Maternal sociodemographic characteristics by birth category

Maternal social and demographic characteristics by birth category are presented in Table 4.5. Estimates of relative risk are located in supplement Table 4b. Due to sample size, many of the relative risk estimates were statistically significant, but the differences in percentage between the groups was small. Notable differences in maternal characteristics between the two groups include: number of previous live births, and planned pregnancy.

Within the singleton term- or near-term children, there were fewer nulliparous women compared to the singleton preterm children. Mothers of term- or near-term singleton children tended to have one or more previous live births. Compared to the mothers of singleton children born at or near-term, the mothers of singleton preterm children and children were less likely to have one and two or more previous live births. Compared to the term- or near-term group, the relative risk of having one previous live birth for the preterm group was 0.73 (95%CI: 0.69, 0.76).

The pregnancies of the term- or near-term singleton children were planned more frequently than the pregnancies of the singleton preterm children (77.0% versus 73.5%). The

relative risk of reporting a pregnancy was unplanned was 1.15 (95% CI:1.08, 1.24) for singleton preterm children compared to singleton term- or near-term children.

Birth outcomes by birth category

Continuous birth outcomes by birth category are presented in Table 4.5.1. Singleton children born at term- or near-term had substantially larger birthweights compared to the singleton preterm children. By definition, the mean gestational age was higher for the children in the term- or near-term group compared to the singleton preterm group.

Categorical birth outcomes are shown in Table 4.5.2, and the corresponding relative risks can be found in the supplement Table 4c. Many of the categorical birth outcome categories were substantially different between the birth categories. Singleton children born at or near term were more frequently born via unassisted vaginal delivery compared to the singleton preterm children (76.6% and 47.5%, respectively), and there were fewer emergency c-sections within the term- or near-term singleton children. Children in the preterm birth category had 4.77 times the risk of being born via emergency c-section compared to the children in the term- or near-term birth group (95% CI: 4.58, 4.96).

The singleton children in the term- or near-term group were less frequently transferred to the neonatal ward compared to the children in the singleton preterm group. They also had fewer instances of intra-cranial bleeding and mechanical ventilation compared to the preterm group. Children in the preterm birth category had 13.99 times the risk of being admitted to the neonatal ward compared to the children in the term- or near-term group (95% CI: 13.67, 14.31). Singleton children who were born prior to 35 weeks gestation had 49.99 (95% CI: 39.74, 62.87),

times the risk of having intracranial bleeding and 42.32 (95% CI: 38.42, 46.62) times the risk of being mechanically ventilated compared to singleton children at 35 weeks gestation or later.

Comparison of Children With CP and Without CP

Maternal characteristics by CP status

There were few large differences in maternal characteristics when comparing the children with CP to the children without (Table 4.6). Children with CP more frequently had mothers who had not previously had a live birth than the children without CP. Compared to children whose mothers had previously had no live births, children born to mothers with one previous live birth had a relative risk of CP of 0.78 (95% CI: 0.60, 1.00) (Table 4d). Children in the CP group also had mothers who more frequently reported being physically inactive during the first part of pregnancy (47.2%) compared to the children without CP (43.5%), though the relative risk estimate was small and crossed the null (relative risk: 1.14 [95% CI: 0.84, 1.56]). Within the children with CP, maternal pre-pregnancy BMI was higher than the maternal pre-pregnancy BMI of children without CP. When children with CP were compared to children without, there were fewer children whose mothers reported a pre-pregnancy BMI of 18.5 to less than 23 kg/ m^2 (CP: 33.7%, no CP: 42.3%), while there was a higher percentages of mothers whose pre-pregnancy BMI was in the highest categories. Children whose mothers had a BMI of 25 to less than 30 kg/ m^2 had a relative risk of CP of 1.49 compared to children of mothers whose BMI was less than 18.5 kg/ m^2, and children whose mothers pre-pregnancy BMI was 30 kg/m^2 or greater had a 1.75 fold greater risk of CP compared to the children whose mothers pre-pregnancy BMI was less than 18.5 kg/ m^2.

Birth outcomes by CP status

Birth outcomes differed to a greater degree than maternal characteristics when children with CP and the children without were compared. The mean birthweight and the mean

gestational age were lower in the CP group compared to the no CP group (difference in means: 219.6 grams and 0.4 weeks, respectively) (Table 4.7.1).

There was a higher frequency of boys in the CP group compared to no CP group (56.9% and 51.2%, respectively) (Table 4.7.2). The CP group required more interventions during birth than the group without CP. There were fewer deliveries via unassisted vaginal birth in CP group compared to the no CP group (51.6% and 74.7%, respectively), and there was a higher percentage of emergency Cesarean section deliveries in the CP group compared to the no CP group (32.1% versus 10.0%). The risk of CP was 1.81 times higher for children who were delivered by assisted vaginal birth than those born via unassisted vaginal delivery (95% CI: 1.28, 2.54) (supplement Table 4e). Children who were born via emergency C-section had 3.12 times the risk of being diagnosed with CP compared to the reference (95% CI: 2.34, 4.16). Children in the CP group more frequently had Apgar Scores of less than five at five minutes post birth compared to children without CP (10.6% and 0.2%, respectively). Children in the CP group were more frequently transferred to the neonatal ward than children in the no CP group (60.3% versus 8.5%), and along with this, there were higher percentages of adverse birth outcomes, such as neonatal seizure and encephalopathy, for the children in the CP group compared to the children without CP. Children who had an Apgar of less than five at five minutes post-birth had 72.83 times the risk of CP compared to children who had a five minute Apgar score of more than five (95% CI: 52.74, 100.56). Children who had been transferred to the neonatal ward had almost 14 times the risk of CP compared to children who had not been transferred (relative risk: 13.54 [95% CI:10.78, 17.00]). Children who experienced intracranial bleeding (relative risk: 68.37 [95% CI: 41.01, 113.96]), had an indicator of encephalopathy (relative risk:54.11 [95%CI:

37.77, 77.53]), had a neonatal seizure (relative risk: 122.32 [95% CI: 93.59, 159.87]), or who were mechanically ventilated (relative risk: 21.43 [95% CI: 14.20, 32.34] also had significantly higher risk of CP than children who did not experience these events.

Discussion

The prevalence of CP for singleton children born at or near term in the combined MOBAND-CP cohort was 1.50 per 1,000 children who survived to one year of age. This is similar to the prevalence rate estimated for children born at term in a meta-analysis that drew on research from around the world (1.35 per 1,000 live births).(4) The inclusion criterion of survival to one year of age and the addition of children born at 35 and 36 weeks gestation to this sample are likely reasons for the small difference in prevalence. The DNBC sample had a lower prevalence of CP for children born at or near term (1.41 per 1,000 children who survived to one year) than the MoBa sample (1.58 per 1,000 children who survived to one year). The prevalence in the DNBC sample was similar to the prevalence estimated from the Danish Cerebral Palsy Registry for term live births that occurred between 1999 and 2007.(15) The Norwegian estimate was higher than term birth prevalence estimate derived from the Medical Birth Registry of Norway (1.15 per 1,000 of live, singleton births between 1967 and 2001).(16) The difference is likely due to the different time frame of data collection and the different data source for the cases.

Prevalence of CP varied within singleton children born at or near term, though the prevalence for the 35 and 36 week age group was still substantially lower than the prevalence of CP for children born prior to 35 weeks gestation. Children born between 35- and 36-weeks gestation had a prevalence of 3.42, while children born 37-39 weeks had a prevalence of 1.60,

and children born 40 weeks and later had a prevalence of 1.34. In a meta-analysis of world-wide estimates of CP, the prevalence of CP within children born 32-36 weeks was 6.8 cases per 1,000 live births (95% CI: 5.6, 9.9), and the prevalence of CP for children born at 37 weeks gestation or later was 1.4 cases per 1,000 live births (95% CI: 1.2, 1.6).(4) Our estimates of prevalence of CP for children born at or near term by gestational age group suggest that prevalence of CP continues to decrease as gestational age approaches term and continues to fall after 37 weeks gestation has been reached. These findings concur with the findings from a study that used the Medical Birth Registry of Norway, that showed that risk of CP decreased from 37 to 40 weeks gestational age at delivery and rose again after 42 weeks gestational age at delivery was reached.(16) We had too few children with CP who were delivered after 42 weeks gestation to estimate the risk of CP among children born post-term.

Of the 209,716 children who survived to age one, more than 94% were singletons born at 35 weeks gestation or later. These children were significantly different from the children of singleton pregnancies born prior to 35 weeks gestation with respect to the prevalence of CP. This was unsurprising because both preterm birth is associated with a greater risk of CP.(36) Mothers of children in the term or near-term birth category were more likely to be multiparous and to have planned their pregnancy compared to mothers in the singleton preterm group. Children in the singleton term or near-term sample had fewer adverse birth events compared to the children in the singleton preterm category as well.

There were few statistically significant differences in maternal characteristics between the singleton children born at or near term with CP compared to those without, though there were patterns that emerged. Differences between the CP and no CP group in maternal pre-

pregnancy BMI were significant. Maternal pre-pregnancy BMI has previously been shown to be associated with CP risk in the MOBAND-CP cohort previously.(47) There was a smaller percentage of women with a pre-pregnancy BMI of 18.5 to less than 23 kg/m^2, and higher proportions of pre-pregnancy BMIs in the overweight category (25 kg/m^2 to <30 kg/m^2) and the obese category (30 kg/m^2 or greater) in the CP group compared to the no CP group. The 2016 MOBAND-CP analysis of BMI found that as pre-pregnancy BMI increased, the prevalence of CP increased.(47) BMI classifications of overweight and obesity were also found to be associated with CP risk in a study that used Swedish registry data.(48)

The percentage of women who had not previously had a live birth was higher for the children with CP compared to those without (50.8% versus 43.8%), and there were fewer pregnancies in which women had one or more previous live births. Primiparous pregnancies have previously been shown to have a higher risk of CP compared to multiparous pregnancies. A case-control study of perinatal arterial ischemic stroke found that within cases of perinatal arterial ischemic stroke, the prevalence of primiparous mothers was 74%, while the prevalence of primiparous women in the group without perinatal arterial ischemic stroke was only 44%.(55) The Swedish registry study of BMI tested multiple variables for inclusion in their causal model and found that as parity increased, the rate of CP decreased in an inverse dose-response manner.(48)

There were small differences in maternal age and maternal occupation between CP group and the no CP group. We could not include a category of maternal age less than 20 years because of small numbers in the CP group, but this finding is consistent with another Scandinavian study.(48) There was no notable differences in employment status between the

mothers of children with CP and mothers of children without. Previously, paternal occupational status was not found to affect the risk of CP in children.(58) Differences in the frequency of marital status (partnered or single) were also not significant. Marital status has previously been shown to be an important risk factor for CP in a study that used data from the Danish and Norwegian medical birth records (57), but we did not replicate this finding in the MOBAND-CP term or near-term singleton children. It is likely that this difference could be explained by the exclusion of children born preterm in our sample.

Planned pregnancy, maternal smoking, and alcohol use during pregnancy were not significantly different between the CP group and the no CP group. The frequencies of planned pregnancies were similar between the two groups. The literature on the risk of CP in relation to alcohol use shows mixed results. One study shows significant increases in odds of CP when mothers were diagnosed with an alcohol disorder during pregnancy(49), while another study showed no association with alcohol use during pregnancy when alcohol was measured via self-reported drinks per week.(50) The measure of alcohol we used was crude and it does not reflect severity of use, including amount of alcohol used, frequency of use, or duration of use. It was a self-reported binary of ever versus never used alcohol during pregnancy. Additionally, the harmonization for the alcohol measure between the two cohorts was partial. Complete harmonization was not possible because of the difference in timing of ascertainment of alcohol use during pregnancy and because the questions used for measuring alcohol use were different. DNBC interviewed women at 12 weeks gestation and had few missing observations for alcohol use. The MoBa cohort ascertained alcohol use at 17 weeks gestation, and more missing data than DNBC. Maternal smoking of ten or more cigarettes per day has been shown

to increase the risk of CP for children by 80% (hazard ratio: 1.8 [95% CI:1.1, 2.9]).(51) Our measure of smoking was similar to the our measure of alcohol use: it was a self-reported binary variable of ever versus never smoking during pregnancy and it does not account for dose.

Within the singleton children born at term or near-term, the CP group had more adverse pregnancy outcomes than the no CP group. Children with CP had a lower mean birthweight and gestational age than children without CP. Fetal growth and gestational age have been associated with risk of CP. Fetal growth restriction was shown to be an important risk factor for children born after 34 weeks gestation.(39) Growth restriction occurred 3.5 times more often for children with CP than control children (95% CI:2.2, 5.5). Gestational age is an important risk factor for CP. The risk of CP changes by gestational week of delivery even for children born at term.(16) A study that linked medical records found that children born at 37 weeks gestation had the highest risk of CP for the children born in the term group (relative risk: 1.9 [95% CI:1.6, 2.4]).(16) In our study, 60.3% of the children in the CP group were transferred to the neonatal ward compared to only 8.5% for the children without CP. There were higher frequencies of encephalopathy conditions (8.3% versus 0.2%), neonatal seizures (15.1% versus 0.2%), and use of mechanical ventilators (13.8% versus 0.9%) for children with CP compared to the children without CP. In a study of cerebral palsy following newborn encephalopathy, 13% of children who survived neonatal encephalopathy developed CP.(126) We found that risk of CP was almost 50 fold higher for children who had evidence of neonatal encephalopathy compared to those who did not. Neonatal seizures have long been associated with CP. Nelson and Ellenberg found the risk of CP was elevated for children who had experienced neonatal seizures compared to those who had not in a study from the Collaborative Perinatal Project.(96) In a

more recent study, 25% of children who survived neonatal seizures developed CP.(127) In our study, children who experienced a neonatal seizure had 122-fold increase in risk of CP compared to those who did not. Mechanical ventilation is a treatment for infant respiratory distress syndrome (IRDS) and other complications. In a study of child health outcomes following IRDS for children born between 32 and 36 weeks gestation, the cumulative incidence of CP to 8 years of age was 1.9% (95 % CI: 1.4, 2.5).(128) The cumulative incidence of CP for children who did not experience IRDS was 0.5%. While we do not have reason for ventilator treatment available, we can assume that IRDS is one of the reasons for mechanical ventilation. We found that mechanical ventilation increased the risk of CP 21-fold within singleton children born at or near term.

Conclusion

The prevalence of CP in our sample of term- or near-term births is similar to other estimates of CP prevalence within term- or near-term births. More than 68% of the CP cases arose from children born at 35 weeks gestation or after. There were significant differences between the singleton term- or near-term children compared to the singleton preterm children in the MOBAND-CP sample. There were few statistically significant differences between the maternal characteristics of pregnancies that resulted in a child with CP and the pregnancies that did not, however, important patterns did emerge, particularly for maternal pre-pregnancy BMI. Birth outcomes differed significantly between the CP group and the group without CP, and the differences were consistent with previous literature.

Chapter 5: DHA

Introduction

DHA is a long chain polyunsaturated fatty acid that humans are exposed to either by metabolism of its precursor, alpha-linolenic acid, or through consumption of fatty-fish products. Fish consumption during pregnancy has been associated with a 30% reduction of odds of CP for each additional serving of fish eaten per week.(85) However, there is some indication that n-3 polyunsaturated fatty acids reduce the capacity of platelets to aggregate (129), which has led to suggestions that too much DHA might result in increased risk of bleeding, though the evidence that n-3 fatty acids increase the risk of bleeds is insufficient.(130) Because of the previous research showing the odds of CP decreased as fish intake increased and the reduction of platelet aggregation associated with n-3 PUFAs, we hypothesized that for children born at or near term, the risk of CP by DHA status would follow a U-shaped curve, and the greatest risk would be found in children whose mothers' prenatal DHA intake was categorized in the first and fourth quartiles.

Methods

We calculated the DHA intake quartile for each pregnancy by cohort from combined food and supplement intake. The level of analysis is the child, and mothers could contribute more than one child. To account for the presence of siblings in the data, we clustered analyses by mother's identification number.

To test our hypothesis, we calculated the prevalence of CP per 1,000 children who survived to one year of age by DHA quartile, clustered by mother. We used Stata 15 (StataCorp, College Station, TX) to calculate the prevalence of CP with the cohorts combined and for each

cohort separately. We used this analysis to determine if combining the cohorts for the DHA analysis would be appropriate. Patterns of CP prevalence by DHA quartile were similar between the two countries, but we decided to generate three sets of models: models for the cohorts combined, and models for each cohort individually.

We described maternal characteristics by DHA quartile with frequencies and percentages calculated for the combined cohort and the two cohorts separately. We used multiple imputation (imputations = 30) to retain the full sample for analysis (see Chapter 3 for details on multiple imputation). We imputed the data by cohort to accommodate differences in data ascertainment for some of the covariates.

We constructed log-binomial models for DHA for the combined cohorts and the cohorts separately. We used the first quartile of DHA as the reference group for all models. We included the following variables in the final models: quartile of total energy intake, previous number of live births, maternal occupation, marital status, alcohol use during pregnancy up to the first interview/ questionnaire, exercise in the early part of pregnancy, and pre-pregnancy BMI.

Sensitivity Analysis

To gauge the degree to which a single unmeasured confounder might affect the results of our models, we calculated the e-value.(125) The e-value estimates the association, expressed as relative risk, an unmeasured confounder must have with both the exposure and the outcome in order to reduce the effect size to the null. We also generated models for singleton children born at 37 weeks gestation or later (i.e. term), singleton children born at any gestational age, and for term or near-term children with complete covariate data.

We performed separate *post hoc* analyses of DHA from food intake and from supplement use in the MoBa cohort to better understand the relationship between DHA and CP. DHA intake separated out by food intake and supplement use data was not available from DNBC for this analysis.

Results

Table 5.1 shows the bivariate distribution of maternal characteristics by DHA status for the combined cohorts. Notable differences in maternal characteristics by DHA quartile included maternal age, alcohol use up to the first interview/questionnaire, maternal smoking up to the first interview/questionnaire, exercise in the early part of pregnancy, and maternal pre-pregnancy BMI. As maternal age increased, the percentage of women who fell into the third and fourth quartile of DHA consumption increased. The relative risk estimates for maternal age in the first quartile show that as the risk being 24 years or younger was lower for the second, third, and fourth DHA quartile and the risk of being 35 years or older increased as DHA quartile increased (relative risk estimates and confidence intervals are in supplement Table 5a). Any use of alcohol up to the first interview/questionnaire increased as DHA quartile increased, and the relative risk of using alcohol in pregnancy increased by DHA quartile. Compared to the first quartile, the risk of using alcohol in the first part of pregnancy was 1.14 times higher in the second quartile (95% CI: 1.11, 1.18), 1.21 times higher in the third quartile (95% CI: 1.17, 1.25), and 1.20 times higher in the fourth quartile (95% CI: 1.17, 1.24). The percentage of smoking decreased as DHA quartile increased. Compared to the first DHA quartile, the risk of smoking was lower in the second, third and fourth quartile. As DHA quartile increased, the percentage of pregnancies in which the mother exercised two or more times per week also increased, and the

percentage of pregnancies in which the mother was physically inactive decreased. This is reflected in the relative risk estimates for being physically inactive and exercising two or more times per week by DHA quartile. There were step-wise patterns in pre-pregnancy BMI as well; the percentages and the relative risks of women who reported having a pre-pregnancy BMI of less than 23 kg/m^2 increased as DHA quartile increased and the percentages and relative risks of those who had a pre-pregnancy BMI of 25 kg/m^2 or higher decreased as DHA quartile increased.

Table 5.2 describes maternal characteristics by DHA quartile in the DNBC. Maternal characteristics that differed across DHA quartiles included maternal age, alcohol use up to the first interview, exercise early in pregnancy, and pre-pregnancy BMI. There were smaller differences in the prevalence of smoking up to the first interview than in the combined cohorts. As maternal age increased, the percentage of pregnancies in the third and fourth quartiles of DHA increased. The relative risk of having a mother whose age was in the first and second categories decreased in step-wise progressions as DHA category increased, while the relative risk of having a mother in the highest age category increased in a step-wise progression as DHA category increased (see supplement Table 5b). Like the combined cohorts, the percentage of pregnancies in which the mother drank alcohol up to the first interview increased as DHA quartile increased. Compared to children whose mothers' DHA intake fell into the first quartile, the risk for use of alcohol early in pregnancy was 15% higher in the second DHA quartile (relative risk 1.15 [95% CI: 1.12, 1.18]), 22% higher in the third quartile (relative risk 1.22 [95% CI: 1.19, 1.25]), and 21% higher in the fourth quartile (relative risk 1.21 [95% CI: 1.18, 1.25]). Compared to the first DHA quartile, the risk of having smoked in the first part of pregnancy was

lower in the second DHA quartile (relative risk: 0.90 [95% CI: 0.86, 0.94]), the third quartile (relative risk: 0.86 [95% CI: 0.0.85, 0.92]), and the fourth quartile (relative risk: 0.90 [95% CI: 0.88, 0.91]). The percentage of pregnancies during which the mother was physically inactive decreased as DHA quartile increased, and the percentage of pregnancies for which mothers were physically active two or more times per week increased. The relative risks of being physically inactive in the first part of pregnancy decreased in a step-wise manner as DHA quartile increased, and the relative risks of exercising two or more times per week increased as DHA quartile increased. The percentage of pregnancies in which the pre-pregnancy BMI was below 23 kg/m^2 also increased as DHA quartile increased, and the proportion of pregnancies for which the pre-pregnancy BMI was greater than or equal to 25 kg/m^2 decreased as DHA quartile increased. The relative risks of having a pre-pregnancy BMI less than or equal to 23 kg/m^2 increased as DHA quartile increased, and the relative risk of having a BMI greater or equal to 25 kg/m^2 decreased as DHA quartile increased.

Table 5.3 shows the maternal characteristics by DHA quartile for MoBa. The most notable differences were found in maternal age at birth, previous number of live births, maternal occupation, smoking in the first trimester, exercise in the first part of pregnancy, and pre-pregnancy BMI. Patterns in MoBa and DNBC were similar for maternal age, exercise in the first part of pregnancy, and pre-pregnancy BMI. The relative risk of being in the youngest age category decreased in a step-wise manner as DHA quartile increased, and the relative risk for being 35 years or older increased in a step-wise manner ad DHA quartile increased (see supplement Table 5c). In MoBa, as DHA quartile increased, the percentage of pregnancies for which the previous number of live births was zero also increased, which was the opposite of

DNBC. The relative risk for having no previous live births for the second quartile compared to the first was 1.17 (95% CI: 1.14, 1.20). The risk of having no previous live births was about 25% higher in the third and fourth DHA quartiles compared to the first as well. There were fewer pregnancies that had been preceded by two or more live births in the high DHA quartiles compared to the low DHA quartiles. Alcohol use up to the first questionnaire was less prevalent in the MoBa Cohort compared to DNBC and there was no meaningful difference by DHA quartile, however there was more missingness in this variable compared to the DNBC (see supplement Table Sample b). As DHA quartile increased, smoking up to the first questionnaire decreased. The relative risk of smoking in the second quartile compared to the first quartile was 0.80 (95% CI: 0.77, 0.83). Compared to the first quartile, the relative risk of smoking up to the first interview in the third quartile was 0.73 (95% CI: 0.71, 0.76), and was 0.72 for the fourth quartile compared to the first (95% CI: 0.69, 0.74). Patterns of percentages and relative risks were similar to those found in the combined cohorts and the DNBC.

Figure 5.1 shows the prevalence of CP by DHA quartile. In both cohorts, the prevalence of CP was highest in the second quartile of DHA intake (see supplement Table 5d for the estimates). The prevalence of CP was lower in DNBC than in MoBa in the lowest three quartiles, but it surpassed the prevalence of MoBa in the fourth quartile. In the combined cohorts sample, the prevalence of CP per 1,000 children who survived to age one was lowest in the third quartile (1.04 cases of CP per 1,000 children), followed by the first quartile (1.19 cases per 1,000 children). The DNBC showed the same pattern as the combined cohorts, but in MoBa, the third and fourth quartile had the same prevalence. The highest prevalence of CP was found in

the second quartile of DHA consumption for the combined cohort and the DNBC and MoBa cohorts separately (2.17, 1.74, and 2.50 cases per 1,000 children, respectively).

Almost 63% of women in the DNBC cohort had an estimated daily intake of DHA that met the recommended daily intake of 200 mg per day, while almost 78.9% of the MoBa cohort met the recommendation. In MoBa, the only cohort for which we had food and supplement source of DHA separated, 44.6% of women met the recommended daily intake through supplement use, while 51.3% met the recommended daily intake through food. The majority of women in MoBa were getting more DHA from food than from supplements (data not shown). Table Supplement Ch5e and Ch5f show the minimum, median, and maximum DHA intake by total DHA quartile and supplement category, respectively.

DHA Models

Table 5.4 presents the frequency of CP, the unadjusted relative risk and the adjusted relative risks for CP, and e-value for the quartiles of DHA in the combined cohorts. Compared to children whose mothers DHA intake fell into the first quartile, children whose mothers' DHA intake was in the second quartile had an 85% increase in risk of CP (adjusted relative risk: 1.85 [95% CI: 1.26, 2.71]). The e-value suggested a single unmeasured confounder would have to have a relative risk of 3.10 for both CP and inclusion in the second DHA quartile in order to remove the effect of DHA in the second quartile on CP risk.

In both unadjusted and adjusted models, the risk of CP of children in the third quartile was lower compared to the first quartile, but the decrease was small and non-significant. The e-value indicates that a weak unmeasured confounder could attenuate the association. The relative risk of CP in the fourth DHA quartile compared to the first quartile was elevated, but the increase was small, the confidence interval crossed the null, and could be explained away by unmeasured confounding.

Table 5.5 shows the frequency of CP by DHA quartile, the unadjusted and adjusted relative risk of CP by DHA quartile, and the e-values of the relative risks in the DNBC. The relative risk of CP for children in the second quartile of DHA exposure compared to the first quartile was similar to the relative risk of the second quartile in the combined cohorts, though the smaller sample led to a marginally statistically significant association and a wide confidence interval that crossed the null value. The relative risk of CP for children in the third quartile compared to the first was virtually null and could be explained away by a weak unmeasured confounder. The elevated risk of CP for children in the fourth DHA quartile compared to the

first was different from the combined cohort analysis. In the fully adjusted model, children whose mothers' DHA intake fell into the fourth quartile had a 79% increase risk of CP compared to children whose mothers' intake of DHA fell into the first category, and this finding was marginally significant.

The frequency of CP, unadjusted and adjusted relative risk of CP by DHA quartile, and the e-values of the estimates for the MoBa cohort are shown in Table 5.6. The relative risk for the second quartile compared to the first quartile was similar to those estimated for the combined cohorts and the DNBC. For children in whose mothers' DHA intake fell into the second quartile, the risk of CP was about 83% higher compared to children whose mothers' intake fell into the first quartile (adjusted relative risk: 1.83 [95% CI: 1.13, 2.95]). The e-value indicated that an unmeasured confounder would have to increase the risk of CP and the risk of being in the second quartile of DHA intake three-fold in order to remove the association. The risk of CP for children in the third quartile of DHA exposure was about 15% lower than it was for children in the first quartile, but the confidence interval crossed the null value and the e-value suggested a weak unmeasured confounder could attenuate the association. In the MoBa cohort, the risk of CP for children in the fourth quartile of DHA exposure was similar to the risk of children in the first quartile.

To better understand if births prior to 37 weeks or birthweight contributed to the consistent pattern of increased risk of CP in the second quartile of DHA exposure, we calculated the mean birthweights and gestational age at birth for children in the combined cohorts according to DHA quartile. Table 5.7 shows the unadjusted means for birthweight and gestational age by DHA quartile in the combined cohorts. Differences in birthweight and

gestational age across DHA quartiles were minimal and there was no evidence of a difference in these two key risk factors for CP in the second quartile of DHA exposure.

Post Hoc Analysis of DHA and CP Risk

There are several possible unmeasured confounders for the association between DHA and CP, including exposure to environmental toxicants like methyl mercury. For children in the MoBa cohort we were able to divide maternal DHA intake into exposures from food and exposures from supplement use. We did this analysis to generate hypotheses that might explain the high risk of CP in the second DHA quartile. Table 5.8 shows the results of unadjusted and adjusted models of CP risk according to quartile of DHA from food intake. These models showed minimal elevation of CP risk in the second quartile of intake that could be attenuated by small amounts of unmeasured or residual confounding, unlike the models in the primary analysis that used a measure of DHA that included intake from food and supplements combined.

Table 5.9 presents the unadjusted and adjusted relative risk of CP across categories of DHA obtained from dietary supplement use. The first category was comprised of children of women who did not use supplements during pregnancy, which accounted for more than 32% of the sample. The low exposure group included children whose mother's DHA intake fell between 32.2% of the sample and the 50th percentile, and the remaining two categories included children in the third and fourth highest quartiles (middle and high exposure, respectively) of intake of DHA via dietary supplements. Children whose mothers' intake fell into the low exposure group had an increased risk that was almost 60% higher than the risk of the children whose mothers did not use dietary supplements containing DHA. Children whose exposure fell

into the third quartile had a modestly elevated risk, but the confidence interval crossed the null. Children whose exposure was in the fourth quartile had risk that was similar to the risk for the children of women unexposed to DHA through supplement use.

Discussion

In the combined cohorts, risk of CP was highest in the second quartile of DHA intake and lowest in the third quartile, though the difference between the first quartile and the third quartile was not statistically significant. The risk of CP for children whose mother of prenatal DHA intake was in the fourth quartile had higher risk than children in the first quartile, but this was not statistically significant either. For children enrolled in the DNBC, the risk of CP was highest in the second quartile, the risk in the third quartile was similar to the risk in the first quartile, and the risk of CP in the fourth quartile of exposure was much higher than in the combined cohorts, though the estimates did not reach statistical significance. For the children enrolled in the MoBa cohort, the risk of CP was higher in the second quartile of exposure compared to the first and lower in the third quartile of prenatal DHA compared to the first quartile. The risk of CP in the fourth quartile of exposure was similar to the first quartile. In the MoBa cohort, the increased risk in the second quartile compared to the first quartile of risk exposure reached statistical significance. The lowest risk of CP in third quartile of exposure aligned with our original hypothesis, but the highest risk of CP belonging to children whose exposure was in the second DHA quartile was unexpected.

Prior research on risk of CP in relation to DHA status is limited, and we formulated our hypotheses based on expected mechanism. One case-control study did find that as servings of fish increased, the risk of CP decreased by 30%.(85) While the case-control study suggests a

linear relationship between fish exposure and CP, our study indicates that CP risk is highest in the second quartile of DHA intake. An important difference in our study is that our measure of DHA included both DHA from food intake and DHA from supplement use. Our post-hoc analysis suggests that intake of DHA from food does not change risk of CP substantially, but the risk changes with intake of DHA from supplements.

Studies of motor function suggest that DHA intake during pregnancy has a beneficial impact on child outcomes. In one study, neurologic optimality scores in 18 month old children born at term were lower for children whose umbilical cord DHA status at birth fell into the lowest quartile compared to children in the highest quartile of DHA.(131) Neurologic optimality scores were derived from measures of motor function. In this study, children in the second quartile of umbilical cord DHA concentration had the highest neurologic optimality scores. This finding contrasts with our results of the second quartile of DHA intake having the greatest risk of CP, though neurologic optimality scores at 18 months of age are not directly translatable to risk of CP. Additionally, the children in this study were health term infants. While we limited our sample to children born at 35 weeks of gestation or later, we included children who experienced adverse birth events. The study found no association between umbilical DHA status and Bayley Psychomotor Index or Bayley Mental Developmental Index scores. (131) A study for seven-year old children found that scores on the Mastrich Motor Test were positively associated with DHA concentrations in cord blood.(89) The total score, which was comprised of both quantitative and qualitative measures of movement, increased as DHA concentration in cord blood increased (beta: 0.13, p-value: 0.01).(89) The study suggested that there was a positive linear association between DHA concentration and motor development. Our study

found the risk of CP changed across quartiles of DHA and there was no evidence of a linear relationship between DHA intake and CP.

DHA status in pregnancy has been associated with other neurodevelopmental outcomes as well. A study from the DNBC found odds of high developmental scores at 18 months were largest for children in the highest quintile of fish intake versus the lowest quartile.(132) The study found the odds of a high total development score were 29% higher for children whose mothers fish intake fell into the highest quintile versus the children whose mothers' intake fell into the lowest quintile. The odds of a high development score also increased in the third and fourth quintiles. Developmental scores were determined by the presence or absence of developmental milestones that spanned motor, social, and cognitive skills.(132) The findings suggests that the greater the exposure to fish, and therefore, n-3 PUFAs like DHA, the better the overall neurodevelopment of the child. In our study, the second, and fourth quartiles of DHA intake during pregnancy were associated with higher risk of CP compared to the first quartile, and the third quartile had the lowest risk of CP compared to the first quartile, though the relative risk for the third and the fourth quartiles compared to the first quartile were not statistically significant. This suggests there may be other factors contributing to the risk of CP that cause the association between DHA and CP to behave differently than other neurodevelopmental outcomes.

Early in the decade of the 2000s, the recommended dietary intake of DHA during pregnancy in the United States and Europe was set at 200 mg per day, and the recommendations were consistent in recommending this be achieve through intake of one to two servings of fatty fish per week, while supplements should be reserved for situations where

the recommendation could not be met through food.(78, 79) In the DNBC, 63% of participants reported intake of fish and supplements that provided 200 mg of DHA per day or more, and in MoBa, the estimate was closer to 80%. The high proportion of women who met the recommendation of 200 mg per day in both countries was striking. In the United States, it was estimated that 95% of pregnant women did not meet the recommended 250 mg of DHA per day.(133) A recent DNBC validity study indicates that the DNBC FFQ adequately identifies DHA intake, but the best results were achieved when the DHA estimates were expressed as a percentage of total PUFAs instead as a raw estimate.(114) Caution should be used when interpreting the estimates of frequency of meeting the recommendation from our study because raw estimates were used to calculate the frequencies.

To better understand the unexpected patterns in CP risk, we separated out DHA from food sources and DHA from supplement sources in the MoBa children. We found the risk of CP in the second, third, and fourth quartiles of DHA from food sources was similar to the risk of CP in the first quartile. Risk of CP was higher for children whose mothers DHA intake from supplements fell into the low exposure group compared to the risk of children in the unexposed group, however the effect size was not as large as the effect size in the total DHA analyses. This suggests the use of supplements containing DHA account for part of the elevated risk, though it does not explain why supplement use affects risk. A validation study of the MoBa FFQ found there was no difference in the levels of erythrocyte DHA between supplement users and non-users., which suggests it may be other properties associated with DHA supplementation that impact the risk of CP.(118)

Trials of DHA supplementation during pregnancy yield mixed results of child development outcomes. One study found no difference in cognitive scores on the Bayley Scale of Infant and Toddler Development when mothers were administered 0.8 g of DHA per day in the last half of pregnancy.(134) Another trial found that supplementation with 2.2 g of DHA and 1.1 g of EPA per day resulted in higher mean scores of hand-eye coordination of children at 2.5 years of age compared to the non-supplemented women.(91) In our study, the estimated mean of grams of DHA per day in the low exposure supplement group was 0.08 grams per day (range: 0.01 to 0.14 grams per day). This is substantially lower than the DHA exposures in trials described above and lower than the recommended intake from the Nordic Nutrition Recommendations. The 2012 Nordic Nutrition Recommendations for pregnant and lactating women recommend n-3 essential fatty acids should make up about 1% of total energy intake, and that 200 mg/day should be DHA.(79)

Fish oil supplementation during pregnancy has been shown to affect birthweight and length of gestation. A trial that randomized 2.7 g of fish oil or olive oil a day to women found that gestation was about four days longer in women who were treated with 2.7 grams of n-3 PUFAs compared to the olive oil treated group. Birthweight was also about 107 g heavier in the fish oil treated group compared to the olive oil group.(135) Women who received a randomized treatment of 2.4 g of n-3 LCPUFAs per day from the second trimester of pregnancy through delivery had gestations that were on average two days longer compared to the olive oil treated group, and birthweights were 97 grams higher.(66) Dose of DHA may matter, because a trial that treated women with 0.4 g of DHA per day from the eighteenth week of gestation through delivery showed no difference in length of gestation or birthweight compared to women who

were assigned to placebo.(136) It is possible that DHA impacts the risk of CP by changing length of gestation and birthweight, but we did not see differences in mean gestational age or birthweight in the combined cohort, even though risk of CP differs by gestational age within children born at term.(16) We also did not see differences in mean gestational age by DHA supplement group in the MoBa cohort eligible children (data not shown).

Quality of fish oil supplements can vary widely in content of PUFAs, oxidation, and contamination. (137-140) It is possible that the DHA content of supplements acts as a proxy for supplement quality, and supplements with lower concentrations of DHA may also have had higher levels of oxidation or environmental contaminants. We were not able to test this in our sample. Future research on the association between DHA supplementation during pregnancy and CP risk would benefit from including supplement quality measures. An additional important step is to access DNBC data for DHA separated by supplement and food source.

Sensitivity Analyses

Results for the sensitivity analyses can be found in Table Sensitivity a, Table Sensitivity b, and Table Sensitivity c. The analyses for singleton children born at term showed a similar pattern to the term or near-term analyses, though effect sizes were larger for the term only children. The analyses for all singleton children regardless of gestational age yielded similar patterns as the term or near-term children, but the effects were modestly attenuated in comparison. The complete case model for the combined cohorts and the DNBC were similar to the term or near-term analyses, though effects were modestly attenuated. The complete case analysis for the MoBa cohort showed results that were different from the imputed term or near-term models. This is likely due to missingness in the MoBa data. Over 20% of the eligible

MoBa observations were dropped due to missing covariate data, and 22 cases of CP were excluded from the analysis.

Strengths

This study has many strengths. It is one of the first studies to use estimated DHA intake during the second trimester of pregnancy to estimate risk of CP. As noted previously, a case-control study found that increasing fish intake during pregnancy reduced the odds of CP by about 30%, but this study did not focus on DHA intake.(85) Our study combines two large pregnancy cohorts that are enhanced by linkage to national registries such as the Danish National Patient Registry, the Norwegian Patient Register, and the CP registries in both Norway and Denmark. Record linkages ensure children who were diagnosed with CP were not excluded due to differential withdraw from the study due to the outcome. The sample size for our combined cohort analyses exceeded 138,000 pregnancies, and unique identification numbers for mothers allowed us to account for clustering when mothers contributed more than one pregnancy to the sample. Because of the completeness of our outcome data, we used multiple imputation to retain our sample size so that observations with missing covariates were not excluded.

Limitations

Our study has limitations which may impact the results. Due to the rare nature of CP, we had to limit the number of categories in each confounding variable to ensure we did not have sparse or empty categories. This results in rough categorization of variables, such as pre-pregnancy body mass index, which may not completely remove confounding related to a variable. It is also likely that we have unmeasured confounders that may impact the association

between DHA quartile and CP. For example, methyl mercury exposure has been found to confound the relationship between fish intake and child cognition.(141) The unexpected result of the greatest risk of CP occurring for children whose mother's DHA intake fell into the second quartile may be due to unmeasured confounding by supplement quality, methyl mercury exposure, or other potential confounders like seasonality of fish intake or year. Finally, our measure of DHA was derived from FFQs administered in the second trimester of pregnancy. The two cohorts used different FFQs that asked women to recall their diet over different period of times. Differences in the questionnaires and recall period limits the ability to harmonize estimates of specific nutrients. We found that even though patterns of CP risk by cohort-specific DHA quartiles looked similar between the two cohorts, the estimated DHA consumption in grams per day were different between to the two cohorts (see supplement Table 5e), which limits the interpretability of the DHA quartile effects.

Conclusion

This study shows there is an association between DHA intake during pregnancy and subsequent risk of CP in offspring, though the observed pattern of risk differed from the hypothesized pattern of risk. The unexpected findings in this study demonstrate the need for more research on the impact of prenatal nutrition on CP risk. Future research should focus on using biological specimens to estimate exposure to DHA during pregnancy, include measures of DHA supplement quality, and include important confounding variables such as methyl mercury exposure.

Chapter 6: ARA and the Ratio of DHA to ARA

Introduction

DHA and ARA are two of the most important fatty acids for fetal brain development. Their precursors, alpha-linolenic acid and linoleic acid, compete for enzymes that result in their metabolism in to DHA and ARA, respectively, and DHA is preferentially transferred across the placenta.(59, 142) ARA is a precursor to prostaglandins, leukotrienes, and products of lipoxygenase and cyclooxygenase processes, which are essential in inflammatory response.(72) ARA is often conceptualized as being a pro-inflammatory fatty acid while DHA is thought to be anti-inflammatory, though this is an oversimplification of their roles in inflammation induction and resolution.(72, 80) Because of the pro-inflammatory effects of ARA, our second hypothesis posited that for children who were born at or near term and survived to one year of age, the risk of CP would be highest for children whose mothers consumed the highest amounts of ARA during pregnancy. Hypothesis two also posited that the risk of CP would decrease as the ratio of DHA to ARA increased, and that the association between infection during pregnancy would be modified by the ratio of DHA to ARA.

Methods

Children were the unit of analysis, and mothers could contribute more than one pregnancy. Twelve participants reported consuming no ARA in the FFQ, and the result was a missing ratio value. We included these observations in the highest DHA/ARA ratio quartile. Analyses were clustered using unique identification numbers for mothers to ensure appropriate standard errors. To test these hypotheses, we first calculated the prevalence of CP per 1,000 children born at or near term and who survived to age one by ARA quartile and DHA to ARA

ratio quartile clustered by mother. We used Stata 16 (StataCorp, College Station, TX) to calculate the prevalence of CP in the combined cohorts and in each cohort separately. These analyses indicated there were substantially different patterns of CP prevalence by ARA and DHA to ARA ratio in the two cohorts. As a result, we decided to model the two cohorts separately.

We described maternal characteristics by ARA quartile and DHA to ARA ratio quartile for each cohort using frequencies and percentages. We used multiple imputation (imputations = 30) to retain the full sample for analysis, which was described in the methods chapter. We imputed the data by cohort to accommodate differences in data ascertainment for some of the covariates.

We estimated the relative risk of CP by ARA quartile and ratio quartile with separate log-binomial models clustered by mother. We used the first quartile of ARA and the first quartile of the ratio of DHA to ARA as reference groups. Due to the decision to analyze the two cohorts separately, we were unable to test the hypothesis that the ratio of DHA to ARA would modify the association between infection and CP, which would only have been tested if the ratio demonstrated an association with CP risk. We included the following covariates in the final models: previous number of live births, maternal occupation, marital status, alcohol use during pregnancy up to the first interview/ questionnaire, exercise in the first trimester, and pre-pregnancy BMI.

Sensitivity Analysis

To determine the impact of an unmeasured confounder on the results of the log-binomial models, we calculated the e-value. The e-value is an estimate of the degree to which an unmeasured confounder would have to be associated with both an outcome and an

exposure on the relative risk scale in order to remove the association between the exposure and the outcome. Details of the e-value can be found in the methods chapter.

We also generated models for singleton children born at term (i.e. 37 weeks gestation or later), all singleton children regardless of gestational age, and for term or near-term children for whom complete data on covariates were available.

Results

Distributions of Maternal Characteristics

Table 6.1 shows maternal characteristics by ARA quartile in the DNBC. Observed differences in percentages across many of the variables were small. There were notable differences in the following variables: maternal age, alcohol use during pregnancy, and exercise during the first trimester. As ARA quartile increased, the percentage of mothers under the age of 25 decreased from 10.2% to 7.8%. The relative risk of being in the youngest maternal age category for the second quartile compared to the first was 0.90 (95% CI: 0.84, 0.96), and the third and fourth ARA quartiles compared to the first also showed had a lower risk of falling into the youngest maternal age group (relative risk: 0.77 for both third and fourth quartiles. See supplement Table 6a for relative risk estimates and confidence intervals). The percentage of pregnancies in which there was use of alcohol prior to the first interview increased from 41.7% to 46.3% increased as ARA quartile increased, and the highest use of alcohol was in the third quartile of ARA intake. The relative risk of alcohol use in the second quartile of ARA compared to the first quartile was 1.07 (95% CI: 1.04, 1.10), and the relative risk increased when the third quartile of ARA intake was compared to the first (relative risk 1.13 [95% CI: 1.10, 1.16]). The relative risk of alcohol use early in pregnancy for the fourth quartile of ARA intake compared to

the first was 1.11 (95% CI: 1.08, 1.14). The percentage of pregnancies for which the mother reported no physical activity in the first trimester decreased as ARA quartile increased from 63.3% to 60.6%. The relative risks for being physically inactive during pregnancy indicated the risks were not meaningfully different and the relative risks were close to the null value. The percentage of pregnancies for which the mother reported exercising two or more times per week in the first trimester increased from 23.9% to 26.3%, though risk for exercising two or more times per week was only elevated in the fourth quartile compared to the first quartile (relative risk: 1.10 [95% CI: 1.05, 1.14]).

Table 6.2 presents maternal characteristics in the MoBa cohort by ARA quartile. Differences by percent were small for many of the variables. Notable differences in maternal characteristics by ARA quartile included maternal age, previous number of live births, maternal occupation, and exercise in the first trimester. In the MoBa cohort, there were fewer children with mothers who were under 25 years of age in the middle two quartiles of ARA, and more pregnancies for which the mother was 30 years or older in the middle two categories of ARA quartile. The relative risk of being in the lowest maternal age category was lower for the second, third, and fourth quartiles of ARA intake compared to the first quartile, and the third quartile had the lowest risk (see supplement Table 6b for relative risk estimates and confidence intervals). The third quartile of ARA had a larger risk of being in the youngest maternal age group compared to the first quartile (relative risk: 1.22 [95% CI: 1.17, 1.27]). A similar pattern was observed with maternal occupation. There were more employed mothers in the middle two ARA quartiles and fewer students. The relative risks of being employed in the second, third, and fourth quartiles of ARA exposure were small and close to the null, however there was a

notable lower risk of being a student in the second quartile of ARA intake compared to the first (relative risk: 0.81 [95% CI: 0.76, 0.85]), and in the third quartile compared to the first quartile (relative risk: 0.81 [95%CI: 0.77, 0.86]). As ARA quartile increased, the percentage of pregnancies for which the mother reported no previous live births decreased from 45.7% to 40.9%, and the percentage of pregnancies for which there were two or more previous live births increased from 15.8% to 18.3%. The relative risk for having two or more live births increased in a step-wise manner as ARA quartile increased. There were more pregnancies for which the mother reported one previous live birth in the middle quartiles compared to the first and the fourth quartile. Physical activity followed a similar pattern to that in the DNBC. As ARA quartile increased, the percentage of pregnancies in which the mother reported doing no physical activity in the first trimester decreased from 34.1% to 29.8%, while the percentage of pregnancies during which the mother reported exercising two or more times increased from 43.4% to 47.6%. The relative risk of being physically inactive early in pregnancy was 0.93 for the second quartile of ARA intake compared to the first quartile (95% CI: 0.90, 0.95), and 0.87 for the third (95% CI: 0.84, 0.89) and fourth (95% CI: 0.85, 0.90) quartiles. The risk of exercising two or more times per week early in pregnancy was slightly higher in the second, third, and fourth ARA quartiles compared to the first.

Table 6.3 presents maternal characteristics by the quartiles of the ratio of DHA to ARA for children born at or near term and who survived to one year in the DNBC. Six observations were missing a value for the ratio because their estimated ARA intake was zero. These observations were added to the highest ratio quartile. Notable differences were observed for maternal age, number of previous live births, alcohol use prior to the first interview, exercise in

the first trimester, and pre-pregnancy BMI. As the ratio quartile increased, the percentage of mothers over the age of 35 increased from 11.0% to 16.9%, and the percentage of mothers 25 to 29 years of age decreased from 43.3% to 36.7%. The relative risk for being 35 years or older increased in a step-wise fashion as ratio quartile increased, and the relative risk of being 25 to 29 years of age showed a step-wise decrease as the ratio quartile increased (see supplement Table 6c for relative risk estimates and confidence intervals). There were differences in the 24 and younger age group and the 30 to 34 year age group, but the patterns were less defined than the patterns in the lowest and highest age groups. For example, the children of women whose DHA to ARA ratio fell into the third quartile had the lowest risk of being in the youngest age group compared to the first ratio quartile (relative risk 0.68 [95% CI: 0.63, 0.73]). The percentage of pregnancies for which the mother reported no previous live births decreased from 52.9% to 47.6% as the ratio quartile increased, and the percentage of pregnancies for which the mother reported two or more previous live births increased from 13.1% to 15.8%. The relative risks of having no previous live births in the second, third, and fourth ratio quartiles compared to the first quartile were similar, but the risk of having two or more previous live births increased as ratio quartile increased. The percentage of children whose mothers reported using alcohol up to the first interview was highest for the third quartile, and the risk of alcohol intake was elevated for the second, third, and fourth ratio quartiles compared to the first. The percentage of mothers who reported no physical activity during the first trimester decreased from 64.7% to 59.7% as the ratio quartile increased, though the relative risks were close to the null. Pregnancies for which mother reported exercising two or more times a week during the first trimester increased from 23.1% to 27.0% as the ratio quartile increased. The

relative risk of exercising two or more times per week early in pregnancy for the fourth ratio quartile compared to the first was 1.17 (95% CI: 1.12, 1.22), and it increased in a step-wise manner across the quartiles. For BMI, the percentage of pregnancies for which the mother's pre-pregnancy BMI was less than 23 kg/m^2 increased from 49.6% to 55.7% as the ratio quartile increased. The relative risk for having a pre-pregnancy BMI in the lowest category for the fourth quartile of ARA intake versus the first was 1.12 (95% CI: 1.10, 1.15). The percentage of pregnancies with a recorded pre-pregnancy BMI of 25 kg/m^2 or higher decreased from 30.1% to 24.6% as the ratio quartile increased. The relative risks of having a pre-pregnancy BMI in the highest category decreased as ratio quartile increased.

Table 6.4 shows maternal characteristics by quartiles of the ratio of DHA to ARA for children born at or near term and who survived to age one in the MoBa cohort. As with the DNBC ratio variable, six observations were missing due to having a zero value for estimated ARA intake. These observations were added to the highest ratio quartile. Meaningful differences in the percentages were observed for the following variables: maternal age, number of previous live births, smoking during pregnancy up to the first questionnaire, exercise during the first trimester, and maternal pre-pregnancy BMI. The percentage of pregnancies for which the mother was under the age of 25 decreased from 13.7% to 8.4% as the ratio quartile increased while the percentage of pregnancies for which the mother was 35 year of age or older increased from 14.% to 20.4%. The relative risk of being in the youngest age category decreased in a step-wise manner as the ratio quartile increased (see supplement Table 6d for relative risk estimates and confidence intervals). The relative risk of being in the youngest age category for the fourth ratio quartile compared to the first was 0.61 (95% CI: 0.64, 0.72). The

relative risk for having maternal age in the highest category increased in a step-wise fashion as the ratio quartile increased. For the fourth ratio quartile compared the first, the risk of being in the highest age category was 1.36 times higher (95%CI: 1.31, 1.43). As the ratio quartiles increased, the percentage of pregnancies for which the mother had no previous live births increased from 35.7% to 47.4%. The percentages of pregnancies for which there were one or more previous live births decreased as the ratio quartile increased, and the relative risks also increased across ratio quartiles. The relative risk of having no previous live births increased across ratio quartiles. The risk of being unemployed or receiving benefits or a pension was lower for the second, third, and fourth ratio quartiles compared to the first, though the absolute differences in the percentages were small. The lowest risk of being unemployed or receiving benefits or a pension was in the third quartile compared to the first (relative risk: 0.68 [95% CI: 0.64, 0.74]). The percentage of pregnancies during which the mother reported smoking up to the first questionnaire decreased from 26.4% to 18.0% as the ratio quartile increased, and the relative risks decreased across ratio quartile in a step-wise manner. The percentages and risk ratios of pregnancies during which the mother reported no physical activity in the first trimester followed a similar pattern to the smoking pattern. The percentage of pregnancies for which the mother reported exercising two or more times per week during the first trimester increased from 35.7% to 54.1% as the ratio quartile increased. The relative risk for exercising two or more times per week was highest in the fourth quartile compared to the first (relative risk: 1.52 [95% CI: 1.48, 1.55]). As the ratio quartile increased, the percentage of pregnancies that fell into the lowest pre-pregnancy BMI category increased from 41.1% to 52.6%, and the percentage of pregnancies in the highest pre-pregnancy BMI category decreased from 36.8% to

25.5%. The relative risk of being in the lowest BMI category increased across the ratio quartiles. The fourth ratio quartile had the highest risk for being in the lowest BMI category, compared to the lowest ratio quartile (relative risk: 1.28 [95% CI: 1.25, 1.31]). The relative risk having pre-pregnancy BMI in the highest category steadily decreased across ratio quartiles.

Prevalence of CP by ARA and Ratio of DHA to ARA

Figure 6.1 shows the prevalence of cerebral palsy per 1,000 singleton children born at or near term and who survived to one year of age by ARA quartile for the combined cohorts and the DNBC and MoBa cohorts separately. Patterns of CP in the DNBC and MoBa differed substantially. In Denmark, the prevalence of CP decreased as ARA quartile increased, while in MoBa, the prevalence of CP showed a positive association with ARA quartile. This resulted in a flat pattern for the prevalence of CP by ARA quartiles in the combined cohorts. In the DNBC, the prevalence of CP was 1.54 per 1,000 children born at or near term (95% CI: 1.04, 2.39). This decreased to 1.47 in the second quartile (95% CI: 0.98, 2.31), 1.27 in the third quartile (95% CI: 0.82, 2.07), and 0.87 in the fourth quartile (95% CI: 0.51, 1.59). In MoBa, the prevalence of CP in the first quartile was 1.33 (95% CI: 0.91, 2.00), which declined in the second quartile (1.17 [95% CI:0.79, 1.82]), and then sharply rose to 1.64 per 1,000 term or near term children in the third quartile (95% CI: 1.17, 2.36), and continued to rise to 2.09 per 1,000 children in the fourth quartile (95% CI: 1.55, 2.88). In the combined cohorts, the lowest prevalence of CP was 1.30 per 1,000 children in the second quartile and the highest was 1.56 per 1,000 children in the fourth quartile (see supplement Table 6e).

Figure 6.2 presents the prevalence of CP per 1,000 singleton children who were born at or near term and survived to one year by quartile of the DHA to ARA ratio in the combined

cohorts and in the DNBC and MoBa separately. As with ARA, the patterns of CP prevalence by ratio quartile differed between the two cohorts. In the DNBC, as the quartile of the ratio increased, the prevalence of CP increased. The opposite pattern was observed in the MoBa cohort. In the DNBC, the prevalence of CP per 1,000 children was 1.00 in the first quartile (95% CI: 0.62, 1.75), and it decreased slightly in the second quartile (0.87 [95% CI: 0.51, 1.59]), before it climbed to 1.47 in the third quartile (95% CI: 0.98, 2.31), and the fourth quartile (1.80 [95% CI: 1.25, 2.70]). In MoBa, the CP prevalence was 1.63 in the first quartile (95% CI: 1.17, 2.36), which rose to 1.99 in the second quartile (95% CI: 1.47, 2.77), before falling back to 1.63 in the third quartile (95% CI: 1.17, 2.36). The prevalence of CP fell further in the fourth quartile (0.97 [95% CI: 0.63, 1.58]) (see supplement Table 6e).

Models

The frequency of CP, unadjusted and adjusted relative risks for CP by ARA quartile for children born at or near term and who survived to one year in the DNBC are presented in Table 6.5. There was no difference in the unadjusted or adjusted risk between the first and the second quartile. The adjusted risk of CP was reduced by 15% in the third quartile of ARA compared to the first quartile, and the fourth quartile had a reduced risk of 41% compared to the first quartile, though confidence intervals were wide and the estimates were not statistically significant. The e-values suggest that the relationships between an unmeasured confounder, the outcome, and the exposure would have to have relative risks of 1.21 or greater in order to attenuate the effect of ARA exposure in the second quartile, and the relative risks of 2.78 or greater in order to attenuate the association for the fourth quartile. Because the confidence intervals are wide, the e-value limits are the null value, which means the

associations between the unmeasured confounder, exposure, and outcome could be small and still remove the effect.

Table 6.6 shows the frequency of CP, unadjusted and adjusted relative risks of CP by ARA quartile for children born at or near term and who survived to age one in the MoBa cohort. In contrast to the DNBC, the risk of CP increased as ARA quartile increased. The risk of CP was lower in the second quartile compared to the first, though the confidence interval crossed the null. The relative risk of CP for the third quartile compared to the first ARA quartile was about 24% higher, though again the confidence interval crossed the null. Quartile four had an adjusted risk of CP that was 58% higher compared to the first quartile and was marginally statistically significant, which aligned with the original hypothesis. The e-value suggested an unmeasured confounder would have to have to have associations with both the outcome and the fourth ARA quartile that had a relative risk of 2.54 in order to remove the association.

Table 6.7 presents the unadjusted and adjusted CP risk ratios by DHA to ARA quartile for children born at or near term and who survived to one year of age enrolled in the DNBC. As the ratio of DHA to ARA increased, the prevalence of CP increased. Unadjusted and adjusted risks in the second and third quartiles compared to the first quartile were not significantly different, though the third quartile's point estimate indicated there was a 57% increase in risk compared to the first quartile. Unadjusted risk in the fourth quartile was not statistically different from the first quartile, but the adjusted risk ratio did reach statistical significance. Risk of CP was increased by 95% in the fourth ratio quartile compared to the first quartile (adjusted risk ratio: 1.95 [95% CI: 1.04, 3.64]). The e-value suggests that an unmeasured confounder would have to have associations with both CP and the fourth quartile of the ratio that produced risk ratios of

3.31 or higher in order to attenuate the estimated effect. The lower limit of the e-value indicates the relative risk of the unmeasured confounder and CP, and the unmeasured confounder and fourth quartile ratio could be as low as 1.24.

Table 6.8 contains the unadjusted and adjusted CP risk ratios for DHA/ARA ratio for children born at or near term and who survived to age one in the Norwegian Mother and Child Cohort. As the DHA to ARA ratio increased, the risk of CP decreased except from the first to the second quartile. Unadjusted and adjusted risk ratios did not reach statistical significance for the second and third quartiles compared to the first quartile. The e-values suggested a weak unmeasured confounder could account for the differences in the point-estimates. The fourth quartile compared to the first quartile had a marginally significant difference in risk. The risk ratio for CP in the fourth quartile versus the first was 0.59 (95% CI: 0.33, 1.04). The e-value indicated that the relative risks for an unmeasured confounder and CP and the unmeasured confounder and the DHA/ARA ratio in the fourth quartile would have to be 2.84 in order to reduce the relative risk for the fourth ratio quartile to the null, though the wide confidence intervals suggest that even a weak unmeasured confounder could attenuate the estimated risk.

Discussion

We hypothesized that within term or near-term children, the risk of CP would be highest for those whose mothers' ARA intake during pregnancy was in the highest quartile. We hypothesized this because ARA is a precursor to eicosanoids, which are chemical mediators of inflammation, several of which are pro-inflammatory.(143) We also hypothesized that the risk of CP would decrease as the ratio of DHA to ARA increased. The risk of CP by ARA intake and the ratio of DHA to ARA varied between the two cohorts, even though the distribution of

estimated ARA intake in the two cohorts was similar (see supplement table 6f and 6g). In the DNBC, as quartile of ARA increased, the risk of CP decreased. This was opposite of the hypothesized effect. Estimates were not statistically significant, but the third quartile point estimated suggested a 15% decrease in risk compared to the first quartile, and the fourth quartile point-estimate suggested a 41% decrease in risk compared to the first quartile. In the MoBa cohort, as ARA quartile increased, risk of CP also increased, which agreed with our hypothesis. Compared to the first quartile, the relative risk of CP was 24% higher in the third quartile (not statistically significant), and the 58% higher in the fourth quartile (95% CI: 0.94, 2.64).

In the DNBC, the third and fourth quartiles of DHA to ARA ratio were associated with greater risk of CP compared to the first quartile, which was opposite of the expected result. The third quartile had a 57% increase in risk of CP compared to the first quartile (not statistically significant), and the fourth quartile had a 95% increase in risk of CP (95% CI: 1.04, 3.64). In the MoBa cohort, we found that the second quartile had a slightly elevated risk of CP compared to the first (adjusted relative risk: 1.19 [95% CI: 0.74, 1.90]), but there was no difference in risk in the third quartile compared to first. The risk of CP in the fourth quartile of DHA to ARA ratio was 41% lower than in the first quartile in the MoBa cohort, though this result was only marginally statistically significant. It is possible that ARA estimated from FFQs is more indicative of dietary patterns than of actual exposure to ARA *in utero*. There is evidence that dietary patterns for pregnant Danish and Norwegian women differ in terms of ARA supply. A study that used principal component analysis in the DNBC identified red meat as a variable that loaded heavily in one of the components(144), which is a primary source of ARA. A study from the

MoBa cohort which used principal component analysis did not show red meat as a variable that loaded heavily onto a factor.(145)

There is a paucity of epidemiologic research on the effect of dietary intake of ARA during pregnancy on CP risk. Studies in infants born at term demonstrated that the umbilical artery concentrations of ARA were lower in children with abnormal movements compared to children with normal movements.(94) In this study, movements were classified by complexity, variation, and fluency of movement. Children were classified as having abnormal movement if the complexity and variation of their movements was low.(94) There is evidence that infant movements in children born at term are less predictive of CP than in children born pre-term.(146) Follow-up of the children for whom general movements were analyzed did not find associations between ARA concentration and the Bayley Psychomotor Development Index at 18 months of age.(131) In our study, the risk of CP decreased as ARA quartile increased in the DNBC, though the estimates did not reach statistical significance. The finding was opposite for children in the MoBa cohort. While the results from the DNBC seem to concur with the findings from the study of general movement in infants, the MoBa cohort results do not, and it is difficult to translate the results of a study of general movement in infants to risk of developing CP.

Animal models have suggested that ARA is important for motor development, though many of these models supplement with ARA after birth.(86, 87) A model in which compared wild-type mice to delta-6-desaturase knock-out mouse pups that were either in the control group or supplemented with ARA showed that the control knock-out mice had lower body weights than wild type mice, but the ARA supplemented knock-out mice had similar body

weights.(86) Compared to wild-type mice, the quantity and quality of motor activity in the knock-out control mice was lower, and while the ARA supplemented knock-out mice had improved quantity of motor activity, the quality of the movements was diminished.(86) The opposite effect of ARA on CP risk in the two cohorts does not clarify the association between ARA and CP risk and these results indicate that more research in this field is necessary.

There is more epidemiologic evidence that prenatal ARA and DHA are both necessary for optimal neurodevelopment, though research of prenatal intake of the two fatty acids and their effects on CP is still sparse. Studies that have used ratios also tend to use sums of n-6 fatty acids including linoleic acid, ARA, and docosapentaenoic acid, along with sums of n-3 fatty acids including DHA, eicosapentaenoic acid, and alpha-linolenic acid. General movement quality was assessed in infants whose mothers were supplemented with either DHA only or DHA and ARA, and found that the infants whose mothers were supplemented with both had no difference in general movement quality compared to the control group, while the DHA only group demonstrated lower quality general movements.(147) The association between the ratio of n-6 to n-3 PUFAs and child neurodevelopment has been assessed using the Bayley Scale of Infant Development- II (69), the Ages and Stages questionnaire, and Peg Moving Test-5 (PMT-5).(90) The ratio of total n-6 to total n-3 PUFAs was negatively associated with performance on the Bayley Psychomotor Development Index (PDI) in six month old children (beta estimate: -0.2, p-value: 0.04), as was the ratio of linolenic acid to alpha-linoleic acid (beta estimate: -0.2, p-value: 0.04), which are the precursors to ARA and DHA, respectively.(69) Infants whose mothers were in the second, third, or fourth quartile of linoleic acid/alpha-linolenic acid ratio had two to three times the risk of delayed PDI performance compared to the children whose mothers ratio was

in the first quartile.(69) There is little evidence that the Bayley scales can predict motor dysfunction and cerebral palsy in older children,(148) though an analysis from the Neonatal Brain Hemorrhage Study showed that many children diagnosed with disabling CP had Bayley motor scores of less than 50.(149) The Ages and Stages Questionnaire administered at age three years found a negative association with pre-natal total n-6 to total n-3 fatty acid ratio for children who were never breastfed, and this same study also showed a marginally significant positive association between the PMT-5 and total n-6 to total n-3 ratio.(90) These studies suggest that balance between total n-6 and total n-3 PUFA intake during pregnancy, as indicated by the ratio, is important for optimal neurodevelopment, but their results are again difficult to apply to CP. Our study used the ratio of DHA, an n-3 PUFA, to ARA, an n-6 PUFA because we expected these two PUFAs to be the most important for optimal brain development. We also expected the ratio of DHA to ARA to reduce the risk of CP as it increased because of the shared metabolism pathway of DHA and ARA,(71) and the expectation that the anti-inflammatory effects of DHA would balance out the pro-inflammatory effects of ARA.(143)

Post-natal mouse models of neurodevelopment support that both DHA and ARA are necessary for optimal movement quality.(87) An expansion of the mouse models that demonstrated ARA increased movement quantity but not quality in delta-six desaturase knock-out mice showed that supplementation with both ARA and DHA in these mice enhanced motor quality and quantity beyond that of control wild-type mice.(87) The results of the DNBC analysis of DHA to ARA ratio suggest that the risk of CP increases as the ratio of these two increases, while the MoBa cohort analysis indicates that a higher intake of DHA compared to ARA is protective for CP. These analyses fail to clarify the effect the balance of prenatal DHA and ARA

might have on CP risk in term or near-term children, but they do point to a need for more research in this area that incorporates the precursor fatty acids of DHA and ARA.

Sensitivity Analyses

Results for the sensitivity analyses can be found in Table Sensitivity a, Table Sensitivity b, and Table Sensitivity c. In the DNBC, the term analyses for ARA produced similar results as the term or near-term analyses. The MoBa term analysis for ARA showed larger effect sizes for the third and fourth quartile compared than the effect sizes for the same categories in the term or near-term analysis. The ratio analyses for the DNBC and the MoBa cohort for term children were similar to those of the term or near-term analyses. The ARA and ratio analyses for all singleton children regardless of gestational age had smaller effect sizes than the effect sizes for the term or near-term children. In some cases, the direction of risk changed, but the estimates were close to the null and confidence intervals were wide. The complete case analyses yielded similar effect sizes as the term or near-term analyses.

Strengths

This is one of the first studies to try to understand the relationship between prenatal intake of ARA and DHA, and the subsequent risk of CP in children born at or near term. There are few studies of maternal diet and subsequent risk of CP in children.(85) The MOBAND-CP study is the largest prospective pregnancy cohort study of CP in the world. Because CP is a rare event, the size and prospective designs of the DNBC and MoBa cohorts make them valuable assets for CP research. Even though the two cohorts were not combined for these analyses, each cohort had a substantial sample size, which yielded 199 cases of CP for children born at or near term. The DNBC contributed 77 cases of CP, and the MoBa cohort contributed 122 cases.

We used multiple imputation to retain the full sample size in each cohort, which allowed us to detect statistically significant differences when the relative risks were around 1.80 or greater. CP cases were ascertained using the CP registries in each country, both of which used standardized definitions from the SCPE Network registry consortium to identify cases. Because we analyzed the cohorts separately, data harmonization challenges did not affect the unique analyses.

Limitations

There are several limitations to this study. ARA measured via FFQ may not be an accurate representation of actual exposure of a fetus to ARA during pregnancy, and the estimated values of ARA were lower than we anticipated. The precursor to ARA, linoleic acid, is common in Western diets, and it is readily converted to ARA in the human body.(70, 150) Though estimated quartile ranges of ARA were similar between the two cohorts (see Supplement Table 6f), harmonization of the ARA variables for the two cohorts was limited by differences in the period of recall for the two FFQs (*i.e.,* previous month in the DNBC and the previous four to five months in MoBa). Future work may want to combine ARA and linoleic acid measures from the FFQ. We did not analyze the combined cohorts for these analyses because of the differences in the risk of CP by ARA quartile and by ratio quartile. Even with the large sample sizes for each cohort, the number of cases of CP was small, which required us to collapse some of the categories for our control variables including maternal occupation, previous number of live births, and maternal pre-pregnancy body mass index. This may result in residual confounding of our exposure and outcome relationships, though we tried to collapse categories in ways that made sense and retained previously identified associations with CP. For

example, we collapsed the categories of number of previous live births by combining two previous live births and three or more previous live births, and the risk of CP decreases as the number of previous live births decreases.(48) Similar considerations were made for pre-pregnancy BMI.(47) Finally, data harmonization challenges makes comparing the results between the two cohorts challenging, though we used only variables that were considered fully or partially harmonized in our analyses. Incomplete harmonization can result in covariates that fail to completely adjust for confounding.

Conclusion

While this study did not clarify the relationship between ARA and CP, or the association between the ratio of DHA to ARA and CP, it provides a starting point for future research on prenatal exposure to PUFAs during pregnancy and risk of CP. ARA measured through FFQ may be more indicative of dietary patterns than of actual exposure to ARA, and research using FFQs should consider both ARA and its precursor, linoleic acid, when studying prenatal exposure and CP risk. Evidence from the neurodevelopment community suggests that the balance between ARA and DHA is important for optimal neurodevelopment, but there remains important work to be done to better understand the impact of ARA and the ratio of DHA to ARA on CP risk.

Chapter 7: Infection and Effect Modification by DHA

Introduction

Previously, a study of self-reported infections during pregnancy found an association between self-reported fever during pregnancy and CP in the DNBC (51), and hospital-reported genito-urinary tract infections were associated with CP in a Danish registry-based cohort study.(101) DHA exhibits anti-inflammatory properties (72), and is important for the resolution of inflammation.(75) We hypothesized that for children born at or near term, the risk of CP would be higher for those whose mothers reported an infectious event during pregnancy compared to those whose mothers did not. We also hypothesized that the association between infection and CP would be modified by DHA status. Specifically, within each quartile, the children of mothers who reported an infectious event during pregnancy would have higher risk of CP than the children of mothers who did not report an infectious event, but the relative risk would narrow as DHA quartile increased.

Methods

We defined infection as the presence of self-reported urinary tract infection or fever up to the second interview or questionnaire. The second interview and questionnaire were administered around the 30th gestational week. We excluded the third interview/questionnaire for these exposures because there was substantial missingness in the UTI and fever variables due to non-response in the third interview/questionnaire. The third data collection point was six months post-birth. The percentage of MOBAND-CP participants that missed participating in the third interview or questionnaire and who were eligible for the nutrition and infection analyses was 13.5%.

The unit of analysis was children, and we accounted for mothers who contributed multiple pregnancies by using maternal identification number to cluster the analyses. To test our hypotheses, we calculated the prevalence of CP per 1,000 children who survived to one year of age by infection status, reported fever and UTI in interviews conducted prior to 28 weeks of gestation. We used Stata 16 (StataCorp, College Station, TX) STRATE to calculate the prevalence of CP for the combined cohorts and in each separate cohort. We described and tested, using percentages and relative risks clustered by mother, differences in maternal characteristics by infection status, UTI status, and fever up to 28 weeks gestation. We calculated the relative risk for each category of the covariates by infection, fever, and UTI status.

To facilitate interpretation of the effect modification analyses, we included covariates that were used in the fatty acid analyses in our models. We used multiple imputation (imputations = 30) to retain the full sample for analysis. The methods chapter includes details of the imputation model. We imputed the data by cohort to accommodate differences in ascertainment for some of the covariates.

We used log-binomial models clustered by mother to estimate relative risk of CP. We generated separate models for infection, UTI, fever, and the interaction between the three infection variables and DHA in the combined cohorts. We included the following variables as covariates: previous number of live births, maternal occupation, marital status, alcohol use during pregnancy up to the first interview/ questionnaire, exercise in the first part of pregnancy, and pre-pregnancy BMI. The effect modification models also included quartiles of total energy intake. Because of the conflicting results by cohort for the ARA and ratio of DHA to

ARA analyses, we did not test the hypothesis that the ratio of DHA to ARA would modify the association between prenatal infection and CP risk.

Sensitivity Analysis

To estimate the association a single unmeasured confounder would have to have with both CP and the infection exposure in order to reduce the effect estimate to the null, we calculated the e-value for the models of the main effect.(125) See the methods chapters for details on the e-value calculation. We also modeled the risk of CP in singleton children born at term, singleton children regardless of gestational age, and children in the term or near-term sample who had complete data for covariates.

Post Hoc Effect Modification Analysis

We separated out DHA from food sources and from supplement sources and generated source-specific effect modification models. Details on the categorization of food and supplement DHA exposure can be found in Chapter 5 under the results of the *post hoc* analysis.

Results

Description of Infection

Table 7.1 shows the prevalence of infection along with maternal characteristics for children born at or near term and who survived to one year of age in the combined cohorts. About 30% of the pregnancies had a self-reported infection. The missing category for infection included only DNBC observations, as the two variables used to generate the infection variable were complete for the MoBa cohort. Notable differences were observed for number of

previous live births, maternal occupation, alcohol use during pregnancy, smoking during pregnancy, exercise in the first part of pregnancy, and pre-pregnancy body mass index.

For children for whom the mother reported an infection during pregnancy, there was a higher percentage that had one previous live birth compared to the children for whom no infection was reported (38.0% versus 35.2%). However, the relative risks for one previous live birth and two or more previous live births were close to null when the infection group was compared to the group that did not report prenatal infection (see supplement Table 7a for the relative risk estimates and confidence intervals). The missing category had a higher percentage of women that had no previous live births compared to the category with no infection reported (52.7% versus 45.7%). When the missing group was compared to the no-infection group, the relative risk for having one previous live birth was 1.15 (95% CI: 1.08, 1.23), and the relative risk for having two or more previous live births was 0.88 (95% CI: 0.79, 0.97).

About 78% of the infection group reported being employed during pregnancy, versus 80% in the group that reported no infection, while 74% of the missing group reported being employed. The relative risk for being employed during pregnancy for the infection group compared to the no infection group was 0.97 (95% CI: 0.97, 0.98), while the relative risk of being employed in the group missing infection data compared to the no infection group was 0.93 (95% CI: 0.89, 0.96). There was a higher risk of being a student for the group that reported infections during pregnancy compared to the group that did not report an infection (relative risk: 1.14 [95% CI: 1.10, 1.17]). There was also a higher risk of being a student in the group that was missing infection data compared to the group that did not report having an infection (relative risk: 1.50 [95% CI: 1.29, 1.76]).

More women reported using alcohol early in pregnancy in the infection and the missing groups compared to the no infection group (infection: 23.9%, missing: 45.0%, no infection: 18.4%). The risk of alcohol use early in pregnancy was 1.30 times higher in the group that reported a prenatal infection compared to the group that did not (95% CI: 1.27, 1.33) and was 2.45 times higher in the group that was missing pregnancy infection data (95% CI: 2.27, 2.65). There was a higher proportion of women who reported smoking in the first part of pregnancy in the infection group and the missing group compared to the no infection group (infection: 24.1%, missing: 26.1%, no infection: 21.5%). Compared to the group that did not report an infection during pregnancy, the infection group had a risk of smoking early in pregnancy that was 1.12 fold higher (95% CI: 1.11, 1.13), and the risk for the group that was missing infection data was 1.22 fold higher (95% CI: 1.08, 1.37).

The infection and the missing groups had higher proportions of women who reported no physical activity in the first part of pregnancy compared to the no infection group, and lower percentages of women who reported exercising two more times per week. For the group that reported an infection during pregnancy, there was an elevated risk of being physically inactive in the first part of pregnancy compared to the group that reported no infections (relative risk: 1.12 [95% CI: 1.11, 1.13]), while the risk of being physically inactive for the group that was missing infection data was 50% higher compared to the no infection group (relative risk: 1.47 [95% CI: 1.40, 1.55]). The risk of exercising once a week and two times a week or more was also lower for the group that was missing infection data compared to the no infection group. The risks for the pre-pregnancy BMI categories were similar between the infection and the no infection groups, but the group missing infection data was more likely to have a pre-pregnancy

BMI in the lowest category compared to the no infection group (relative risk: 1.13 [95% CI: 1.06, 1.20]).

Description of Urinary Tract Infection

Table 7.2 gives the prevalence of UTI along with maternal characteristics for children born at or near term and who survived to one year of age in the combined cohorts. About 10% of the pregnancies had a self-reported UTI. The UTI data in the MoBa cohort was complete, and the missing group for the UTI variable included DNBC observations only. Many of the differences by UTI status were important. There was a higher percentage of pregnancies in the UTI group for which the mother was 24 years of age or younger compared to the no UTI group (13.0% versus 9.2%). The group that reported a UTI during pregnancy had a risk of being in the youngest age group 1.41 times higher than the group that did not report a UTI (95% CI: 1.34, 1.47) (see supplement Table 7b for relative risk estimates).

The UTI group had the highest percentage of pregnancies for which there were no previous live births, and the no UTI group had the lowest percentage (50.4% and 44.6% respectively). The risk for having no previous live births was 1.13 times higher in the UTI group compared to the group that did not report a UTI (95% CI: 1.11, 1.15). The group that was missing data on UTI status during pregnancy also had an elevated risk of having no previous live births (relative risk: 1.12 [95%CI: 1.05, 1.21]).

The percentage of pregnancies during which the mother was employed was lower in the UTI group and the missing group compared to the no UTI group (UTI: 75.9%, missing: 71.6%, no UTI: 79.8%). There were higher percentages of pregnancies for which the mother reported being unemployed, receiving benefits, or being a student for the UTI and the missing groups

compared to the no UTI group. There were elevated risks of being unemployed or receiving benefits or a pension, and for being a student in the group that reported a UTI and the group that was missing UTI data compared to the group that did not report a UTI.

There was a higher percentage of unplanned pregnancies for the UTI group compared to the no UTI group (UTI: 17.2% versus 14.5%), and the relative risk was about 20% higher for the group that reported a UTI during pregnancy compared to the group that did not. Alcohol use up to the first interview/questionnaire was most common for the pregnancies missing data about UTI (45.3%). This difference in the missing observations' alcohol use is due to the missing UTI observations coming only from the DNBC, and there was a substantial difference in reported alcohol use up to the first interview/ questionnaire between the DNBC and the MoBa cohort (see supplement Table Sample b). The prevalence of smoking up to the first interview/questionnaire was higher in the UTI group and the missing group than the no UTI group (UTI: 25.5%, missing: 27.0%, no UTI: 21.9%). The relative risk of smoking early in pregnancy was 1.16 times higher in the group that reported a UTI during pregnancy compared to the no UTI group (95% CI: 1.13, 1.20), and was 1.24 times higher in the group that was missing UTI data (95% CI: 1.10, 1.39).

The group that reported a UTI and the group that did not report a UTI during pregnancy were similar across the categories of exercise and pre-pregnancy BMI. The group that was missing data on UTI during pregnancy was more likely to have been physically inactive during the first part of pregnancy compared to the no UTI group (relative risk: 1.45 [95% CI: 1.37, 1.53]). The group missing UTI data was also less likely to report having done any exercise in the first part of pregnancy compared to the group that did not report a UTI during pregnancy.

Description of Fever

Table 7.3 shows the prevalence of fever along with maternal characteristics by fever status in the term or near-term sample of the combined cohorts (relative risk estimates are located in supplement Table 7c). About 20% of the pregnancies had a self-reported fever. The MoBa cohort had complete data for the fever variable, and the missing category for fever was comprised of observations from the DNBC. Important differences were noted for the following variables: previous number of live births, alcohol use during the first part of pregnancy, and exercise in the first part of pregnancy.

The group that reported a fever during pregnancy had fewer pregnancies with no previous live births (41.4% versus 46.3%) and had more pregnancies preceded by one live birth compared to the no fever group (40.5% versus 34.7%). The risk of no previous live births was lower in the group that reported fever during pregnancy (relative risk: 0.89 [95% CI: 0.88, 0.91]), and the risk of one previous live birth was 1.17 times higher compared to the group that did not report a fever during pregnancy (95% CI: 1.15, 1.18).

There was a higher percentage of women in the fever group that reported using alcohol in the first part of pregnancy compared to the no fever group (25.7% versus 18.5%). Women who reported a fever during pregnancy were 1.39 times as likely to have used alcohol in the first part of pregnancy compared to women who did not report a fever (95% CI: 1.35, 1.42). The high percentage of women in the missing category that reported using alcohol up to the first interview/questionnaire reflected differences in the cohorts' timing of data collection and the alcohol questions asked in each cohort. More of the women in the fever group reported no physical activity in the first part of pregnancy (49.0%), while 43.3% of the no fever group

reported no physical activity. The fever group was 1.13 times a likely to have reported no physical activity early in pregnancy compared to the fever group (95% CI: 1.12, 1.15), and was less likely to report exercising two or more times a week (relative risk: 0.89 [95% CI: 0.88, 0.91]). The group that was missing data for fever during pregnancy was 1.39 times more likely to be physically inactive early in pregnancy than the group that did not report fever (95% CI: 1.27, 1.53), and was less likely to exercise two or more times per week (relative risk: 0.66 [95% CI: 0.55, 0.81]).

CP Prevalence and Infection Models

Figure 7.1 shows the prevalence of CP for the combined cohorts and the DNBC and the MoBa cohort by infection status. In the combined cohort, the prevalence of CP for children whose mothers did not report any type of infection was 1.39 per 1,000 children who survived to age one (95% CI: 1.18, 1.66), while the prevalence in the group that did report some type of infection was 1.56 (95% CI: 1.22, 2.01). The unadjusted relative risk of CP for the children who were exposed to infection *in utero* compared to those who were not was 1.12 (95% CI: 0.83, 1.51). The adjusted relative risk for CP for children exposed to infection compared to those who were not was 1.13 (95% CI: 0.84, 1.53) (Table 7.4).

In the DNBC, the group that reported no infection had a prevalence of 1.25 per 1,000 children (95% CI: 0.95, 1.69), while the group that reported infection was 1.35 (95% CI: 0.95, 1.99). The unadjusted relative risk of CP in the DNBC was 1.08 (95% CI: 0.68, 1.71), and the adjusted relative risk was 1.06 (95% CI: 0.67, 1.70).

In the MoBa cohort the prevalence of CP for children who were not exposed to infection *in utero* was 1.48 per 1,000 (95% CI: 1.21, 1.84), and the children who were exposed had a

prevalence of 1.79 (95% CI: 1.29, 2.55) (see supplement Table 7d). The unadjusted relative risk of CP for children who were exposed to infection versus children who were not was 1.21 (95% CI: 0.81, 1.79), and the adjusted relative risk was 1.21 (95% CI: 0.81, 1.80).

Figure 7.2 shows the prevalence of CP for children born at or near term who survived to age one by prenatal UTI status. Overall, the prevalence of CP was higher for the children whose mothers reported a UTI during pregnancy compared to the children who were not exposed. In the combined cohorts, the prevalence of CP in the unexposed children was 1.41 per 1,000 (95% CI: 1.22, 1.65), while the prevalence in the exposed was 1.60 per 1,000 (95% CI: 1.07, 2.51). Children who were exposed to UTI during pregnancy had a slightly elevated risk of CP compared to those who did not, though the confidence interval included the null (unadjusted relative risk: 1.13 [95% CI: 0.73, 1.76]). The adjusted relative risk for CP was 1.11 (95% CI: 0.71, 1.73) (Table 7.4).

The DNBC prevalence of CP for unexposed children was 1.28 per 1,000 (95% CI: 1.01, 1.63) and the prevalence in the exposed was 1.23 per 1,000 (95% CI: 0.63, 2.75). The unadjusted relative risk of CP for children who were exposed compared to children who were not was 0.96 (95% CI: 0.46, 1.99), and the adjusted relative risk was null also (relative risk: 0.93 [95% CI: 0.44, 1.95]).

In the MoBa cohort, the prevalence in the unexposed was 1.52 per 1,000 (95% CI: 1.26, 1.84), and the prevalence in the unexposed was 1.94 (95% CI: 1.17, 3.47) (see supplement Table 7d). The unadjusted relative risk for CP for the MoBa cohort children whose mothers reported a UTI during pregnancy compared to children whose mothers did not report a UTI was 1.28 (0.73, 2.23), and the adjusted relative risk was 1.25 (95% CI: 0.72, 2.19).

Figure 7.3 shows the prevalence of CP for children born at or near term and who survived to age one by maternal self-reported fever prior to 28 weeks gestation. In the combined cohort, there was a small difference between the unexposed and the exposed. The unexposed group had a prevalence of 1.40 cases per 1,000 children (95% CI: 1.20, 1.65), while the exposed group had a prevalence of 1.59 (95% CI:1.21, 2.13). The unadjusted relative risk for CP for children exposed to fever during pregnancy was 1.13 (95% CI: 0.82, 1.57), and the adjusted relative risk was 1.16 (95% CI: 0.84, 1.61) (Table 7.4).

In the DNBC, there was a difference in prevalence between the exposed and the unexposed. In the unexposed, the prevalence of CP was 1.18 cases per 1,000 children (95% CI: 1.01, 1.63), while in the exposed the prevalence was 1.58 (95% CI: 0.69, 2.75). The unadjusted relative risk of CP for children exposed to fever *in utero* compared to those who were not was 1.35 (95% CI: 0.84, 2.15). The adjusted effect size was modestly attenuated (relative risk: 1.34 [95% CI: 0.84, 2.15]).

The difference in prevalence between the exposed and unexposed was much smaller in the MoBa cohort compared to the DNBC. For the unexposed in the MoBa cohort, the prevalence was 1.55 cases of CP per 1,000 children (95% CI: 1.28, 1.89), and the prevalence in the exposed was 1.59 (95% CI: 1.05, 2.53) (see supplement Table 7d). The unadjusted and adjusted relative risk of CP for children exposed to fever during pregnancy compared to those who were not were close to the null value (unadjusted relative risk: 1.03 [95% CI: 0.64, 1.65], adjusted relative risk: 1.04 [95% CI: 0.65, 1.66]).

E-values for the adjusted relative risk estimates in the combined cohorts indicated that a single unmeasured confounder that increased the risk of CP and increased the risk of infection, UTI, or fever by about 50% would be able to attenuate the infection effect sizes to the null.

Effect Modification Models

Tables 7.5, 7.6, and 7.7 show the results from the analysis of effect modification by DHA quartile on the association between CP and infection, UTI, and fever, respectively, in the combined cohorts. There were no substantial differences in percentages of infection, UTI, and fever by DHA quartile (Table 7.8). Risk of CP by infection varied by DHA quartile, indicating there is effect modification of the relationship between infection and CP by DHA status (Table 7.5). Within the first quartile of DHA, children whose mothers reported an infection during pregnancy had lower risk of CP than those whose mothers did not (adjusted relative risk: 0.59 [95% CI: 0.27, 1.49]). For children in the second, third, and fourth quartiles of DHA exposure, and the risk by infection status was highest in the third quartile of DHA exposure. The risk of CP was higher for those whose mothers who reported infection compared to those whose mothers did not, however only the relative risk for the third quartile was significant (adjusted relative risk: 1.92 [95%CI: 1.00, 3.71]).

The risk of CP by UTI status varied by quartile of DHA exposure during pregnancy (Table 7.6). Within the first quartile of DHA exposure, the children of women who reported a UTI during pregnancy had a lower risk of CP compared the children whose mothers did not report a UTI though the confidence interval was wide and included the null (adjusted relative risk: 0.43 [95% CI: 0.10, 1.79]). The risk of CP in the second and third quartiles was higher for children whose mothers reported a UTI during pregnancy compared to the children whose mothers did not. The highest relative risk for CP by UTI status was for children in the third DHA quartile. The relative risk of CP in the third quartile of DHA exposure was 2.61-fold higher for the children whose mothers reported UTI compared to those who did not (95% CI: 1.18, 5.73). The relative

risk for CP in the fourth quartile was lower for children whose mothers reported an UTI compared to those whose mothers did not, but the confidence interval was wide and crossed the null.

The risk of CP by fever varied across quartiles of DHA status, though confidence intervals included the null (Table 7.7). For children in the first quartile of DHA exposure, the risk of CP was lower for children whose mothers reported fever compared to the children whose mothers did not (adjusted relative risk: 0.65 [95% CI:0.28, 1.55]). The risk of CP for children in the second, third, and fourth quartiles was higher for children exposed to fever compared to those who were not exposed, though effects were not statistically significant. The highest relative risk estimate was in the third quartile (adjusted relative risk: 1.78 [95% CI: 0.89, 3.55]).

Post Hoc Analysis of Effect Modification

Because only MoBa children were included in the *post hoc* analysis, there was insufficient power for an interaction analysis to achieve statistical significance. There were trends that indicated supplements containing DHA may drive the effect modification analysis results. Table 7.9 presents the results of the effect modification analysis of DHA from food on the three infection variables. The risk of CP for children exposed to infection, UTI, and fever varied by quartile of DHA from food, though there was no clear pattern and confidence intervals included the null. The UTI analysis showed the most variation across quartiles of DHA intake, and the variation was less pronounced for fever.

Table 7.10 shows the results of the effect modification analysis of DHA from supplements on the three infection variables. There was not much difference in risk of CP for children whose mother reported an infection during pregnancy across categories of DHA from

supplements. The results for the UTI analysis were most similar to our main analyses. In the moderate category DHA from supplement use, children who were exposed to a UTI had 2.3 times the risk of CP compared to children who were not exposed (95% CI: 0.89, 5.14), which reflected the results of our main analyses. For children in the high category of DHA exposure via supplements, children exposed to fever during pregnancy had about 1.5 times the risk of CP compared to children who were unexposed.

Discussion

We hypothesized that infection during pregnancy would increase the risk of CP for children born at or near term in the combined cohorts. We defined infection as presence of either self-reported urinary tract infection or fever reported up to the third interview/questionnaire. We also hypothesized that DHA intake during pregnancy would modify the association between infection, UTI, fever and CP. The results for infection, UTI, and fever were null, with small effect sizes that could be attenuated by weak unmeasured confounding, or even residual confounding that was the result of crude categorization of covariates. The analyses of effect modification indicated that the risk of CP for children whose mothers reported an infectious event during pregnancy compared to those whose mothers did not report an infectious event varied by quartile of DHA exposure. For all the infection exposures, we expected the relative risk to narrow as the quartile of DHA increased, but we found that the risk widened from the first quartile to the third quartile, and then narrowed in the fourth quartile. The highest relative risks of CP by infection, UTI, and fever status was in the third quartile of prenatal DHA exposure.

Infection during pregnancy is an established risk factor for CP. A study from the DNBC demonstrated that the children whose mothers had vaginal infections or fever during pregnancy had a higher hazard ratio for developing CP compared to the children whose mothers did not.(51) We did not have a vaginal infection variable harmonized at the time of analysis, but a fever variable was available. In the DNBC study, the hazard ratio for children whose mothers self-reported vaginal infections compared to those who whose mothers did not was 1.52 (95% CI: 1.04, 2.24), and the hazard ratio for fever was 1.53 (95% CI: 1.06, 2.21). The study did not find an association between CP and pyelonephritis or cystitis.(51) This study provides an interesting comparison to our study, as it was conducted in the DNBC and used self-reported infection variables. Both studies found null associations between self-reported UTI indicators and CP, though the effect in the DNBC study showed a modestly protective effect (adjusted hazard ratio: 0.74 (0.40, 1.38), while we found a modestly increased risk (adjusted relative risk: 1.11 [95% CI: 0.71, 1.73]). The DNBC study found a 50% increased risk of CP for children whose mothers reported a fever during pregnancy, while we found a small, non-significant increase in risk in the combined cohorts of about 13%. Our relative risk estimate for the children in the DNBC indicated that for children whose mothers reported a fever during pregnancy, the risk of CP was about 35% higher compared to the children whose mothers did not. The MoBa children's risk of CP after exposure to fever was null, and this attenuated the effect estimate of the combined cohorts toward the null. Other important differences in the studies include the fact the authors of the DNBC study used hazard ratios which require time-to-event, while we used log-binomial models. Time to event in the case of CP indicates time to diagnosis and not the event of brain damage. There are likely differences in the definition of

fever between the two studies due to harmonization process for variables included in MOBAND-CP data. The fever and UTI variables were only partially harmonized in the MOBAND-CP data, which likely contributes to the difference in the effects the two studies found. The MoBa fever data was more refined than the DNBC fever data. In order to harmonize the data, the MoBa fever data was made into a single, binary variable, which likely resulted in misclassification. The DNBC study did not specify the number of interviews used to calculate infection status, which makes it difficult to compare the exposures.(51)

Other observational studies have established that UTI is a risk factor for CP. A retrospective study that used the Danish National Birth Registry and the Danish National Hospital Record found that the children whose mothers had a genitourinary tract infection during pregnancy had an increased risk of CP compared to those whose mothers did not (hazard ratio: 1.9 [95% CI: 1.4, 3.3]).(101) Within mothers of children born at term, genitourinary tract infections identified at hospitalization were associated with a two-fold higher odds of CP compared to the children of mothers who had no infections at delivery.(43) Genitourinary tract infections identified at hospitalization for delivery for mothers of children born at term were associated with a two-fold higher odds of CP compared to the children of mothers who had no infections at delivery.(43) A Swedish case-control study found that bacterial growth in urine increased the odds of CP for children by two-fold (odds ratio: 2.1 [95% CI: 1.1, 4.1]), and any infection during pregnancy increased the odds of CP by nearly three-fold (odds ratio: 2.9 [95% CI: 1.0, 3.8]). The literature on the interaction between pregnancy intake of PUFAs like DHA and infection is sparse. In our study we found null associations between

infection, UTI, and fever and CP. We did, however, find that prenatal DHA intake modified the effect of infection and UTI on risk of CP.

There is little literature that studies the effect modification of prenatal DHA on the association between infection and CP, and this is an area of CP research that deserves more attention as both DHA and ARA are important for the resolution of inflammation.(72, 80) Most of the results for the effect modification analyses were not statistically significant, which was anticipated, however effect estimates suggest that prenatal DHA exposure modifies the association between infection, UTI, and fever. In all three analyses, the pattern of risk we noted across the DHA quartiles was different from what we hypothesized, and additional exploration of why the for children in the first quartile of prenatal DHA exposure who experienced an infectious event during pregnancy had a lower risk of CP compared to the children in the same quartile who did not experience an infectious event is needed. It is also interesting that the third quartile of DHA had the largest relative risks of CP by infection status, when we expected the relative risk to narrow as DHA quartile increased. Our *post hoc* analysis of DHA exposure via supplement use indicated that for UTI, children in the moderate category who were exposed to a UTI during pregnancy had 2 times the risk of CP compared to children in the third DHA quartile who were unexposed.

In the fourth quartile of DHA exposure, the risk of CP following report of a prenatal infection narrowed as we expected. Children exposed to UTI in the fourth quartile of DHA exposure had lower risk of CP compared to children who were not exposed to UTI in the fourth quartile of DHA exposure. Our *post hoc* analysis showed that from the third to the fourth quartile of DHA exposure from food, the risk of CP narrowed. Contrary to our general analysis,

the effect estimate showed a 50% increase in risk for CP for children exposed to UTI compared to children who were not. The supplement analysis had similar results to our total DHA effect modification analysis, and for children in the high category of DHA exposure, the risk of CP was lower for those exposed to UTI compared to children who were unexposed. Women who are high supplement users may be different from the general population, and the reduced risk is likely the result of residual confounding for healthy lifestyle. An important next step will be to access estimated DHA intake from food and supplements in the DNBC to expand the analysis and determine if the results change with the inclusion the second cohort.

Sensitivity Analyses

We generated models for singleton children born at 37 weeks gestation or later, singleton children regardless of gestational age, and for children with complete data for all covariates. We also generated models for UTI up to interview/questionnaire one, and for UTI from interview/questionnaire one to interview/questionnaire two. Results for the sensitivity analyses can be found in the supplement tables: Table Sensitivity a, Table Sensitivity b, Table Sensitivity c, Table Sensitivity d, and Table Sensitivity e.

For the infection and fever analyses, results for the 37-week gestational age analysis were similar to our main analyses. For children born at 37 weeks or later, the risk of CP by self-reported UTI was null. The results of the effect modification analyses in children born at 37 weeks gestation or later were similar to those of the term or near-term sample.

The infection and fever models for all singleton children produced null results. The UTI model and the effect modification models yielded similar results to the term or near-term models.

The complete case analyses infection and fever produced relative risk estimates that were null as well. The UTI models and effect modification models reflected the term or near-term models.

UTI up to the first interview/questionnaire were not statistically significant, but the effect estimates were larger than the estimates for UTI at any time through the second interview/questionnaire (adjusted relative risk: 1.43 [95% CI: 0.86, 2.40]). The effect modification analysis for UTI at first interview/questionnaire followed a similar pattern as UTI at any point through interview/questionnaire two.

The analysis for UTI between the first and second interview/questionnaire showed a modestly elevated risk of CP for children who were exposed versus not exposed (adjusted relative risk: 1.21 [95% CI: 0.20, 2.06]), though it was not statistically significant. The effect modification analysis yielded similar results to the models of UTI at any point up to the second interview/questionnaire.

Future research should explore the effect modification by studying exposure to environmental chemicals associated with DHA exposure including methyl mercury. Prenatal methyl mercury exposure has been shown to confound the relationships between prenatal fish intake and cognition in children (141), and recall in children.(151) Accounting for environmental contaminants like methyl mercury that have been shown to disrupt fetal brain development may help to clarify the associations found in this study.

Strengths

This study used the combined data of two of the largest prospective pregnancy cohorts in the world to assess the risk of CP for children born at or near term by pregnancy infection

status. It is one of the first studies to assess PUFA status during pregnancy's impact on the association between infections during pregnancy and CP. This study included 199 children with CP, 138,251 pregnancies, and 124,760 mothers. While larger prospective cohorts can be organized through linkages of medical records and other population registries, these studies may miss minor infections that did not result in a doctor's visit and they cannot assess dietary exposures in pregnancy. The MOBAND-CP study is the largest CP study to date that makes prenatal dietary and self-reported infection measures available. CP cases were identified using the CP registries in each country, both of which used standardized definitions from the SCPE Network registry consortium. We used multiple imputation address missingness in infection variables and our control covariates. There was little missingness in our infection, urinary tract infection, and fever variables, but there was substantially more missing data in the covariates used for model adjustment. Multiple imputation allowed us to retain the 20% of the observations that would have been dropped in a complete case analysis.

Limitations

There are several limitations to this study. First, harmonization of all infection variables in the two cohorts was not possible due to differences in data collection. As a result, there were several infection variables, such as respiratory infection, that we could not include, and harmonization for prenatal fever and UTI was only partial. Harmonization of the two FFQs micronutrient data was partial because the two cohorts used different FFQs that asked women to recall their diet over different period of times. Differences in the focus of the questionnaires and recall period limits the ability to harmonize estimates of specific nutrients. Additionally, harmonization was partial for many of the covariates, which may result in incomplete

adjustment for confounding. For example, alcohol use during the first part of pregnancy was partially harmonized, and the underlying distributions of alcohol use up to the first interview in the DNBC and up to the first questionnaire in the MoBa cohort were very different. However, these variables have been used in another MOBAND-CP research study.(108) . Even with the large sample size of the combined cohorts, the small number of CP cases required us to collapse some of the categories for our control variables which may result in residual confounding. Collapsed categories included maternal occupation, previous number of live births, and maternal pre-pregnancy body mass index. We collapsed categories of covariates in ways consistent with previous findings on CP risk. For example, we collapsed the categories of number of previous live births by combining two previous live births and three or more previous live births. The risk of CP decreases as the number of previous live births increases.(48) Similar considerations were made for pre-pregnancy BMI.(47) Due to missing data from non-response to the third interview/questionnaire, we could not include UTI or fever in the last part of pregnancy which may be an important period of risk for CP. Importantly, our measures of infection relied on maternal self-report, which increases the likelihood of misclassification. As the data were prospectively collected, we have no reason to believe this misclassification would be biased, but misclassification error can attenuate effect sizes.

Conclusions

In our study, the hypothesis that infection during pregnancy would increase risk of CP was not supported. We did find evidence to support the hypothesis that DHA would modify the association between infection during pregnancy and CP, though the hypothesized pattern of narrowing relative risk of CP by infection status across DHA quartile was not observed. This study serves a first effort to understand the association between prenatal DHA intake, infection, UTI, fever, and CP, and will hopefully encourage further research in this area.

Chapter 8: Conclusions and Future Directions

Prevention of CP during pregnancy is an especially important and understudied topic. Prenatal nutritional, such as PUFA intake, is one field of study that may yield important prevention methods, such as the use of prenatal iodine for the prevention of endemic cretinism, which shares characteristics with CP.(152) Fetal brain development depends on the availability of PUFAs like DHA and ARA, but we do not yet understand if maternal intake of PUFAs during pregnancy impacts risk of CP directly, if it is a modifier of other risk factors like infections, or if it is an artifact of other pregnancy exposures and behaviors.

Our analyses indicated that the associations between our selected PUFAs and CP did not align with what we expected. For example, the risk of CP in both cohorts was highest in the second quartile of DHA consumption, even though the estimated ranges of DHA by quartile differed by cohort. Differences in the focus of the FFQs used in each cohort may have contributed to the different ranges (109, 117), but the use of quartiles to categorize exposure allowed the two sets of data to be used together. Misclassification of PUFA status may result from estimation via FFQ, and the differences in the length of recall for the two cohorts' FFQs makes full harmonization of the dietary data difficult. Residual confounding from partially harmonized covariates like alcohol use early in pregnancy may also contribute to the unexpected findings. Future research should address why the second quartile of DHA consumption seems to carry a high burden of risk for CP. Subgroup analysis of children with unexplained CP, such as children with CP who did not have a low Apgar score five minutes post birth, may also provide more insight into the impact of DHA on risk of CP. The *post hoc* analysis of DHA food and DHA supplement use indicated that at supplements that supply low levels of

DHA may contribute to the bump in risk in the second total DHA quartile. Using biological specimens collected during pregnancy or at delivery, such as cord blood, to measure PUFA exposure may provide insight into the relationship between DHA and CP. Along these lines, our *post hoc* analysis of DHA from food and from supplement use needs to be refined and expanded to determine if DHA from supplements does carry risk as our analyses suggested.

ARA exposure had different CP risk patterns in the two cohorts making it difficult to use the combined cohort data even though the ranges of ARA within each quartile were similar in the two cohorts. In the DNBC, the risk of CP decreased as ARA quartile increased, which was opposite the anticipated effect, while in the MoBa cohort, the risk of CP increased as ARA quartile increased, which was the pattern we expected. As with ARA, the ratio of DHA to ARA had opposite effects on risk of CP in the DNBC and the MoBa cohort. In the DNBC, as the ratio quartile increased, the risk of CP increased, while in MoBa, as the ratio quartile increased, the risk of CP decreased. We expected the risk of CP to decrease as ratio of DHA to ARA increased. Different approaches to measuring n-6 PUFAs is an important next-step in better understanding their relationship with CP. Though conversion of linoleic acid into ARA is inefficient in humans(84), using the ARA pre-cursor fatty acid, linoleic acid, measured in FFQs may be an informative addition for understanding the effect of n-6 PUFA exposure on risk of CP.

We found evidence to support effect modification of the relationship between CP and infection by DHA in this study, but the question is by no means settled. We included fewer measures of infection than we had originally intended due to data harmonization challenges and the rareness of infection indicators like clinical chorioamnionitis. Partial harmonization of the infection variables we used resulted in measures of infection that were more crude than

the measures available in the individual cohorts. This may have contributed to the null associations we found. Self-reported infection measures leave room for misclassification. Exploration of medical record abstraction for respiratory, urinary tract, vaginal, and other infections facilitated by the data linkages of the DNBC and the MoBa cohort to medical registries should be explored. Inflammation profiles in biological specimens and calculation of the Diet Inflammatory Index® may also be fruitful approaches to understanding the role of infection and, more broadly, inflammation during pregnancy in the occurrence of CP in children.

Micronutrients like PUFAs are not consumed in isolation, and future PUFA research needs to consider additional micronutrients that may be associated with CP, such as iodine.(152) Dietary patterns may also be important contributors to risk of CP, and analyses that address mixtures of food or micronutrient intake are important next steps in understanding the association between prenatal diet and CP. Principal component analysis has been used to identify diet patterns in both the DNBC(144) and the MoBa(145), and these types of analyses may provide insight into diet patterns that increase and decrease risk of CP. Indices like the Dietary Inflammatory Index® may useful understanding the association between prenatal diet, inflammation, and CP.(153)

PUFAs may also interact with other exposures like preterm birth(154), methyl-mercury(141), and phthalates(155). There is evidence that DHA exposure may lengthen gestation and increase birthweight(66), which are two components of important risk factors for CP: preterm birth and intra-uterine growth restriction. Exploring mediation models of CP risk with preterm birth may be important for prevention of CP. Disruption of fetal brain

development by environmental toxicants is another important line of research. Understanding the ways in which these toxicants change availability of PUFAs for fetal brain development may provide insight into mechanisms of CP. Our post-hoc analyses of DHA obtained from supplements suggest that there may be quality issues that impact CP risk, and quality of supplements containing DHA may be compromised by environmental chemicals.(140) Supplement use may also reflect health habits of mothers.

This study provided a first look at the exposures of DHA, ARA, and the ratio of DHA to ARA and focused on singleton children born at or near-term children. While it is important to identify risk factors for CP for this group, prevention research would benefit from analyses that include children born preterm as well, given that prevention of CP ideally starts before such outcomes occur. The added benefit of including all children regardless of gestational age is the ability to estimate causal effects and determine if any of the effect of the fatty acids might be directed through preterm birth. As it stands, this work is an important step in identifying how CP risk changes with exposure to two nutritionally important PUFAs for children born at or near term.

APPENDICES

APPENDIX A:

Tables and Figures Chapter 1

Table 1.1. Estimates of CP prevalence by region

Region	Years	Prevalence estimate	Population	Data source	Study
World	1996-2010	2.11/1,000 live births	19 studies dates ranged from 1996 to 2010	Meta-analysis of studies found in MEDLINE and EMBASE	Oskoui, Coutinho, Dykeman, *et al* (2013)
USA					
Metropolitan Atlanta, Georgia	1985-2002	1.9/1,000 in 1985 to 2.2/ 1,000 in 2002, though no change in spastic CP rate (1.78/1,000 survivors)	1-year survivors	Metropolitan Atlanta Developmental Disabilities Surveillance Program Numerator: cases ascertained through school and health records Denominator: linked birth and death records	Van Naarden Braun, Doernberg, Schieve, *et al* (2016)

Table 1.1 (Cont'd)

USA					
Alabama, Georgia, Missouri, and Wisconsin	2008	Overall: 3.1/1,000 8 year olds Alabama: 3.2/1,000 Georgia: 3.6/1,000 Missouri: 2.5/1,000 Wisconsin: 2.6/1,000	8 year-old children	Autism and Developmental Disabilities Monitoring Network Denominator: number of 8-year old children residing in an area according to the National Center for Health Statistics Vintage 2009	Christensen, Van Naarden Braun, Doernberg, *et al* (2014)
Metropolitan Atlanta, Georgia	1991-2010	1991-2010: 3.5/1,000 8 year-olds 1991: 2.9/1,000 2010: 3.4/1,000	8 year-old children	Metropolitan Atlanta Developmental Disabilities Surveillance Program Numerator: cases ascertained through school and health records Denominator: National Center for Health Statistics intercesnal population estimates vintage 1991-1996 and 2002-2008; decennial population estimates for 2000 and 2010	Van Naarden Braun, Christensen, Doernberg, *et al* (2015)

Table 1.1 (Cont'd)

USA					
15 States	2013-2015	1.78 cases per 1,000 Medicaid patients	Children through age 20 who participated in managed Medicaid plans	Numerator: Managed Medicaid patients through age 20 with CP diagnosis Denominator: Managed Medicaid plan patients through age 20 who have been enrolled in a Medicaid plan for at least 11 months	Pulgar, Bains, Gooch, *et al* (2019).
Nation-wide	2009-2016	3.2 cases per 1,000 3 to 17 year old children	Children 3 to 17 years old in the United States	The National Health Interview Survey participants age 3 to 17 years old	McGuire, Tian, Yeargin-Allsopp *et al*. (2019)
Europe					
Europe	1980-2003	1980: 1.90/1,000 live births 2003: 1.77/1,000 live births	Live births	20 registers participating in the Surveillance of Cerebral Palsy in Europe Numerator: cerebral palsy cases reported to registers Denominator: live births in participating countries	Sellier, Platt, Andersen, *et al* (2016)

Table 1.1 (Cont'd)

Europe					
Norway	1996-1998	2.1/1,000 live births	Live births	The Cerebral Palsy Registry of Norway Numerator: children diagnosed with cerebral palsy according to the definition of Surveillance of Cerebral Palsy in Europe who were reported to the registry Denominator: Live births in Norway from 1996 to 1998	Andersen, Irgens, Haagaas, *et al* (2008)
Norway	1999-2010	Overall: 2.35/1,000 live births 1999: 2.62/1,000 2010: 1.89/1,000	Live births	The Cerebral Palsy Registry of Norway and the Norwegian Patient Register Numerator: children diagnosed with cerebral palsy according to the definition of Surveillance of Cerebral Palsy in Europe who were reported to the registry Denominator: All children registered in the Medical Birth Registry of Norway between 1999 and 2010	Hollung, Vik, Lydersen, *et al* (2018)

Table 1.1 (Cont'd)

Europe					
Norway	2008-2010	3.0/1,000 live births	Live births	The Norwegian Patient Register Numerator: children with diagnosis of Cerebral Palsy born between 1999-2008 Denominator: all children identified in the Norwegian Patient Register between 2008 and 2010 who were born between 1999 and 2008	Suren, Bakken, Aase, *et al* (2012)
Denmark	1999-2007	1999-2001: 1.8/1,000 live births 2002-2004: 1.9/1,000 2005-2007: 1.8/1,000	Live births	The Danish Cerebral Palsy Registry Numerator: Physician diagnosed cerebral palsy cases reported to the registry Denominator: Live births from the Danish National Birth Register in the birth years 1999-2007	Hoei-Hansen, Laursen, Langhoff-Roos, *et al* (2019)

Table 1.1 (Cont'd)

Term Births					
World	1996-2010	1.35/1,000 live births	9 studies, 37 weeks gestation and older	Meta-analysis of studies found in MEDLINE and EMBASE	Oskoui, Coutinho, Dykeman *et al* (2013)
Norway	1967-2001	1.15/1,000 live births	Singleton births born at 37 to 44 weeks of gestation	Numerator: Cerebral palsy cases were individuals born at term with a CP diagnosis born between 1967 and 2001 Denominator: The Medical Birth Registry of Norway singleton births from 1967 to 2001 born between 37 and 44 weeks gestation	Moster, Wilcox, Vollset, *et al* (2010)
Denmark	1999-2007	1999-2001: 1.4/1,000 live births 2002-2004: 1.2/1,000 2005-2007: 1.1/1,000 63% of cerebral palsy cases born at 37 weeks or after	Live births	The Danish Cerebral Palsy Registry Numerator: Physician diagnosed cerebral palsy cases reported to the registry Denominator: Live births from the Danish National Birth Register in the birth years 1999-2007	Hoei-Hansen, Laursen, Langhoff-Roos, *et al* (2019)

Table 1.2. Fatty acid concentrations in common food items

Fatty Acid	Common Food Sources	Typical Concentration in 100g of source food
DHA	Salmon oil Cod liver oil Sardines oil Caviar Mackerel Salmon (depending on type of salmon)	18 g 11 g 11 g 4 g 3 g 1.5 g
α-linolenic acid	Flax seed oil Chia seeds Canola oil	53 g 18 g 9g
ARA	Beef Lamb	0.01-0.37 g 0.03-0.21 g
Linoleic acid	Soy oil Corn oil Sunflower seeds	50 g 50 g 34 g
Source: USDA Food Composition Tables: http://ndb.nal.usda.gov Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid		

Table 1.3. Pubmed search terms and number of articles returned for CP and PUFAs

Search Terms	Number of Articles Returned	Notes
Cerebral palsy AND docosahexaenoic acid	5 results	One medical hypothesis and one review, three relevant articles
Cerebral palsy AND arachidonic acid	6 results	Returned same medical hypothesis and review article. Mostly animal studies
Cerebral palsy AND fish AND pregnancy	11 results	Mostly pertained to methylmercury exposure
Cerebral palsy AND fish	24 results	Mostly pertained to methylmercury exposure

Figure 1.1. Metabolic pathways of omega-6 and omega-3 fatty acids

Omega-6 Series		**Omega-3 Series**
18:2		18:3
Linoleic Acid		alpha-Linolenic Acid
↓	Δ^{6-}Desaturase	↓
18:3		18:4
↓	Elongase	↓
20:3		20:4
↓	Δ^{5}-Desaturase	↓
20:4		20:5
Arachidonic Acid		Eicosapentaenoic Acid
	Elongase	↓
		22:5
	Δ^{4}-Desaturase	↓
		22:6
		Docosahexaenoic Acid

Adapted from Lauritzen *et al* (71) and Hadley *et al* (70)

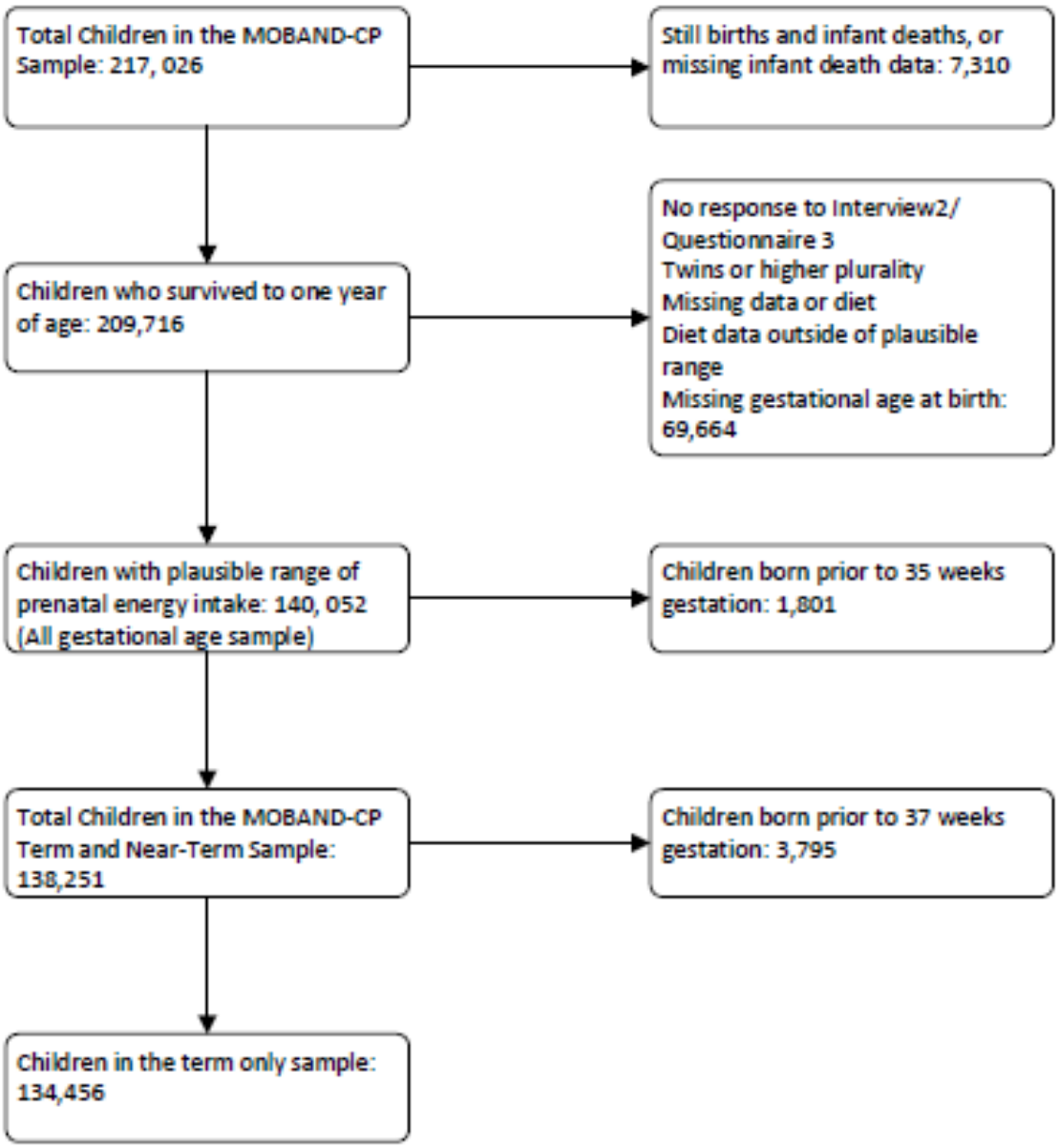
APPENDIX B:

Tables and Figures Chapter 3

Table 3.1. Power calculations for exposures

Odds Ratio	Power DHA	Power ARA	Power DHA/ARA	Power Fever	Power Urinary Tract Infection
1.5	74.4%	75.9%	73.9%	72.0%	63.9%

Figure 3.1. Flow chart of the inclusion and exclusion of children for the fatty acid analyses



APPENDIX C:

Tables and Figures Chapter 4

Table 4.1. List of characteristics used to compare birth categories

Cerebral Palsy Characteristics	**Maternal Characteristics**	**Birth Outcomes**
Prevalence of cerebral palsy	Maternal Age	Child Sex
Cerebral palsy subtype	Previous live births	Birth weight
Gross Motor Function Classification Scale	Previous stillbirths	Gestational age
Cerebral palsy with visual impairment	Maternal occupation	Mode of delivery
Cerebral palsy with mental impairment	Marital status	Apgar score under 5 at five minutes
Cerebral palsy with epilepsy	Planned or unplanned pregnancy	Transfer to neonatal ward
	Alcohol use during pregnancy	Any conditions of encephalopathy
	Smoking during pregnancy	Intra-cranial bleeding
	Diabetes	Neonatal seizures
	Exercise early in pregnancy	Mechanical ventilation
	Maternal pre-pregnancy body mass index	

Table 4.2. List of characteristics of children with and without CP included in term or near-term sample

Maternal Characteristics	**Birth Outcomes**
Maternal Age	Child Sex
Previous live births	Birth weight
Previous stillbirths	Gestational age
Maternal occupation	Mode of delivery
Marital status	Apgar score under 5 at five minutes
Planned or unplanned pregnancy	Transfer to neonatal ward
Alcohol use during pregnancy	Any conditions of encephalopathy
Smoking during pregnancy	Intra-cranial bleeding
Diabetes	Neonatal seizures
Maternal exercise in the first trimester	Ventilation
Maternal BMI	

Table 4.3. Prevalence of cerebral palsy per 1,000 children who survived to age one

Combined Cohorts	**Prevalence per 1,000 (95% CI)**
Term or near-term singleton children	1.50 (1.34, 1.69)
Preterm singleton children	22.59 (18.5, 27.89)
By Cohort: Term or near-term singleton children only	
DNBC	1.41 (1.19, 1.68)
MoBa	1.58 (1.36, 1.84)
Combined Cohorts Term or Near-Term Children by Gestational Age	
35-36 weeks gestation	3.42 (2.28, 5.59)
37-39 weeks gestation	1.60 (1.35, 1.92)
40 weeks gestation and higher	1.34 (1.15, 1.57)
Abbreviations: CI, confidence interval; DNBC, Danish National Birth Cohort; ,MoBa, Norwegian Mother and Child Cohort	

Table 4.4. CP Characteristics by birth category

CP Characteristics: Percentages	Term or Near Term	Preterm	Relative Risk (95% CI) Reference is Term or Near Term
Sample Size	197,765	3,984	
Cases of CP	297	90	
For Cases of CP			
CP Subtypes			
Spastic Unilateral	42.4	26.7	0.63 (0.44, 0.91)
Spastic Bilateral	38.0	72.2	1.90 (1.56, 2.30)
Dyskinetic	11.1	1.1	0.10 (0.01, 0.72)
Ataxic	5.4	(-)	(-)
Non-classifiable	3.0	(-)	(-)
GMFCS			
1	50.5	36.7	0.73 (0.54, 0.97)
2	11.4	14.4	1.26 (0.70, 2.29)
3	4.7	14.4	3.06 (1.49, 6.28)
4	11.1	13.3	1.20 (0.65, 2.23)
5	18.5	20.0	1.08 (0.67, 1.74)
Missing	3.7	1.1	0.30 (0.04, 2.30)
CP with Visual Impairment			
Yes	23.6	23.3	0.99 (0.65, 1.52)
No	62.6	58.9	0.94 (0.77, 1.08)
Missing	13.8	17.8	1.29 (0.76, 2.18)
CP with Mental Impairment			
Yes	33.0	35.6	1.08 (0.78, 1.49)
No	46.8	43.3	0.93 (0.71, 1.21)
Missing	20.2	21.1	1.05 (0.66, 1.65)
CP with Epilepsy			
Yes	31.3	24.4	0.78 (0.52, 1.17)
No	60.9	68.9	1.13 (0.96, 1.33)
Missing	7.7	6.7	0.86 (0.36, 2.05)

Abbreviations: CP, cerebral palsy; GMFCS, Gross Motor Function Classification System

Denominator includes singleton children with CP who survived to one year of age

Table 4.5. Maternal characteristics by birth category

Maternal Characteristics: Percentages	Term or Near Term	Preterm	Relative Risk (95% CI) Reference is Term or Near Term
Sample Size	197,765	3,984	
Maternal Age			
24 years and under	10.8	13.8	1.27 (1.18, 1.38)
30-34 years	35.4	33.5	0.95 (0.91, 0.99)
25-29 years	37.7	33.4	0.89 (0.85, 0.93)
35 years and older	16.1	19.3	1.20 (1.12, 1.28)
Previous Live Births			
0	43.8	55.2	0.73 (0.69, 0.76)
1	36.7	26.6	0.82 (0.75, 0.90)
2 or more	17.1	14.7	1.03 (0.86, 1.22)
Missing	2.5	3.5	1.39 (1.18, 1.64)
Previous Stillbirths			
0	99.6	98.9	
1 or more	0.4	1.1	2.64 (1.96, 3.56)
Occupational Status			
Employed	71.9	68.3	0.95 (0.93, 0.97)
Unemployed	6.8	7.3	1.07 (0.95, 1.19)
Student	10.9	11.4	1.05 (0.96, 1.14)
Receiving benefits or pension	1.3	2.4	1.84 (1.50, 2.25)
Missing	9.0	10.6	1.18 (1.07, 1.29)
Marital Status			
Spouse or partner	89.2	86.7	
Single	2.0	2.7	1.34 (1.11, 1.63)
Missing	8.7	10.6	1.21 (1.10, 1.33)
Planned Pregnancy			
Yes	77.0	73.5	
No	14.4	16.6	1.15 (1.08, 1.24)
Missing	8.6	9.8	1.15 (1.04, 1.26)

Table 4.5 (Cont'd)

Alcohol Use During Pregnancy			
Yes	19.9	16.0	0.80 (0.75, 0.86)
No (less than 0.5 units per week)	67.2	68.3	
Missing	12.9	15.7	1.22 (1.14, 1.31)
Smoking During Pregnancy			
Yes	22.2	26.3	1.19 (1.13, 1.25)
No	69.5	63.8	
Missing	8.3	9.9	1.20 (1.09, 1.31)
Diabetes			
No	98.7	97.0	
Any	1.3	3.0	2.28 (1.90, 2.73)
Exercise Early in Pregnancy			
Physically non-active	43.0	43.5	1.01 (0.98, 1.05)
One time per week	14.9	14.4	0.97 (0.90, 1.04)
Two times per week	10.1	9.8	0.98 (0.89, 1.07)
Three or more times per week	22.2	21.2	0.95 (0.90, 1.01)
Missing	9.7	11.1	1.14 (1.04, 1.25)
Maternal BMI kg/m^2			
<18.5 kg/m^2	3.3	4.5	1.38 (1.19, 1.59)
18.5-<23 kg/m^2	42.5	37.2	0.88 (0.84, 0.91)
23-<25 kg/m^2	17.4	16.3	0.94 (0.88, 1.01)
25-<30 kg/m^2	18.9	19.2	1.01 (0.95, 1.08)
30 kg/m^2 or greater	8.0	11.0	1.38 (1.26, 1.51)
Missing	10.0	11.7	1.17 (1.07, 1.28)
Abbreviations: BMI, body mass index Denominator includes children who survived to one year of age			

Table 4.6.1. Birth outcomes with continuous variables by birth category

Birth Outcomes: Means	Term or Near Term	Preterm
Mean Birthweight	3,623.80	2,122.10
Birthweight standard error	1.20	13.50
Missing birthweight	492	57
Gestational age	40.1	32.4
Gestational age standard error	0.00	0.04
Denominator includes all singleton children who survived to age one		

Table 4.6.2. Birth outcomes with categorical variables by birth category

Birth Outcomes: Percentages	Term or Near Term	Preterm	Relative Risk (95% CI) Reference is Term or Near Term
Sex			
Male	51.2	53.2	1.04 (1.01, 1.07)
Female	48.8	46.8	
Mode of Delivery			
Vaginal	76.6	47.5	0.62 (0.60, 0.64)
Assisted (Forceps or Vacuum)	9.5	3.2	0.34, 0.29, 0.40)
Elective or unspecified C-section	5.1	7.5	1.48 (1.32, 1.65)
Emergency C-section	8.6	41.2	4.77 (4.58, 4.96)
Missing	0.2	0.6	2.56 (1.70, 3.85)
Apgar Score less than 5			
Yes	0.2	1.4	6.88 (5.23, 9.06)
No	99.3	97.1	
Missing	0.4	1.5	3.35 (2.58, 4.35)
Transfer to the neonatal ward			
Yes	5.9	82.1	13.99 (13.67, 14.31)
No	94.0	17.6	
Missing	0.1	0.3	2.96 (1.69, 5.18))
Encephalopathy (all conditions together)			
Yes	0.2	0.6	2.69 (1.77, 4.08)
No	99.8	99.4	
Intra-cranial bleeding			
Yes	0.1	3.6	49.99 (39.74, 62.87)
No	99.9	96.4	
Neonatal seizures			
Yes	0.2	0.5	2.36 (1.49, 3.73)
No	99.8	99.5	
Mechanical Ventilator			
Yes	0.4	17.3	42.32 (38.42, 46.62)
No	99.5	82.4	
Missing	0.1	0.3	2.96 (1.69, 5.18)
Abbreviations: C-section, Caesarian section; Denominator includes all children who survived to age one			

Table 4.7. Maternal characteristics by cerebral palsy status

Maternal Characteristics: Percentages	CP	No CP	Relative Risk of CP (95% CI)
Sample Size	297	197,468	
Maternal Age			
24 years and under	11.2	10.7	Reference
30-34 years	33.9	35.1	0.91 (0.61, 1.34)
25-29 years	35.8	37.8	0.95 (0.64, 1.40)
35 years and older	19.0	16.4	1.16 (0.75, 1.78)
Previous Live Births			
0	50.8	43.8	Reference
1	32.0	36.7	0.78 (0.60, 1.00)
2	15.4	17.1	0.84 (0.59, 1.20)
Missing	2.0	2.5	0.72 (0.32, 1.62)
Previous Stillbirths			
0*	98.9	99.6	Reference
1 or more	1.1	0.4	1.58 (0.39, 6.33)
Occupational Status			
Employed	71.1	72.0	Reference
Unemployed	6.7	6.8	0.78 (0.47, 1.30)
Student	10.8	10.8	0.95 (0.65, 1.38)
Receiving benefits or pension	3.4	1.3	2.29 (1.18, 4.46)
Missing	8.0	8.9	0.97 (0.64, 1.45)
Marital Status			
Spouse or partner	88.3	89.3	Reference
Single	3.0	2.0	1.17 (0.55, 2.47)
Missing	8.7	8.7	1.05 (0.71, 1.56)
Planned Pregnancy			
Yes	78.2	77.2	Reference
No	14.0	14.2	0.92 (0.66, 1.29)
Missing	7.8	8.5	0.97 (0.64, 1.46)

Table 4.7 (Cont'd)

Alcohol Use During Pregnancy			
Yes	17.0	19.8	0.87 (0.64, 1.18)
No (less than 0.5 units per week)	70.9	67.4	Reference
Missing	12.2	12.9	0.96 (0.68, 1.36)
Smoking During Pregnancy			
Yes	23.6	22.2	1.00 (0.76, 1.32)
No	68.6	69.5	Reference
Missing	7.8	8.3	0.97 (0.64, 1.48)
Diabetes			
No	96.3	98.6	Reference
Any	3.7	1.4	2.60 (1.39, 4.88)
Exercise in the First Part of Pregnancy			
Physically non-active	47.2	43.5	1.17 (0.84, 1.62)
One time per week	16.1	14.8	1.03 (0.70, 1.53)
Two times per week	9.6	10.0	0.88 (0.64, 1.20)
Three or more times per week	18.1	22.0	Reference
Missing	8.9	9.7	0.97 (0.64, 1.46)
Maternal BMI kg/m^2			
<18.5 kg/m^2	2.5	3.3	0.96 (0.52, 1.77)
18.5-<23 kg/m^2	33.7	42.3	Reference
23-<25 kg/m^2	19.0	17.4	1.37 (1.05, 1.80)
25-<30 kg/m^2	23.6	18.9	1.57 (1.22, 2.02)
30 kg/m^2 or higher	10.6	8.1	1.63 (1.17,2.67)
Missing	10.6	10.0	1.33 (0.95, 1.86)

Abbreviations: BMI, body mass index;
Denominator includes singleton children born at 35 weeks gestation or later who survived to one year of age

Table 4.8.1. Birth outcomes with continuous variables by cerebral palsy status

Birth Outcomes: Means	CP	No CP
Mean Birthweight	3,404.50	3,624.10
Birthweight standard error	38	1.21
Missing birthweight	5	487
Gestational age	39.7	40.1
Gestational age standard error	0.1	0.00
Abbreviations: CP, cerebral palsy Denominator includes singleton children born at 35 weeks gestation or later and who survived to one year		

Table 4.8.2. Birth outcomes with continuous variables by cerebral palsy status

Birth Outcomes: Percentages	CP	No CP	Relative Risk of CP (95% CI)
Sex			
Male	56.9	51.2	1.26 (1.00, 1.58)
Female	43.1	48.8	Reference
Mode of Delivery			
Vaginal	51.6	74.7	Reference
Assisted (Forceps or Vacuum)	10.6	9.4	1.81 (1.28, 2.54)
Elective or unspecified C-section	5.5	5.6	1.18 (0.69, 2.03)
Emergency C-section	32.1	10.0	3.12 (2.34, 4.16)
Missing	0.2	0.3	1.82 (0.25, 12.39)
Apgar Score less than 5			
Yes	10.6	0.2	72.83 (52.74, 100.56)
No	88.1	99.3	Reference
Missing	1.4	0.5	1.75 (0.44, 7.04)

Table 4.8.2. (Cont'd)

Transfer to the neonatal ward			
Yes	60.3	8.5	13.54 (10.78, 17.00)
No	39.7	91.5	Reference
Missing	(-)	0.1	(-)
Encephalopathy (all conditions together)			
Yes	8.3	0.2	54.11 (37.77, 77.53)
No	91.7	99.8	Reference
Intra-cranial bleeding			
Yes	13.5	0.1	68.37 (41.01, 113.96)
No	86.5	99.9	Reference
Neonatal seizures			
Yes	15.1	0.2	122.32 (93.59, 159.87)
No	84.9	99.8	Reference
Mechanical Ventilator			
Yes	13.8	0.9	21.43 (14.20, 32.34)
No	86.2	99	Reference
Missing	(-)	0.1	(-)

Abbreviations: C-section, Caesarian section
Denominator includes singleton children born at 35 weeks gestation or later and who survived to one year

APPENDIX D:

Tables and Figures Chapter 5

Table 5.1. Maternal characteristics for the term or near-term sample by DHA status for the combined cohorts in the MOBAND-CP study

Maternal Characteristics (%)	DHA Quartile 1	DHA Quartile 2	DHA Quartile 3	DHA Quartile 4
Sample Size	34,558	34,565	34,563	34,565
Maternal Age				
24 years and under	12.9	9.7	8.2	7.7
25-29 years	38.3	37.0	35.3	32.9
30-34 years	35.7	38.5	39.5	39.7
35 years and older	13.1	14.8	17.1	19.8
Number of Previous Live Births				
0	43.3	45.2	46.5	45.9
1	37.2	36.6	35.2	35.0
2 or more	16.9	15.6	15.4	16.2
Missing	2.7	2.6	2.9	2.9
Previous Stillbirths				
0	99.6	99.6	99.5	99.6
1 or more	0.4	0.4	0.5	0.4
Maternal Occupation				
Employed	77.8	80.1	80.7	79.0
Unemployed or receiving benefits or pension	9.5	7.6	7.0	8.1
Student	11.5	11.2	11.3	11.9
Missing	1.2	1.1	1.0	1.0
Marital Status				
Spouse or partner	96.9	97.6	97.6	97.3
Single	2.2	1.6	1.6	1.9
Missing	0.9	0.8	0.8	0.9

Table 5.1 (Cont'd)

Planned Pregnancy				
Yes	83.3	84.9	85.5	84.7
No	16.0	14.5	13.9	14.6
Missing	0.7	0.7	0.6	0.6
Alcohol Use Early in Pregnancy				
Yes	17.7	20.2	21.4	21.3
No (less than 0.5 units per week)	77.0	74.4	72.9	73.0
Missing	5.4	5.4	5.7	5.7
Smoking Early in Pregnancy				
Yes	25.9	21.8	20.7	20.6
No	73.7	77.9	79.0	79.0
Missing	0.4	0.3	0.4	0.4
Diabetes				
No	98.7	98.7	98.7	98.7
Any Diabetes (including gestational)	1.3	1.3	1.3	1.3
Exercise During First Trimester				
Physically inactive	51.2	45.6	41.9	39.5
One time per week	16.9	17.6	17.1	15.9
Two times per week	29.7	35.1	39.5	43.0
Missing	2.2	1.8	1.5	1.6
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	44.5	48.0	51.5	54.0
23-<25 kg/m^2	18.6	19.4	19.4	19.1
25 kg/m^2 or higher	34.4	30.6	27.1	25.1
Missing	2.4	1.9	2.0	1.9
Denominators include pregnancies that resulted in a child who survived to 1 year of age Abbreviations: CP, cerebral palsy; DHA, docosahexaenoic acid; MOBAND-CP, MOthers and BAbies of Norway and Denmark- Cerebral Palsy				

Table 5.2. Maternal characteristics for the term or near-term sample by DHA status for the Danish National Birth Cohort

Maternal Characteristics Frequency (%)	DHA Quartile 1	DHA Quartile 2	DHA Quartile 3	DHA Quartile 4
Sample Size	14,960	14,965	14,962	14,965
Maternal Age				
24 years and under	11.9	8.9	7.1	7.0
25-29 years	43.5	40.6	38.1	35.9
30-34 years	33.7	37.2	39.2	39.2
35 years and older	10.9	13.3	15.6	17.9
Number of Previous Live Births				
0	52.3	48.6	47.7	46.9
1	34.2	37.2	37.1	37.3
2 or more	13.5	14.3	15.2	15.8
Missing	(-)	(-)	(-)	(-)
Previous Stillbirths				
0	99.6	99.6	99.4	99.5
1 or more	0.4	0.4	0.6	0.5
Maternal Occupation				
Employed	75.8	77.7	77.7	75.2
Unemployed or receiving benefits or pension	11.2	9.1	8.7	10.5
Student	13.0	13.2	13.5	14.3
Missing	0.0	0.0	0.0	0.0
Marital Status				
Spouse or partner	97.9	98.4	98.5	98.2
Single	2.1	1.5	1.5	1.8
Missing	0.0	0.0	0.0	0.0
Planned Pregnancy				
Yes	89.1	90.0	90.0	89.0
No	10.9	10.0	10.0	11.0
Missing	0.0	0.0	0.0	0.0

Table 5.2 (Cont'd)

Alcohol Use Early in Pregnancy				
Yes	39.3	45.1	47.8	47.7
No (less than 0.5 units per week)	60.7	54.8	52.1	52.2
Missing	0.1	0.1	0.1	0.1
Smoking Early in Pregnancy				
Yes	25.9	23.3	22.8	23.3
No	74.1	76.7	77.2	76.7
Missing	0.0	0.0	0.0	0.0
Diabetes				
No	98.7	98.9	98.9	98.8
Any Diabetes (including gestational)	1.3	1.1	1.1	1.2
Exercise In the First Part of Pregnancy				
Physically inactive	65.8	62.9	60.2	58.9
One time per week	12.2	13.7	14.0	13.1
Two times per week or more	22.0	23.4	25.8	28.0
Missing	0.0	0.0	0.0	0.0
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	48.4	50.9	55.1	57.0
23-<25 kg/m^2	18.3	19.2	18.1	18.2
25 kg/m^2 or higher	31.6	28.6	25.2	23.3
Missing	1.7	1.4	1.6	1.5

Denominators include pregnancies that resulted in a child who survived to 1 year of age
Abbreviations: CP, cerebral palsy; DHA, docosahexaenoic acid

Table 5.3. Maternal characteristics for the term or near-term sample by DHA status for the Norwegian Mother and Child Cohort

Maternal Characteristics Frequency (%)	DHA Quartile 1	DHA Quartile 2	DHA Quartile 3	DHA Quartile 4
Sample Size	19,598	19,600	19,601	19,600
Maternal Age				
24 years and under	13.7	10.3	9.0	8.2
25-29 years	34.3	34.2	33.1	30.6
30-34 years	37.3	39.4	39.6	40.1
35 years and older	14.7	16.1	18.3	21.2
Number of Previous Live Births				
0	36.4	42.6	45.7	45.2
1	39.4	36.2	33.7	33.2
2 or more	19.5	16.5	15.5	16.5
Missing	4.7	4.6	5.2	5.1
Previous Stillbirth				
0	99.6	99.7	99.6	99.6
1 or more	0.4	0.3	0.4	0.4
Maternal Occupation				
Employed	79.3	82.0	83.0	81.9
Unemployed or receiving benefits or pension	8.3	6.3	5.6	6.2
Student	10.4	9.7	9.6	10.1
Missing	2.1	2.0	1.8	1.8
Marital Status				
Spouse or partner	96.1	97.0	96.9	96.6
Single	2.4	1.7	1.8	1.9
Missing	1.5	1.3	1.4	1.5

Table 5.3 (Cont'd)

Planned Pregnancy				
Yes	78.8	81.0	82.1	81.4
No	20.0	17.8	16.9	17.4
Missing	1.2	1.1	1.0	1.1
Alcohol Use Early in Pregnancy				
Yes	1.2	1.1	1.2	1.1
No (less than 0.5 units per week)	89.4	89.4	88.7	88.9
Missing	9.4	9.4	10.1	9.9
Smoking Early in Pregnancy				
Yes	25.9	20.7	19.1	18.6
No	73.5	78.8	80.3	80.8
Missing	0.6	0.5	0.6	0.6
Diabetes				
No	98.6	98.6	98.5	98.6
Any Diabetes (including gestational)	1.4	1.4	1.5	1.4
Exercise Early in Pregnancy				
Physically inactive	40.2	32.4	27.9	24.7
One time per week	20.5	20.5	19.5	18.1
Two times per week	35.5	44.0	50.0	54.4
Missing	3.8	3.1	2.7	2.8
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	41.6	45.9	48.7	51.7
23-<25 kg/m^2	18.9	19.6	20.3	19.7
25 kg/m^2 or higher	36.5	32.1	28.6	26.4
Missing	3.0	2.4	2.3	2.2
Denominators include pregnancies that resulted in a child who survived to 1 year of age Abbreviations: CP, cerebral palsy; DHA, docosahexaenoic acid				

Table 5.4. Unadjusted and adjusted relative risk of cerebral palsy by DHA quartile in the combined cohorts

DHA- Combined Cohorts	Frequency (%) CP	Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk* (95% CI)	E-value (limit)
Quartile 1	41 (0.12)	Reference		
Quartile 2	75 (0.22)	1.83 (1.25, 2.68)	1.85 (1.26, 2.71)	3.10 (1.83)
Quartile 3	36 (0.10)	0.88 (0.56, 1.37)	0.90 (0.57, 1.41)	1.46 (1.00)
Quartile 4	47 (0.14)	1.15 (0.75, 1.74)	1.19 (0.78, 1.83)	1.67 (1.00)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first part of pregnancy
Abbreviations: CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid
Imputations = 30

Table 5.5. Unadjusted and adjusted relative risk of cerebral palsy by DHA quartile in the Danish National Birth Cohort

DHA- DNBC	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (lower limit)
Quartile 1	15 (0.10)	Reference		
Quartile 2	26 (0.17)	1.73 (0.92, 3.27)	1.84 (0.97, 3.49)	3.08 (1.00)
Quartile 3	13 (0.09)	0.87 (0.41, 1.82)	0.97 (0.46, 2.03)	1.21 (1.00)
Quartile 4	23 (0.15)	1.53 (0.80, 2.94)	1.79 (0.91, 3.50)	2.98 (1.00)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CP, cerebral palsy; CI, confidence interval; DNBC, Danish National Birth Cohort

Imputations = 30

Table 5.6. Unadjusted and adjusted relative risk of cerebral palsy by DHA quartile in the Norwegian Mother and Child Cohort

DHA- MoBa	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (lower limit)
Quartile 1	26 (0.13)	Reference		
Quartile 2	49 (0.25)	1.88 (1.17, 3.03)	1.83 (1.13, 2.95)	3.06 (1.51)
Quartile 3	23 (0.12)	0.89 (0.51, 1.55)	0.85 (0.48, 1.50)	1.63 (1.00)
Quartile 4	24 (0.12)	0.92 (0.53, 1.61)	0.90 (0.51, 1.56)	1.46 (1.00)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CP, cerebral palsy; CI, confidence interval; MoBa, Norwegian Mother and Child Cohort study

Imputations = 30

Table 5.7. Unadjusted means for birthweight and gestational age by DHA quartile in the combined cohorts

Mean (SE)	DHA Quartile 1	DHA Quartile 2	DHA Quartile 3	DHA Quartile 4
Birthweight	3621.41 (2.83)	3630.70 (2.77)	3629.16 (2.75)	3627.31 (2.79)
Number missing birthweight	59	67	58	68
Gestational age	40.05 (0.01)	40.09 (0.01)	40.10 (0.01)	40.10 (0.01)
Abbreviations: DHA, docosahexaenoic acid; SE, standard error				

Table 5.8. Risk of CP by DHA quartile from food intake in the Norwegian Mother and Child Cohort

DHA from Food	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (lower limit)
Quartile 1	30 (0.15)	Reference		
Quartile 2	33 (0.17)	1.10 (0.67, 1.80)	1.13 (0.69, 1.84)	1.51 (1.00)
Quartile 3	32 (0.16)	1.04 (0.63, 1.71)	1.07 (0.65, 1.76)	1.34 (1.00)
Quartile 4	28 (0.14)	0.93 (0.56, 1.56)	0.97 (0.58, 1.63)	1.21 (1.00)

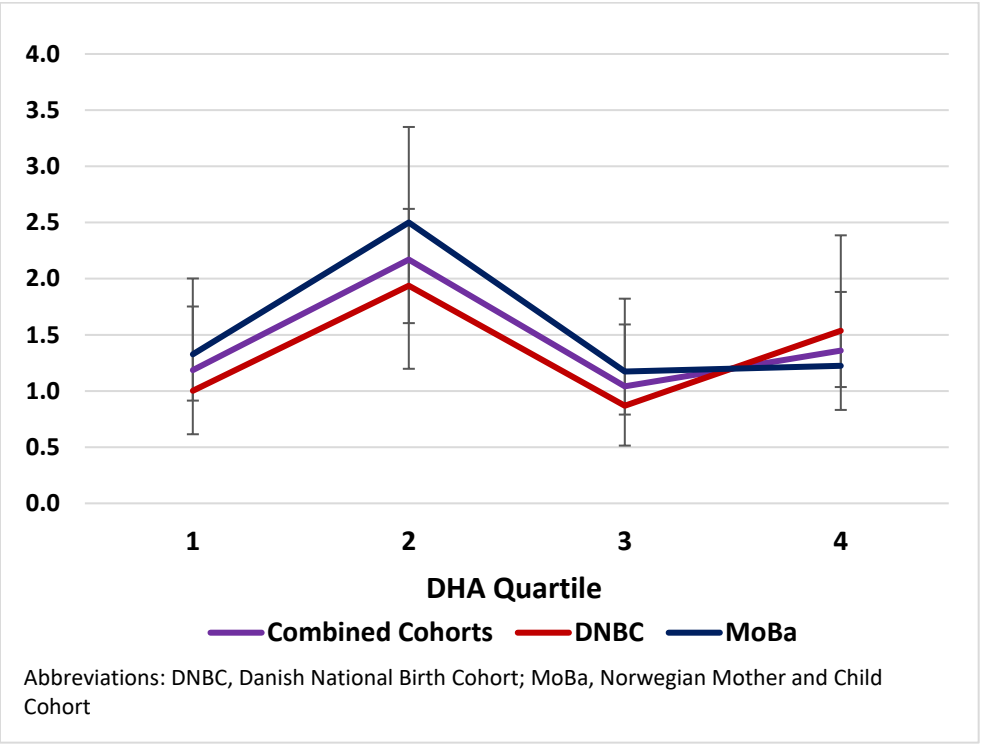
*Adjusted for total energy intake, previous live births, maternal occupation, marital status, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester
Abbreviations: CP, cerebral palsy; CI, confidence interval; MoBa, Norwegian Mother and Child Cohort study
Imputations = 30

Table 5.9. Risk of CP by DHA quartile from dietary supplements in the Norwegian Mother and Child Cohort

DHA from Supplements	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (lower limit)
Unexposed (non-users)	33 (0.13)	Reference		
Low Exposure	31 (0.22)	1.66 (1.01, 2.72)	1.59 (0.97, 2.63)	2.56 (1.00)
Middle Exposure	34 (0.17)	1.32 (0.82, 2.13)	1.25 (0.76, 2.04)	1.81 (1.00)
High Exposure	25 (0.13)	0.98 (0.58, 1.64)	0.95 (0.56, 1.61)	1.29 (1.00)

*Adjusted for previous live births, maternal occupation, marital status, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester
Abbreviations: CP, cerebral palsy; CI, confidence interval; MoBa, Norwegian Mother and Child Cohort study
Imputations = 30

Figure 5.1. Prevalence of cerebral palsy per 1,000 children who survived to age one by DHA quartile and cohort



Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

APPENDIX E:

Tables and Figures Chapter 6

Table 6.1. Maternal characteristics for term or near-term sample by ARA status for the Danish National Birth Cohort

Maternal Characteristics Frequency (%)	ARA Quartile 1	ARA Quartile 2	ARA Quartile 3	ARA Quartile 4
Sample Size	14,949	14,975	14,967	14,961
Maternal Age				
24 years and under	10.2	9.1	7.8	7.8
25-29 years	40.5	39.2	39.0	39.5
30-34 years	35.7	37.6	38.0	38.0
35 years and older	13.7	14.0	15.2	14.7
Previous Number of Live Births				
0	50.2	47.5	47.0	50.7
1	35.2	37.5	37.6	35.5
2 or more	14.6	15.1	15.4	13.8
Previous Number of Stillbirths				
0	99.6	99.5	99.6	99.4
1 or more	0.4	0.5	0.4	0.6
Maternal Occupation				
Employed	75.6	77.3	77.5	75.9
Unemployed or receiving benefits or pension	10.7	9.8	9.5	9.6
Student	13.7	12.9	13.0	14.5
Missing	0.0	0.0	0.0	0.0
Marital Status				
Spouse or partner	97.9	98.2	98.5	98.4
Single	2.1	1.7	1.5	1.5
Missing	0.0	0.1	0.0	0.0
Planned Pregnancy				
Yes	10.9	10.5	10.1	10.3
No	89.0	89.5	89.9	89.7
Missing	0.0	0.0	0.0	0.0

Table 6.1 (Cont'd)

Alcohol Use Early in Pregnancy				
Yes	58.2	55.3	52.6	53.7
No (less than 0.5 units per week)	41.7	44.6	47.3	46.2
Missing	0.1	0.1	0.1	0.1
Smoking During Pregnancy				
Yes	24.7	23.3	23.6	23.6
No	75.3	76.6	76.4	76.4
Missing	0.0	0.0	0.0	0.0
Diabetes				
No	98.8	98.9	98.8	98.9
Any Diabetes (including gestational)	1.2	1.1	1.2	1.1
Exercise During First Trimester				
Physically non-active	63.3	62.2	61.6	60.6
One time per week	12.7	13.7	13.4	13.1
Two times per week	23.9	24.1	24.9	26.3
Missing	0.0	0.0	0.0	0.0
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	51.7	52.1	52.8	54.8
23-<25 kg/m^2	18.2	18.5	18.4	18.8
25 kg/m^2 or higher	28.6	27.9	27.3	24.9
Missing	1.6	1.5	1.5	1.5
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: ARA, arachidonic acid; CP, cerebral palsy				

Table 6.2. Maternal characteristics for the term or near-term sample by ARA status for the Norwegian Mother and Child Cohort

Maternal Characteristics Frequency (%)	ARA Quartile 1	ARA Quartile 2	ARA Quartile 3	ARA Quartile 4
Sample Size	19,593	19,605	19,565	19,636
Maternal Age				
24 years and under	12.4	9.6	8.8	10.4
25-29 years	34.6	33.1	32.0	32.5
30-34 years	37.4	40.2	40.3	38.6
35 years and older	15.6	17.0	19.0	18.6
Previous Number of Live Births				
0	45.7	42.4	40.8	40.9
1	33.6	36.3	37.2	35.5
2 or more	15.8	16.6	17.4	18.3
Missing	4.9	4.8	4.6	5.3
Previous Number of Stillbirths				
0	99.6	99.7	99.6	99.6
1 or more	0.4	0.3	0.4	0.4
Maternal Occupation				
Employed	80.2	82.9	82.5	80.6
Unemployed or receiving benefits or pension	6.6	6.2	6.6	7.1
Student	11.3	9.1	9.2	10.2
Missing	2.0	1.9	1.7	2.1
Marital Status				
Spouse or partner	96.3	96.7	96.8	96.7
Single	2.3	1.9	1.7	1.9
Missing	1.4	1.4	1.4	1.3
Planned Pregnancy				
Yes	18.8	17.2	17.4	18.8
No	80.1	81.6	81.6	80.0
Missing	1.1	1.2	1.0	1.2

Table 6.2 (Cont'd)

Alcohol Use Early in Pregnancy				
Yes	89.2	89.2	89.5	88.6
No (less than 0.5 units per week)	1.0	1.1	1.3	1.3
Missing	9.8	9.7	9.2	10.1
Smoking Early in Pregnancy				
Yes	21.2	20.7	20.4	22.1
No	78.3	78.7	79.1	77.4
Missing	0.5	0.6	0.6	0.6
Diabetes				
No	98.8	98.6	98.5	98.4
Any Diabetes (including gestational)	1.2	1.4	1.5	1.6
Exercise Early in Pregnancy				
Physically non-active	34.1	31.6	29.6	29.8
One time per week	19.2	19.6	20.3	19.5
Two times per week	43.4	45.8	47.2	47.6
Missing	3.3	3.1	2.9	3.2
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	47.4	47.1	46.8	46.5
23-<25 kg/m^2	19.3	19.7	19.4	20.1
25 kg/m^2 or higher	30.7	30.7	31.4	30.8
Missing	2.6	2.4	2.3	2.5
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: ARA, arachidonic acid; CP, cerebral palsy				

Table 6.3. Maternal characteristics for the term or near-term sample by quartile of DHA to ARA ratio for the Danish National Birth Cohort

Maternal Characteristics Frequency (%)	Ratio Quartile 1	Ratio Quartile 2	Ratio Quartile 3	Ratio Quartile 4
Sample Size	14,961	14,962	14,962	14,967
Maternal Age				
24 years and under	10.7	8.8	7.3	8.1
25-29 years	43.3	40.0	38.1	36.7
30 -34 years	35.0	37.4	38.7	38.2
35 years and older	11.0	13.8	15.9	16.9
Previous Number of Live Births				
0	52.9	47.9	47.0	47.6
1	34.0	37.5	37.7	36.6
2 or more	13.1	14.6	15.3	15.8
Previous Number of Stillbirths				
0	99.6	99.5	99.5	99.6
1 or more	0.4	0.5	0.5	0.4
Maternal Occupation				
Employed	75.7	77.8	77.6	75.2
Unemployed or receiving benefits or pension	10.6	9.3	9.0	10.7
Student	13.7	12.9	13.3	14.1
Missing	0.0	0.0	0.0	0.0
Marital Status				
Spouse or partner	98.2	98.5	98.3	98.1
Single	1.8	1.5	1.7	1.9
Missing	0.0	0.0	0.0	0.1
Planned Pregnancy				
Yes	89.2	90.2	89.8	89.0
No	10.8	9.8	10.2	11.0
Missing	0.0	0.0	0.0	0.0

Table 6.3 (Cont'd)

Alcohol Use Early in Pregnancy				
Yes	40.6	46.2	47.2	45.8
No (less than 0.5 units per week)	59.3	53.7	52.7	54.1
Missing	0.0	0.1	0.1	0.1
Smoking Early in Pregnancy				
Yes	25.2	23.7	23.3	23.0
No	74.8	76.2	76.6	77.0
Missing	0.0	0.0	0.0	0.0
Diabetes				
No	98.8	98.9	99.0	98.8
Any Diabetes (including gestational)	1.2	1.1	1.0	1.2
Exercise Early in Pregnancy				
Physically non-active	64.7	62.5	60.8	59.7
One time per week	12.2	13.6	13.9	13.3
Two times per week	23.1	23.9	25.3	27.0
Missing	0.0	0.0	0.0	0.0
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	49.6	51.6	54.5	55.7
23-<25 kg/m^2	18.6	18.9	18.1	18.2
25 kg/m^2 or higher	30.1	27.9	26.0	24.6
Missing	1.6	1.6	1.4	1.5
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; DHA, docosahexaenoic acid				

Table 6.4. Maternal characteristics by quartile of DHA to ARA ratio for the Norwegian Mother and Child Cohort

Maternal Characteristics Frequency (%)	Ratio Quartile 1	Ratio Quartile 2	Ratio Quartile 3	Ratio Quartile 4
Sample Size	19,598	19,599	19,599	19,603
Maternal Age				
24 years and under	13.7	9.9	9.3	8.4
25-29 years	34.1	33.6	33.2	31.3
30-34 years	37.3	39.6	39.6	40.0
35 years and older	14.9	16.9	18.0	20.4
Previous Number of Live Births				
0	35.7	41.5	45.2	47.4
1	39.8	36.5	34.1	32.1
2 or more	19.8	17.1	15.8	15.3
Missing	4.7	4.8	4.9	5.2
Previous Number of Stillbirths				
0	99.6	99.7	99.6	99.7
1 or more	0.4	0.3	0.4	0.3
Maternal Occupation				
Employed	79.3	82.0	82.8	82.0
Unemployed or receiving benefits or pension	8.5	6.2	5.8	6.0
Student	10.0	9.9	9.6	10.1
Missing	2.2	1.9	1.7	1.9
Marital Status				
Spouse or partner	96.1	97.1	96.9	96.5
Single	2.4	1.6	1.7	2.1
Missing	1.5	1.3	1.4	1.4
Planned Pregnancy				
Yes	78.7	81.2	81.6	81.9
No	20.1	17.8	17.3	17.0
Missing	1.3	1.0	1.1	1.1

Table 6.4 (Cont'd)

Alcohol Use During Pregnancy				
Yes	1.2	1.3	1.1	1.1
No (less than 0.5 units per week)	89.2	89.4	88.9	89.0
Missing	9.6	9.3	10.0	10.0
Smoking During Pregnancy				
Yes	26.4	20.6	19.2	18.0
No	73.0	78.9	80.2	81.3
Missing	0.6	0.5	0.6	0.6
Diabetes				
No	98.6	98.5	98.6	98.6
Any Diabetes (including gestational)	1.4	1.5	1.4	1.4
Exercise During First Trimester				
Physically non-active	39.8	32.2	28.1	24.9
One time per week	20.5	20.7	19.1	18.2
Two times per week	35.7	44.2	50.0	54.1
Missing	4.0	2.9	2.7	2.9
Maternal Pre-Pregnancy Body Mass Index				
<23 kg/m^2	41.1	45.7	48.4	52.6
23-<25 kg/m^2	19.1	20.0	19.9	19.6
25 kg/m^2 or higher	36.8	32.1	29.4	25.4
Missing	3.1	2.2	2.3	2.4
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; DHA, docosahexaenoic acid				

Table 6.5. Unadjusted and adjusted relative risk of cerebral palsy by ARA quartile for the Danish National Birth Cohort

ARA- DNBC	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (limit)
Quartile 1	23 (0.15)	Reference		
Quartile 2	22 (0.15)	0.96 (0.53, 1.71)	0.97 (0.54, 1.73)	1.21 (1.00)
Quartile 3	19 (0.13)	0.83 (0.45, 1.51)	0.85 (0.46, 1.56)	1.63 (1.00)
Quartile 4	13 (0.09)	0.57 (0.29, 1.12)	0.59 (0.30, 1.15)	2.78 (1.00)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester
Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; CI, confidence interval; DNBC, Danish National Birth Cohort
Imputations = 30

Table 6.6. Unadjusted and adjusted relative risk of cerebral palsy by ARA quartile for the Norwegian Mother and Child Cohort

ARA- MoBa	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (limit)
Quartile 1	26 (0.13)	Reference		
Quartile 2	23 (0.12)	0.88 (0.51, 1.55)	0.89 (0.50, 1.57)	1.50 (1.00)
Quartile 3	32 (0.16)	1.23 (0.74, 2.07)	1.24 (0.73, 2.10)	1.79 (1.00)
Quartile 4	41 (0.21)	1.57 (0.96, 2.57)	1.58 (0.94, 2.64)	2.54 (1.00)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester
Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; CI, confidence interval; MoBa, Norwegian Mother and Child Cohort
Imputations = 30

Table 6.7. Unadjusted and adjusted relative risk of cerebral palsy by quartile of ratio of DHA/ARA for the Danish National Birth Cohort

DHA/ARA Ratio-DNBC	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (limit)
Quartile 1	15 (0.10)	Reference		
Quartile 2	13 (0.09)	0.87 (0.41, 1.82)	0.90 (0.43, 1.88)	1.46 (1.00)
Quartile 3	22 (0.15)	1.47 (0.76, 2.83)	1.57 (0.81, 3.02)	2.52 (1.00)
Quartile 4	27 (0.18)	1.80 (0.96, 3.38)	1.95 (1.04, 3.64)	3.31 (1.24)

*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid

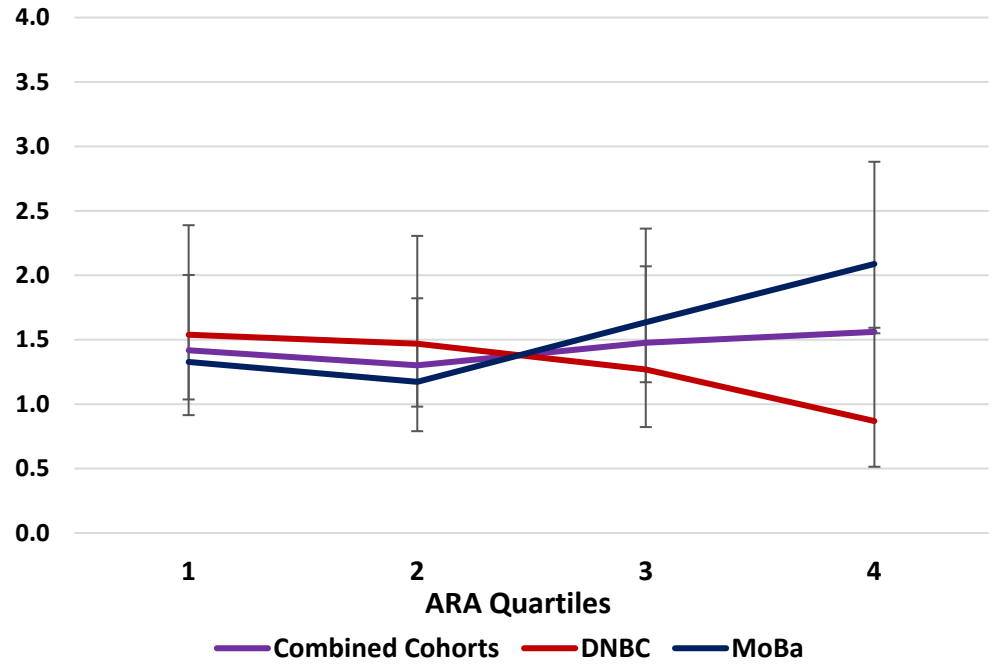
Imputations = 30

Table 6.8. Unadjusted and adjusted relative risk of cerebral palsy by quartile of ratio of DHA/ARA for the Norwegian Mother and Child Cohort

DHA/ARA Ratio-MoBa	Frequency (%) CP	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (limit)
Quartile 1	32 (0.16)	Reference		
Quartile 2	39 (0.20)	1.22 (0.76, 1.94)	1.19 (0.74, 1.90)	1.67 (1.00)
Quartile 3	32 (0.16)	1.00 (0.61, 1.63)	0.97 (0.59, 1.60)	1.21 (1.00)
Quartile 4	19 (0.10)	0.59 (0.34, 1.05)	0.59 (0.33, 1.04)	2.84 (1.00)

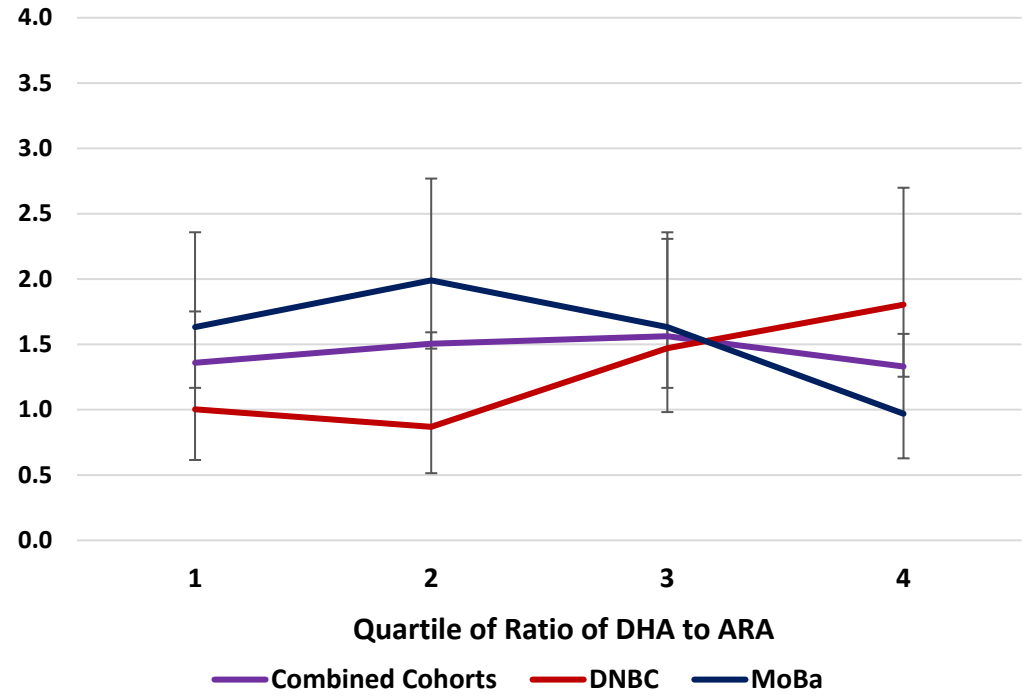
*Adjusted for total energy intake, previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester
Abbreviations: ARA, arachidonic acid; CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid
Imputations = 30

Figure 6.1. Prevalence of cerebral palsy per 1,000 children who were born at or near term and survived to age one by ARA quartile



Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

Figure 6.2. Prevalence of cerebral palsy per 1,000 children who survived to age one by quartile of ratio of DHA to ARA



Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

APPENDIX F:

Tables and Figures Chapter 7

Table 7.1. Maternal characteristics for the term or near-term sample by infection status in the combined cohorts in the MOBAND-CP study

Maternal Characteristics Frequency (%)	Infection	No infection	Missing
Total	40,520	96,934	797
Infection overall	29.3	70.1	0.6
Maternal Age			
24 years and under	10.5	9.3	8.3
25-29 years	36.2	35.7	35.5
30-34 years	37.9	38.5	39.4
35 years and older	15.5	16.5	16.8
Previous Number of Live Births			
0	43.9	45.7	52.7
1	38.0	35.2	30.9
2 or more	16.0	16.0	16.4
Missing	2.2	3.1	(-)
Previous Number of Stillbirths			
0	99.6	99.6	99.6
1 or more	0.4	0.4	0.4
Maternal Occupation			
Employed	77.9	80.1	74.2
Unemployed or receiving benefits or pension	8.7	7.7	9.2
Student	12.5	11.0	16.6
Missing	0.9	1.2	0.1
Marital Status			
Spouse or partner	97.1	97.5	98.0
Single	2.1	1.7	1.9
Missing	0.8	0.8	0.1
Planned Pregnancy			
Yes	84.0	84.8	88.8
No	15.4	14.5	11.0
Missing	0.5	0.7	0.1

Table 7.1 (Cont'd)

Alcohol Use Early in Pregnancy			
Yes	23.9	18.4	45.0
No (less than 0.5 units per week)	71.5	75.7	54.8
Missing	4.7	6.0	0.1
Smoking During Pregnancy			
Yes	24.1	21.5	26.1
No	75.6	78.2	73.5
Missing	0.3	0.4	0.4
Diabetes			
No	98.6	98.7	98.1
Any Diabetes (including gestational)	1.4	1.3	1.9
Exercise During First Trimester			
Physically non-active	48.1	42.9	63.2
One time per week	16.1	17.2	12.5
Two times per week	34.3	38.0	24.1
Missing	1.5	1.9	0.1
Maternal Pre-Pregnancy Body Mass Index			
<23 kg/m^2	49.1	49.6	56.0
23-<25 kg/m^2	18.4	19.4	17.2
25 kg/m^2 or higher	30.4	28.9	25.0
Missing	2.0	2.1	1.9
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: CP, cerebral palsy; MOBAND-CP, MOthers and BAbies of Norway and Denmark-Cerebral Palsy			

Table 7.2. Maternal characteristics for the term or near-term sample by urinary tract infection status in the combined cohorts of the MOBAND-CP study

Maternal Characteristics Frequency (%)	UTI	No UTI	Missing
Total	13,739	123,735	777
UTI Overall	9.9	89.5	0.56
Maternal Age			
24 years and under	13.0	9.2	9.7
25-29 years	36.6	35.8	36.8
30-34 years	35.2	38.7	35.5
35 years and older	15.1	16.3	18.0
Previous Number of Live Births			
0	50.4	44.6	50.2
1	31.6	36.5	33.8
2 or more	15.4	16.1	16.0
Missing	2.6	2.8	(-)
Previous Number of Stillbirths			
0	99.6	99.6	99.5
1 or more	0.4	0.4	0.5
Maternal Occupation			
Employed	75.9	79.8	71.6
Unemployed or receiving benefits or pension	9.5	7.9	10.9
Student	13.6	11.2	17.4
Missing	1.0	1.1	0.1
Marital Status			
Spouse or partner	96.2	97.5	97.9
Single	2.7	1.7	1.9
Missing	1.1	0.8	0.1
Planned Pregnancy			
Yes	82.2	84.8	87.1
No	17.2	14.5	12.7
Missing	0.6	0.7	0.1

Table 7.2 (Cont'd)

Alcohol Use Early in Pregnancy			
Yes	20.2	20.0	45.3
No (less than 0.5 units per week)	74.0	74.5	54.6
Missing	5.8	5.6	0.1
Smoking Early in Pregnancy			
Yes	25.5	21.9	27.0
No	74.2	77.8	72.6
Missing	0.3	0.3	0.4
Diabetes			
No	98.3	98.7	97.8
Any Diabetes (including gestational)	1.7	1.3	2.2
Exercise Early in Pregnancy			
Physically non-active	46.5	44.2	64.0
One time per week	16.4	17.0	10.9
Two times per week	35.3	37.1	25.0
Missing	1.8	1.8	0.1
Maternal Pre-Pregnancy Body Mass Index			
<23 kg/m^2	48.2	49.6	55.1
23-<25 kg/m^2	18.4	19.2	16.5
25 kg/m^2 or higher	31.4	29.1	26.4
Missing	2.0	2.1	2.1
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: CP, cerebral palsy; MOBAND-CP, MOthers and BAbies of Norway and Denmark-Cerebral Palsy			

Table 7.3. Maternal characteristics for the term or near-term sample by fever prior to 28 weeks gestation status in the combined cohorts of the MOBAND-CP study

Maternal Characteristics Frequency (%)	Fever	No Fever	Missing
Total	30,233	107,719	299
Fever	21.9	77.9	0.2
Maternal Age			
24 years and under	9.7	9.6	6.7
25-29 years	36.1	35.8	31.4
30-34 years	38.8	38.2	46.5
35 years and older	15.5	16.4	15.4
Previous Number of Live Births*			
0	41.4	46.3	49.5
1	40.5	34.7	34.4
2 or more	16.2	15.9	16.1
Missing	1.9	3.0	(-)
Previous Number of Stillbirths			
0	99.6	99.6	99.7
1 or more	0.4	0.4	0.3
Maternal Occupation*			
Employed	78.3	79.7	79.9
Unemployed or receiving benefits or pension	8.6	7.9	7.4
Student	12.3	11.2	12.7
Missing	0.8	1.2	(-)
Marital Status*			
Spouse or partner	97.3	97.3	98.3
Single	1.9	1.8	1.7
Missing	0.7	0.8	(-)
Planned Pregnancy*			
Yes	84.5	84.6	90.0
No	15.0	14.7	10.0
Missing	0.5	0.7	(-)

Table 7.3 (Cont'd)

Alcohol Use During Pregnancy*			
Yes	25.6	18.5	46.5
No (less than 0.5 units per week)	70.2	75.6	53.5
Missing	4.2	5.9	(-)
Smoking During Pregnancy*			
Yes	23.8	21.8	23.4
No	76.0	77.8	76.6
Missing	0.3	0.4	(-)
Diabetes			
No	98.7	98.7	98.7
Any Diabetes (including gestational)	1.3	1.3	1.3
Exercise During First Trimester*			
Physically non-active	49.0	43.3	60.2
One time per week	16.0	17.1	14.7
Two times per week	33.6	37.7	25.1
Missing	1.3	1.9	(-)
Maternal Pre-Pregnancy Body Mass Index			
<23 kg/m2	49.5	49.5	58.2
23-<25 kg/m2	18.3	19.4	17.1
25 kg/m2 or higher	30.1	29.1	23.4
Missing	2.0	2.1	1.3
Denominators include children born at 35 weeks gestation or later and who survived to one year of age Abbreviations: CP, cerebral palsy; MOBAND-CP, MOthers and BAbies of Norway and Denmark-Cerebral Palsy			

Table 7.4. Unadjusted and adjusted relative risk of cerebral palsy by infection, urinary tract infection, and fever status in the combined cohorts

Infection variable category	Unadjusted Risk Ratio (95% CI)	Adjusted Risk Ratio* (95% CI)	E-value (lower limit)
Infection			
No	Reference		
Yes	1.12 (0.83, 1.51)	1.13 (0.84, 1.53)	1.51 (1.00)
Urinary Tract Infection			
No	Reference		
Yes	1.13 (0.73, 1.76)	1.11 (0.71, 1.73)	1.46 (1.00)
Fever			
No	Reference		
Yes	1.13 (0.82, 1.57)	1.16 (0.84, 1.61)	1.59 (1.00)
*Adjusted for previous live births, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester Abbreviations: CI, confidence interval;			

Table 7.5. Unadjusted and adjusted relative risk of cerebral palsy by infection status within DHA quartiles in the combined cohorts

DHA status*Infection	Percent CP Exposed (95% CI)[a]	Percent CP Unexposed (95% CI)[a]	Unadjusted Risk Ratio (95% CI)	Adjusted* Risk Ratio (95% CI)
Quartile 1 Infection versus No Infection	0.08 (0.02, 0.13)	0.14 (0.09, 0.18)	0.59 (0.27, 1.27)	0.59 (0.27, 1.49)
Quartile 2 Infection versus No Infection	0.24 (0.15, 0.34)	0.21 (0.15, 0.27)	1.16 (0.72, 1.89)	1.18 (0.73, 1.93)
Quartile 3 Infection versus No Infection	0.15 (0.08, 0.23)	0.08 (0.05, 0.12)	1.89 (0.98, 3.65)	1.92 (1.00, 3.71)
Quartile 4 Infection versus No Infection	0.14 (0.07, 0.22)	0.13 (0.09, 0.18)	1.10 (0.60, 2.04)	1.12 (0.60, 2.07)

*Adjusted for total energy intake, previous live births, marital status, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CI, Confidence Interval; CP, cerebral palsy; DHA, docosahexaenoic acid

Imputations = 30

[a]Percent derived across 30 imputations and the frequency was not used

The reference group in each row is the no infection group for the specified quartile

Table 7.6. Unadjusted and adjusted relative risk of cerebral palsy by urinary tract infection status within DHA quartiles in the combined cohorts

DHA Status * Urinary Tract Infection	Percent CP Exposed (95% CI)[a]	Percent CP Unexposed (95% CI)[a]	Unadjusted Risk Ratio (95% CI)	Adjusted* Risk Ratio (95% CI)
Quartile 1 UTI versus No UTI	0.05 (0.00, 0.13)	0.13 (0.09, 0.17)	0.44 (0.11, 1.83)	0.43 (0.10, 1.79)
Quartile 2 UTI versus no UTI	0.28 (0.11, 0.45)	0.21 (0.16, 0.26)	1.25 (0.62, 2.51)	1.23 (0.61, 2.48)
Quartile 3 UTI versus no UTI	0.23 (0.07, 0.38)	0.09 (0.06, 0.12)	2.66 (1.21, 5.83)	2.61 (1.18, 5.73)
Quartile 4 UTI versus no UTI	0.11 (0.00, 0.20)	0.14 (0, 0.18)	0.64 (0.2, 2.07)	0.63 (0.19, 2.03)

*Adjusted for total energy intake, previous live births, marital status, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid; UTI, urinary tract infection

Imputations = 30

[a]Percent derived across 30 imputations and the frequency was not used

The reference group in each row is the no UTI group for the specified quartile

Table 7.7. Unadjusted and adjusted relative risk of cerebral palsy by fever status within DHA quartiles in the combined cohorts

DHA Status*Fever before 28 weeks of gestation	Percent CP Exposed (95% CI)[a]	Percent CP Unexposed (95% CI)[a]	Unadjusted Risk Ratio (95% CI)	Adjusted* Risk Ratio (95% CI)
Quartile 1 Fever versus No Fever	0.08 (0.02, 0.15)	0.13 (0.09, 0.17)	0.64 (0.27, 1.51)	0.65 (0.28, 1.55)
Quartile 2 Fever versus No Fever	0.24 (0.13, 0.35)	0.21 (0.16, 0.27)	1.13 (0.67, 1.92)	1.16 (0.69, 1.97)
Quartile 3 Fever versus No Fever	0.15 (0.07, 0.24)	0.09 (0.05, 0.13)	1.73 (0.87, 3.46)	1.78 (0.89, 3.55)
Quartile 4 Fever versus No Fever	0.16 (0.07, 0.24)	0.13 (0.09, 0.17)	1.19 (0.62, 2.29)	1.22 (0.63, 2.36)

*Adjusted for total energy intake, previous live births, marital status, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid; Imputations = 30

[a]Percent derived across 30 imputations and the frequency was not used

The reference group in each row is the no fever group for the specified quartile

Table 7.8. Infection, urinary tract infection, and fever by quartile of DHA

DHA Quartile	Infection			Urinary Tract Infection			Fever		
	No Infection	Infection	Missing	No UTI	UTI	Missing	No Fever	Fever	Missing
Quartile 1	70.4	29.0	0.5	89.2	10.3	0.5	78.6	21.2	0.2
Quartile 2	70.3	29.1	0.6	89.6	9.8	0.6	78.1	21.7	0.2
Quartile 3	69.9	29.5	0.6	89.7	9.7	0.6	77.4	22.3	0.3
Quartile 4	69.8	29.3	0.6	89.5	9.9	0.6	77.5	22.3	0.2

Abbreviations: CI, confidence interval; DHA, Docosahexaenoic acid; DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort; UTI, urinary tract infection

Table 7.9. *Post hoc* analysis of effect modification of association between infection variables and DHA from food

Infection	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
Quartile 1 Infection versus No Infection	0.20 (0.08, 0.34)	0. 14(0.08, 0.19)	1.56 (0.73, 3.32)	1.56 (0.73, 3.33)
Quartile 2 Infection versus No Infection	0.13 (0.03, 0.23)	0.18 (0.11, 0.25)	0.71 (0.29, 1.71)	0.71 (0.29, 0.58)
Quartile 3 Infection versus No Infection	0.21 (0.08, 0.34)	0.14 (0.08, 0.20)	1.47 (0.69, 3.12)	1.46 (0.69, 3.11)
Quartile 4 Infection versus No Infection	0.17 (0.05, 0.28)	0.14 (0.08, 0.19)	1.24 (0.55, 2.83)	1.23 (0.54, 2.8)
UTI	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
Quartile 1 UTI versus No UTI	0.26 (0.03, 0.50)	0.14 (0.09, 0.20)	1.88 (0.72, 1.26)	1.84 (0.71, 1.59)
Quartile 2 UTI versus No UTI	0.06 (0.00, 0.17)	0.18 (0.12, 0.24)	0.33 (0.04, 2.38)	0.32 (0.04, 2.36)
Quartile 3 UTI versus No UTI	0.23 (0.00, 0.45)	0.15 (0.09, 0.21)	1.51 (0.53, 4.31)	1.46 (0.51, 4.19)
Quartile 4 UTI versus No UTI	0.22 (0.00, 0.43)	0.14 (0.08, 0.19)	1.59 (0.55, 4.57)	1.55 (0.54, 4.46)
Fever	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
Quartile 1 Fever versus No Fever	0.19 (0.04, 0.33)	0.14 (0.09, 0.21)	1.26 (0.52, 3.08)	1.28 (0.52, 3.13)
Quartile 2 Fever versus No Fever	0.15 (0.02, 0.25)	0.17 (0.11, 0.24)	0.87 (0.34, 2.26)	0.89 (0.34, 2.30)
Quartile 3 Fever versus No Fever	0.18 (0.04, 0.32)	0.15 (0.09, 0.21)	1.15 (0.47, 2.81)	1.16 (0.48, 2.83)
Quartile 4 Fever versus No Fever	0.12 (0.03, 0.24)	0.15 (0.09, 0.21)	0.83 (0.29, 2.41)	0.83 (0.29, 2.41)

*Adjusted for total energy intake, previous live births, marital status, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

Abbreviations: CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid; UTI, urinary tract infection

Imputations = 30

[a]Percent derived across 30 imputations and the frequency was not used

The reference group in each row is the no fever group for the specified quartile

Table 7.10. *Post hoc* analysis of effect modification of association between infection variables and DHA from supplements

Infection	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
No Use Infection versus No Infection	0.11 (0.03, 0.20)	0.13 (0.08, 0.19)	0.84 (0.36, 1.93)	0.85 (0.37, 1.95)
Low Infection versus No Infection	0.23 (0.07, 0.40)	0.21 (0.12, 0.30)	1.10 (0.49, 2.47)	1.10 (0.49, 2.48)
Moderate Infection versus No Infection	0.23 (0.10, 0.37)	0.15 (0.09, 0.22)	1.50 (0.73, 3.08)	1.50 (0.73, 3.07)
Quartile Infection versus No Infection	0.17 (0.05, 0.29)	0.11 (0.06, 0.17)	1.50 (0.65, 3.48)	1.48 (0.64, 3.43)
UTI	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
No Use 1 UTI versus No UTI	0.16 (0.00, 0.32)	0.13 (0.08, 0.17)	1.26 (0.44, 3.58)	1.26 (0.44, 3.59)
Low UTI versus No UTI	0.16 (0.00, 0.38)	0.22 (0.14, 0.31)	0.72 (0.17, 3.02)	0.70 (0.17, 2.94)
Moderate UTI versus No UTI	0.34 (0.06, 0.61)	0.16 (0.10, 0.21)	2.19 (0.91, 5.29)	2.13 (0.89, 5.14)
High UTI versus No UTI	0.11 (0.00, 0.28)	0.13 (0.08, 0.18)	0.90 (0.21, 3.83)	0.87 (0.20, 3.86)
Fever	**Percent CP Exposed (95% CI)[a]**	**Percent CP Unexposed (95% CI)[a]**	**Unadjusted Risk Ratio (95% CI)**	**Adjusted* Risk Ratio (95% CI)**
No Use Fever versus No Fever	0.07 (0.00, 0.16)	0.14 (0.09, 0.19)	0.52 (0.16, 1.70)	0.53 (0.16, 1.72)
Low Fever versus No Fever	0.24 (0.05, 0.44)	0.21 (0.12, 0.30)	1.17 (0.48, 2.85)	1.19 (0.49, 2.91)
Moderate Fever versus No Fever	0.18 (0.04, 0.32)	0.17 (0.11, 0.23)	1.05 (0.43, 2.52)	1.05 (0.44, 2.53)
High Fever versus No Fever	0.18 (0.04, 0.33)	0.12 (0.06, 0.17)	1.56 (0.62, 3.90)	1.56 (0.62, 3.93)

*Adjusted for total energy intake, previous live births, marital status, maternal occupation, alcohol use up to the first interview or questionnaire, maternal body mass index, and exercise during the first trimester

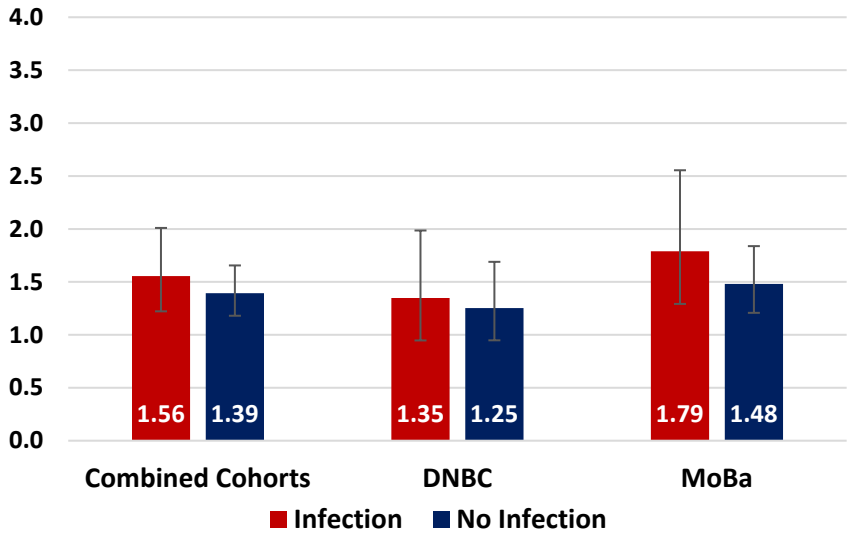
Abbreviations: CP, cerebral palsy; CI, confidence interval; DHA, docosahexaenoic acid; UTI, urinary tract infection

Imputations = 30

[a]Percent derived across 30 imputations and the frequency was not used

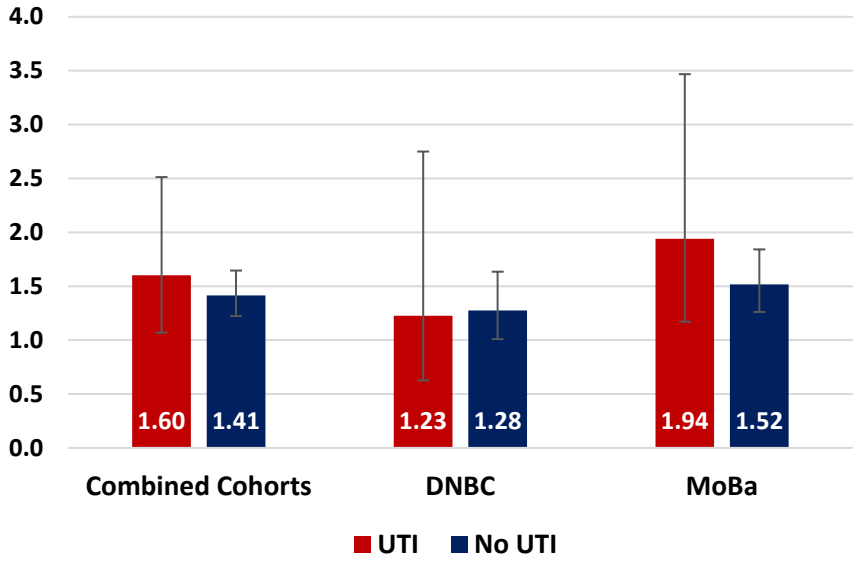
The reference group in each row is the no fever group for the specified quartile

Figure 7.1. Prevalence of cerebral palsy per 1,000 children born at or near term who survived to age one by infection and cohort



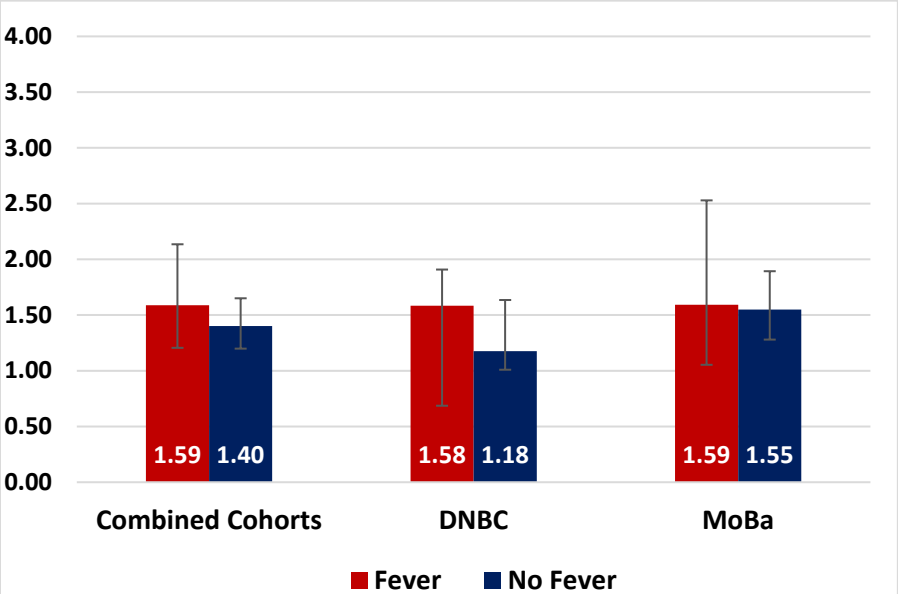
Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

Figure 7.2. Prevalence of cerebral palsy per 1,000 children born at or near term who survived to age one by urinary tract infection status and cohort



Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

Figure 7.3. Prevalence of cerebral palsy per 1,000 children born at or near term who survived to age one by fever status and cohort



Abbreviations: DNBC, Danish National Birth Cohort; MoBa, Norwegian Mother and Child Cohort

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