THE EFFECT OF OMEGA-3 AND OMEGA-6 POLYUNSATURATED FATTY ACIDS ON ILLNESS IN CHILDREN UP TO 4 YEARS OF AGE

by

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Abstract

Supplementation of omega-3 (n-3) and omega-6 (n-6) long-chain polyunsaturated fatty acids (LCPUFA) during the first year of life has been associated with a decreased incidence of illness in children. The need to determine the most effective dose of n-3 and n-6 LCPUFA for infants and children remains, especially in regards to docosahexaenoic acid (DHA).

This study was a double-blind, 2-phase, randomized, controlled, parallel-group, prospective trial. The primary outcome of the study was to evaluate whether DHA and ARA supplemented infant formula affected visual evoked potential acuity in term infants. For the purposes of this thesis project, the outcome is illness as recorded through adverse events during the original study from birth to 4 years of age. One-hundred and fifty-nine infants were randomized into 4 groups of which 91 were eligible for medical record review. Infants were given milk-based infant formula containing no DHA (control formula, n=19), 0.32% DHA (n=25), 0.64% DHA (n=19), or 0.96% of total fatty acids as DHA (n=28). The study was controlled for ARA (0.64% of the total fatty acids) with a dose response to DHA – the control group received no DHA or ARA.

Significantly fewer illnesses were seen in children supplemented with DHA and ARA when compared to the control during the first four years of life. Subjects receiving the formula that contained 0.96% DHA from fatty acids were significantly less likely to be diagnosed with any illness during the first year of life ($p = 0.01$) when compared to the control group. These subjects were also less likely to be

diagnosed with respiratory diagnoses ($p = 0.05$), any allergy diagnoses ($p = 0.003$), combined wheeze/asthma/skin diagnoses ($p = 0.003$), and skin allergic illnesses ($p = 0.02$). Additionally, the 0.64% DHA group experienced fewer ears, eyes, nose, and throat diagnoses ($p = 0.05$) during the first year of life.

In conclusion, DHA and ARA supplementation of infant formula was associated with a reduced incidence of overall diagnoses, respiratory diagnoses, allergic diagnoses, skin diagnoses, combined wheeze/asthma/skin allergic diagnoses, as well as skin allergic illness diagnoses in children up to 4 years of age.

Acknowledgements

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In-Text Figures and Tables

Chapter 1

Introduction

Several previous studies have suggested that omega-3 polyunsaturated fatty acids cause a reduction of illness, such as infections, respiratory illness, asthma, and dermatologic conditions, in children (1-7). Dietary fats, like omega-3 and omega-6 fatty acids, play a role in regulating immune function and inflammation (8). It is these functions that suggest the importance of long-chain polyunsaturated fatty acids (LCPUFA) in preventing and reducing illness in children. While two previous studies have addressed this topic in infants(2,5), only one(2) used random assignment and that study was done in a higher socioeconomic population and provided only one level of formula LCPUFA in addition to the control. The most similar study was the study conducted by Pastor et al (5). In this study, 1,342 infants were enrolled, and the experimental group was given 17 mg DHA and 34 mg ARA per 100 kilocalories of formula. Although this study had adequate power, the study was open-label, and thus the researchers were aware of the treatment assignment. Also, the control group did not seem to truly be controlled, since those infants consumed formula containing 0 to 16 mg DHA and 0 to 13 mg ARA. The other studies relating to the effect of DHA and ARA consumption on illness(1-7) did not investigate the effect in infants from birth to 12 months, the effect of consumption of differing amounts of DHA with 34 mg ARA, the effect on a greater variety of illnesses, or the effect on infants in the United States population.

Statement of Purpose

The purpose of this study is to determine if infants who consume DHA and ARA fortified formula are less likely to have reports of infections, asthma episodes, respiratory illnesses, and dermatologic conditions. Additionally, I plan to determine the effect of dose of formula containing DHA and ARA on illness in infants from birth until 4 years of age.

Research Questions

- If an infant consumes infant formula fortified with DHA and ARA from birth to 12 months, will they be less likely to have episodes of illness such as infections, asthma, respiratory illnesses, and dermatologic conditions during the first 12 months of life when compared to the control group receiving no DHA or ARA?
- If an infant consumes infant formula fortified with DHA and ARA from birth to 12 months, will they be less likely to have episodes of illness such as infections, asthma, respiratory illnesses, and dermatologic conditions during the first 4 years of life?
- Will higher doses of DHA (0.64% or 0.96% fatty acids from DHA)with ARA intake consumed in infant formula cause fewer episodes of illness compared to lower doses of DHA (no DHA or 0.32% fatty acids from DHA) with ARA intake consumed from infant formula?

Chapter 2

Review of Literature

Docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) are long-chain polyunsaturated fatty acids (LCPUFAs) that are consumed directly from the diet, or they can be produced by the body from α-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6), respectively (8). Docosahexaenoic acid (DHA) is found in the membrane phospholipids of the retina and brain, and has been found to rapidly accumulate in these tissues during early infancy (9). Specifically, DHA is found in high concentrations in the gray matter of brain tissue, and in the rod and cone outer segment membranes of the retina (10). The realization that high levels of DHA are found in these tissues and that DHA is found in human milk has led to the hypotheses that DHA is important for visual and neurological development for the developing fetus and during early infancy (9). Visual acuity development has been studied frequently, but few studies have looked at indications of the developing immune system.

DHA and Lactation

DHA is found in human milk, and the amount varies greatly as a result of differing maternal diets. DHA supplementation of lactating women has been found to increase breast milk DHA content (11). Even though DHA can be produced by the body from its precursor, alpha-linolenic acid (ALA), supplementation of the precursor does not increase breast milk DHA content (12). Genetics also play a role in the amount of DHA in breast milk and the human body. Lactating women with the 347S

variant of the apolipoprotein A-IV gene have been shown to have about 40% more DHA in their milk compared to lactating women with the 347T variant (13).

DHA Concentrations in Breast Milk

As noted above, the DHA content of women's milk is influenced by factors including dietary intake and genetics (13,14). In a meta-analysis, Brenna et al. (14) analyzed 65 studies detailing the fatty acid composition of human breast milk to estimate average fatty acid profiles. Analysis of the 65 studies involving 2474 women gave a mean concentration of DHA in breast milk of 0.32% ± 0.22% of total fatty acids with a range of 0.06-1.4%. The mean concentration of ARA was 0.47 ±0.13% of total fatty acids with a range of 0.24-1.0%. These profiles and ranges showed that DHA was highly variable compared to ARA found in breast milk (14).

Meneses et al. (15) studied the relationship between maternal age and milk DHA. They asked if adolescents would have lower LCPUFA levels in breast milk, since adolescents typically have lower dietary intakes of LCPUFA when compared to adults. Among Brazilian adolescents aged 14-19 years, they found that there was no significant difference of individual LCPUFA in breast milk when compared to Brazilian adults of the same socioeconomic status. This result was interesting since the adolescents' mean dietary intakes of n-3 and n-6 LCPUFA were lower when compared to the lactating adults. The study did not provide data as to the dietary intake of specific LCPUFA, but the researchers did mention that there was a low intake of n-3 LCPUFA among the adolescents since only 10% reported a regular intake of fish and fish products. The researchers theorized that the transfer of

essential fatty acids and LCPUFA to the mammary glands could take precedence over transfer to maternal tissues, and this is what could have caused the adolescent mothers to have breast milk with similar LCPUFA profiles when compared to adults with higher mean dietary intakes of n-3 and n-6 LCPUFA. This was a cross-sectional study involving 30 Brazilian adolescents with a mean age of 16.6 years. Inclusion criteria included a healthy full term singleton pregnancy, exclusively or predominately breastfeeding, and 2-6 years post menarche (mean of 5.0 years). 'Predominately breastfeeding' was defined as breast feeding plus sparse use of water, tea/herbal infusions, and water diluted fruit juices. Breast milk and blood samples were obtained between 30 and 120 days postpartum after an 11 hour fast.

Multiple studies (11,13,15-17) have shown that DHA levels in breast milk can be increased through supplementation. A study involving pregnant women in Western Australia found that breast milk from women who received fish oil contained higher amounts of DHA and eicosapentaenoic acid (EPA) when compared to controls at 3 days and 6 weeks after delivery (16). This was a randomized controlled, double-blind study involving 98 pregnant women who delivered after 36 weeks of gestation. The experimental group was given 4 fish oil capsules containing 2.2 g DHA and 1.1 g EPA, and the control group was given four 1 g olive oil capsules.

In the Netherlands, a study found that breast milk ARA and DHA responded to maternal supplementation of these fatty acids, and that the amounts of ARA and DHA in breast milk declined with advancing lactation (17). This study examined the influence of supplementation with ARA and DHA or DHA alone from early

pregnancy through 12 weeks of lactation on the composition of milk ARA, DHA, and the ratio of DHA:ARA. One-hundred eighty-two women participated in the study of which 69 breastfed for at least 12 weeks. DHA was more responsive to supplementation than ARA. DHA supplementation resulted in decreased ARA in breast milk.

Dietary Recommendations of DHA during Pregnancy and Infancy

There are currently no US Dietary Reference Intakes (DRI) for DHA. In 2002, the Food and Nutrition Board of the Institute of Medicine concluded that there was a lack of evidence for setting a DRI for n-3 or n-6 fatty acids. Instead, they set Adequate Intakes (AI) based on dietary surveys measuring the median n-3 and n-6 intakes in the United States (10). The current recommended AI for n-3 fatty acids during pregnancy is 1.4 grams/day and 1.3 grams/day during lactation, and is linked to ALA intake rather than to DHA intake. The AI for n-6 fatty acids is 13 grams/day during pregnancy and lactation, and is based on total n-6 fatty acids, primarily LA (18).

Innis describes how estimates of requirements have typically been achieved in the past (10). To estimate a requirement, the necessary information would include the knowledge of "the intake needed to maintain a given circulating level, tissue concentration, or adequacy of molecular function", "the intake of individuals in groups, which are associated with the absence of any signs of deficiency", "the intake needed to maintain balance, considering intake in relation to status", and "studies of subjects maintained in diets containing low or deficient levels of a nutrient followed

by correction of a deficit when measured amounts of that nutrient are provided." These criteria were used to set total n-3 and n-6 AIs.

Interestingly, it was found by Schwartz et al. (19) that infants consuming present-day formula supplemented with LCPUFAs were able to achieve plasma LCPUFA concentrations similar to that of breastfed infants, but those receiving only ALA and LA were not. In this study, the parents chose what formula they wanted to feed their infant, and the categories included breast milk, formula with no LCPUFAs, and formula with LCPUFAs. They were instructed to fully milk feed (formula-feed or breastfeed) their infants until at least 4 months of age.

An AI for DHA has not been set because at the time of the last review (10) in 2002, functional effects of DHA supplements were not generally known. This might not be the case today.

Benefits of DHA from Breast Milk and Supplemented Infant Formula

Polyunsaturated fatty acids (PUFAs), including DHA, modulate gene expression, regulate the production of eicosanoids (that play a role in inflammation), and regulate the physical properties of cell membranes. Because of these properties, PUFAs play a role in the prevention of neurological disorders and in the etiologies of chronic disease (20).

In a prospective cohort study investigating the relationship between DHA and neurophysical functioning, it was found that children who breastfed or were fed DHA-fortified formula for the first 6 months after birth had higher mean full-scale and verbal IQ scores at 4 years when compared to those who were fed mainly

unfortified formulas (21). This study took place in Southampton, UK, and included 241 children who were followed from birth to 4 years of age. The researchers of this study did note that the differences in the children's IQ scores according to the type of formula that they were fed may be more due to confounding by maternal or family characteristics than to the amount of PUFAs they received in the formulas. They determined that this difference could be explained by maternal education and intelligence. The difference could also be explained by the increased cost of infant formula in comparison to the cost of breastfeeding as well as the increased cost of formulas supplemented with DHA.

Birch et al. (22) found that the supplementation of infant formula with DHA and ARA helps to support IQ and visual acuity at 4 years of age similar to that of infants who were breast-fed. In their study, 79 infants were enrolled and were given infant formula supplemented with microalgal and microfungal DHA and ARA, respectively, for the first 17 weeks of life. Of the 79 healthy term infants, 52 were available for testing at 4 years of age. Additionally, 40 healthy term breast-fed infants were enrolled in the study for comparison, of which 32 were available for testing at 4 years of age. The researchers also found that the children who were fed formula with no DHA or ARA had significantly lower visual acuity and verbal IQ scores in comparison to the children who were breast-fed for an average of 43 weeks.

Immunity and Fatty Acid Intake

During the immune response, organisms and substances that cause disease or invade our body are identified, attacked, and eliminated. The immune cells used

during this response are produced in bone marrow and are then stored throughout the body. When these cells enter the bloodstream, they are usually referred to as the white blood cells or leukocytes (8).

There are 2 types of immunity, and these are known as innate and acquired. Innate immunity is the first line of defense against a foreign organism or substance in the body. This type of immunity uses no memory of the foreign agent, and begins with the production of cytokines at the site of the infection and inflammation. Cytokines are specialized chemical mediators that in turn cause movement of immune cells to the injured site. Specialized cells are then activated to identify and destroy bacteria, and remove dead cells and foreign substances (8).

The presentation of antigens causes the activation of acquired immunity. Acquired immunity is specific and is developed throughout the human life. It involves immunological memory and the help of T and B lymphocytes. T lymphocytes are involved with the cell-mediated immune responses and B cells are involved with the humoral immune response. This type of immunity is specific to the recognition of non-self antigens, which in turn allows tailored responses towards specific antigens or pathogen-infected cells (8).

Autoimmunity is "the failure of an organism to recognize its own constituent parts as self, which results in an immune response against its own cells and tissues." Any disease that causes an immune response in your body is called an autoimmune disease (8).

Dietary fats are known to play a role in regulating immune function and inflammation. The functional roles of fatty acids are mostly due to the products of LA and ALA, the n-6 and n-3 LCPUFAs, respectively. DHA and ARA are precursors to lipid mediators that play a role in the activation and resolution of the inflammatory process. ARA is a precursor for eicosanoids that activate pro-inflammatory mediators, and yet at the same time the eicosanoids produce lipoxins that activate anti-inflammatory mediators. Docosanoids are made from DHA that produce resolvins and protectins to activate anti-inflammatory mediators. Many of these mediators are involved in cell signaling processes leading to the activation or resolution of the inflammatory process (8).

The anti- and pro-inflammatory properties of DHA and ARA have led to the research of their effect on illnesses that exhibit inflammatory processes. Several previous studies (1-7) have suggested that n-3 polyunsaturated fatty acids cause a reduction of illness, such as infections, respiratory illness, asthma, and dermatologic conditions, in children.

Illness in Children Supplemented with DHA

Thienprasert et al. (3) conducted a placebo-controlled, randomized, double-blind study and found that fish oil supplementation reduced illness in healthy 9 to 12-year-old Thai schoolchildren. The experimental group receiving the fish oil treatment consumed UHT (ultra heat treatment) chocolate milk that contained 200 mg EPA and 1 g DHA in 2 g oil once a day, five times a week for 6 months. The control group consumed UHT chocolate milk containing 2 g of soybean oil. Episodes and duration

of illness were recorded daily during the intervention period by research assistants. The types of illness identified and recorded included rhinitis, the common cold, influenza, and diarrhea. The researchers found that supplementation of the chocolate milk with EPA and DHA was an effective method at increasing the blood status of the fatty acids in the children. There was a significant difference ($p < 0.012$) when comparing fatty acid status before and after supplementation, as well as when comparing fatty acid status after supplementation between placebo and treatment groups ($p < 0.001$). It was found that participants receiving the fish oil treatment were significantly less likely ($p = 0.006$) to become ill due to any of the recorded illnesses. In particular, they tended to be less likely ($p = 0.089$) to have febrile illnesses. Also, the duration of their illnesses was significantly shorter in the group receiving the fish oil ($p = 0.014$). These findings suggest that the improvement in immune function, or more specifically, host defense could be due to the supplementation of the above mentioned fatty acids in the schoolchildren.

In a study conducted at the University of Kansas Medical Center, researchers found that the incidence of illness was decreased in toddlers who were fed a DHA-supplemented toddler formula for two months (1). Children were randomly assigned to receive a cow-milk based toddler formula that contained 0 mg, 43 mg, or 130 mg DHA per 237 ml. The parents were instructed to provide the study formula as a milk replacement for one meal each day for 60 consecutive days. To determine the incidence of illness, medical records were collected at the end of the study, and parents were questioned at the 2nd and 3rd visit about the health of their child during

the previous month. Among different classes of adverse events reported, an overall group difference ($p = 0.024$) was detected only in the number of participants experiencing one or more respiratory illnesses. The participants receiving the 130 mg DHA supplemented formula experienced significantly fewer events of all illnesses ($p = 0.007$) than those receiving the control (0 mg DHA) formula.

Respiratory Illness

Pastor et al. (5) followed illness in 1,342 infants who were assigned to formulas with or without varying amounts of DHA and ARA. They found that infants receiving DHA and ARA-enriched formula had fewer episodes of bronchitis and bronchiolitis. The study while prospective was not blinded. Three-hundred fifty-seven pediatricians from different areas of Spain enrolled about 5 participants each. One-thousand ninety-four infants received 17 mg DHA and 34 mg ARA for every 100 kilocalories of formula, and 248 infants received levels of DHA and ARA ranging from 0 to 16 mg DHA and 0 to 13 mg ARA per 100 kilocalories of formula. On average, the infants began their assigned formulas at about the age of 1 month. Infants fed lower DHA and ARA amounts had a greater incidence of bronchitis/bronchiolitis at 5 (13.9% compared to 6.1%), 7 (10.8% vs. 5.1%), and 9 (11.3% vs. 5.8%) months of age. Additionally, there were greater occurrences of rhinitis ($p = 0.05$) and upper respiratory infections ($p = 0.05$) at 1 month in the control group compared to the DHA treatment group. At 12 months, a higher incidence of upper respiratory infections was also found in the control group ($p = 0.01$). The results of this study support previous research of LCPUFA roles in protecting against

respiratory symptoms (1-3). It was also noted by investigators (5) that the study design did not allow them to pinpoint whether the reason for the reduction in illness was due to a reduction of infection or inflammatory response.

In a recent study, DHA/ARA supplementation of infant formula was associated with a reduced incidence of upper respiratory infections, common allergic diseases, and wheezing or asthma in children up to 3 years of age (2). Beginning at ≤ 5 days until 12 months of age, infants consumed either a control formula (Enfamil with iron; Mead Johnson Nutrition, Evansville, Indiana), or a formula supplemented with DHA and ARA (Enfamil LIPIL; Mead Johnson Nutrition) containing 0.32% - 0.36% of total fatty acids as DHA and 0.64% - 0.72% of total fatty acids as ARA. Infants were recruited from two Dallas area hospitals, and all were singleton births born between 37 and 40 weeks of gestational age. To be included in the study, the infants had to be healthy term infants and exclusively formula-fed. The original objectives of the study were to evaluate the effect of DHA and ARA supplementation on visual cortex maturity, and to evaluate the effect of LCPUFA on metabolic parameters. The objective of this study was to investigate the incidence of allergic manifestations and common respiratory illnesses in children using chart review. The medical charts of the infants were reviewed by study nurses who were unaware of the infant's formula assignment. The charts were reviewed for diagnoses of upper respiratory infection, wheezing, asthma, reactive airway disease, bronchiolitis/bronchitis, pneumonia, allergic rhinitis, allergic conjunctivitis, otitis media, sinusitis, food allergy, atopic dermatitis, urticaria, and drug allergy. The

medical records of 89 infants were reviewed, of which 51 received the control formula and 38 received the DHA/ARA supplemented formula.

Asthma and Allergies

A descriptive cross-sectional study found that children aged 8-11 years who regularly consumed fresh, oily fish had a decreased risk (odds ratio of 0.26) of current asthma compared to children who did not regularly consume fresh, oily fish (7). Asthma was defined as recent wheeze and airway hyperresponsiveness (AHR) to exercise. Parents of the children completed a questionnaire on occurrence of asthma or wheeze in the previous 12 months, and a food frequency questionnaire that provided information on their fish intake. Eight hundred and eight children were randomly selected from schools in Sydney, Australia, and 468 families completed the questionnaires.

In a study conducted by Hwang et al. (4), it was found that the amounts of red blood cell (RBC) n-3 PUFA, including EPA and DHA, were significantly lower in preschoolers who had atopic dermatitis, allergic rhinitis, and asthma than those without atopic illnesses. The preschoolers with atopy also had significantly higher n-6 PUFA, particularly ARA. The researchers concluded that the n-3 PUFA of the RBC membrane could be a marker for risk of atopy in early childhood, and that fatty acid disturbances may be an indicator of risk for early childhood atopic diseases. This study included 497 children aged 4-6 years from Pusan, South Korea. Twenty-nine percent of the children had atopic disease including atopic dermatitis, allergic rhinitis, and asthma.

N-3 LCPUFA are anti-inflammatory (8). At least one study (6) found that n-3 LCPUFA from fish oil was effective for the treatment of asthma symptoms. Nagakura et al. (6) found that dietary supplementation with fish oil in children was beneficial for children with bronchial asthma in a strictly controlled environment (6). The study was double-blind, randomized, and controlled. For the first 2 months, the children were observed and data were recorded. For the next 10 months, fish oil was administered. During the administration period, 30 children 4-17 years of age were given fish oil capsules (300 mg) containing 34 mg EPA and 36 mg DHA or a placebo of 300 mg olive oil. The children were instructed to take 6-12 capsules/day depending on their weight. This gave a daily range of 17.0-26.8 mg/kg of EPA, and 7.3-11.5 mg/kg DHA. An acetylcholine inhalation test was administered to the children, to test for responsiveness to acetylcholine. The researchers found that asthma symptom scores were decreased and the responsiveness to acetylcholine was decreased in the fish oil group when compared to the control group.

Behavior and Neurodevelopment

In an open-label, proof-of-efficacy pilot study, researchers investigated the effect of high-dose EPA/DHA supplementation on behavior in children with ADHD (23). They found that the supplementation of EPA and DHA led to significant increases in EPA and DHA, and significant decreases in the ARA:EPA ratio. Additionally, significant improvements in behavior were noted with respect to inattention, hyperactivity, oppositional/defiant behavior, and conduct disorder. This pilot study included 9 children in Sudbury, MA who were originally given 2

tablespoons of a liquid EPA/DHA supplement containing 10.8 g EPA and 5.4 g DHA per day. At 4 weeks, the dose of the EPA/DHA supplements was adjusted depending on the ratio of ARA to EPA in the plasma phospholipids to increase the likelihood that the subjects would reach a level normally found in the Japanese population. The goal was to reach an ARA:EPA ratio between 1.5 and 3.

In a meta-analysis by Beyerlein et al. (24), no significant difference in Bayley mental scales or psychomotor developmental indexes were found at 18-months in toddlers given a formula supplemented with LCPUFAs as infants compared to those fed a control formula with no LCPUFAs. The LCPUFA-supplemented formulas contained 0.17-0.5 g DHA and 0.04-0.4 g ARA per 100 g fat. The analysis included data from 870 children from 4 large randomized clinical trials in which children were given infant formula with or without LCPUFAs.

Summary

Several previous studies have shown a reduction of illness in children supplemented with DHA during infancy. However, the need remains to determine the effect of consumption of differing amounts of DHA with a controlled amount of ARA, the effect on a greater variety of illnesses, and the effect on infants in the United States population. The ability to answer these questions would aid the Food and Nutrition Board of the Institute of Medicine in determining the appropriate DRI for DHA and ARA.

Chapter 3

Methods

This was a double-blind, 2-phase, randomized, controlled, parallel-group, prospective trial. The primary outcome of the study was to evaluate whether DHA and ARA supplemented infant formula affected visual evoked potential acuity in term infants. For the purposes of my thesis research, the outcome is illness as recorded through adverse events during the study. An adverse event is defined as "any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the participation in a clinical study whether or not related to study product." Adverse events were recorded from 7 days of age to 4 years of age.

Ethics

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. The protocol, amendments and informed consent were reviewed and approved by the Institutional Review Board Ethics Committee and the Human Subjects Committee of the University of Kansas Medical Center in Kansas City, KS. Written informed consent was obtained for every participant from his/her parent or guardian prior to participation in the clinical trial.

Subject Selection

To be included in this study, participants (the infant) had to meet the following inclusion criteria: singleton birth, 37 – 42 weeks gestational age, birth weight 2490 to 4200g, five to nine days of age at randomization, and solely formula

fed for at least 24 hours prior to randomization. Participants were excluded from the study if they had a history of underlying disease or congenital malformation which was likely to interfere with the normal growth and development of the participant. Other exclusion criteria included chronic illness of the mother likely to influence the growth and development of the participant, if the infant was breast-fed within 24 hours prior to randomization, and any evidence of formula intolerance or poor intake of formula at the time of randomization.

Women who had stated that they had planned to feed infant formula to their newborn were approached for recruitment on the day they were expected to deliver. If they expressed interest in allowing their child to enroll in the study and met the inclusion criteria and none of the exclusion criteria, they were contacted after birth and the study was explained thoroughly. Written informed consent was collected if the mother decided to participate in the study.

Randomization

The infants were randomized into 4 groups and were given milk-based infant formula containing no DHA (control formula), 0.32% DHA, 0.64% DHA, or 0.96% of total fatty acids as DHA. The study was controlled for ARA (0.64% of the total fatty acids) with a dose response to DHA – the control group received no DHA or ARA. The DHA was from an algal source and the ARA was from a fungal source. The formula was consumed from birth until 12 months of age, and the mother was asked to not breastfeed during that time.

Data Collection

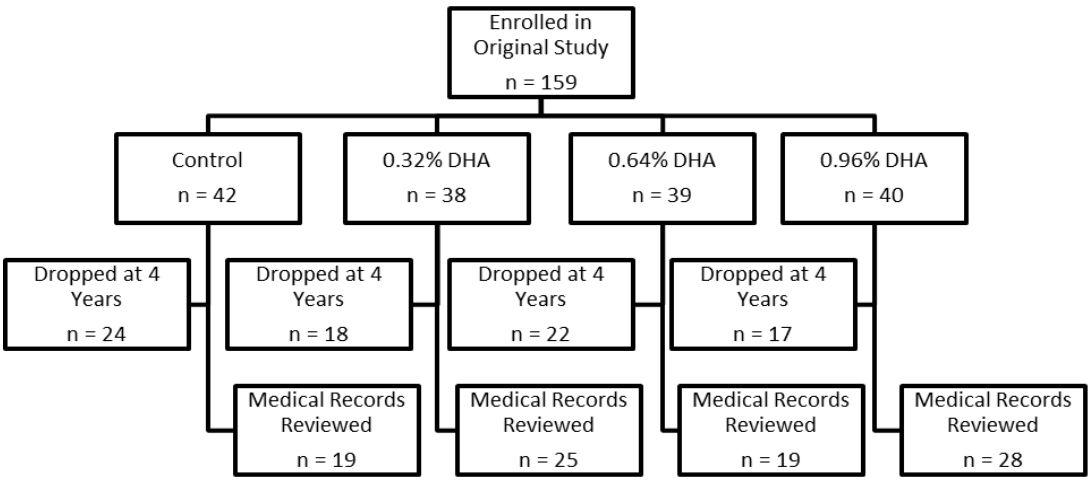
Blood samples measuring DHA and ARA in erythrocyte membranes were taken at 4 months and 12 months. When available, cord blood was also analyzed at birth. Episodes of illness and the length of illness were recorded based on medical doctor visits and caretaker reports. To obtain medical records, consent was obtained from the parent giving permission to request medical records from any hospital or clinic a child had attended (See **Appendix B**). Medical record requests were faxed once a year to all reported hospitals and clinics attended by the child around the time of each child's birthday. At each visit, the caretaker was asked whether their child had been sick or not acting well since his/her last visit (See **Appendix C**). Whether the child was treated at home or by a doctor was recorded, as well as any treatment or medication the child had received. Additionally, parent reports of a doctor visit were verified with medical records requested by the study research assistants. Adverse events were recorded from 42 ± 7 days of age until 4 years ± 7 days of age at each study visit. Visits were at 7-9 days, 6 weeks, 4, 6, 9, 10, 12, and then every 6 months until 4 years of age of the infant. Adverse events were recorded and coded using codes under the following categories: body as a whole (i.e. irritability); eyes, ears, nose, throat (i.e. otitis media); gastrointestinal (i.e. diarrhea); metabolic and nutrition (i.e. weight loss); nervous system (i.e. seizure/convulsion); respiratory (i.e. upper respiratory infection); skin (i.e. diaper rash); urogenital (i.e. urinary tract infection); and other. See **Appendix F** for more details on adverse event codes. Consecutive illnesses were considered separate if they occurred greater than 28 days of each other,

except for allergic diagnoses (any allergy diagnoses) that were considered separate diagnoses after 7 days of duration. Adverse events were then grouped into categories for comparison and statistical analysis (See **Appendix E**).

Subjects

A total of 159 subjects participated in the original DHA study, of which 91 children had adequate medical records and were eligible for medical record review. A medical record was considered adequate if the subject had not dropped from the current follow-up study. Additionally, medical records of dropped subjects were reviewed if the age of the child at the last medical record received was at least 3 years or greater. As seen in **Figure 1**, there were 19 participants in the control group, 25 participants in the 0.32% DHA group, 19 participants in the 0.64% DHA group, and 28 participants in the 0.96% DHA group that were eligible for medical record review.

Figure 1. Consort Diagram



Statistical Analysis

Characteristics of the study population were analyzed using the Chi-squared test and ANOVA, reported as n (%) and mean ± standard deviation, respectively. The incidence of illness was calculated using Chi-squared comparing the combined treatment groups to the control group. Statistical differences found between the average number of illnesses per child was calculated using an equal variance T-test comparing each of the treatment groups to the control group. Odds ratio was calculated using the Fisher exact probability test. Results were considered significant if there was a p value less than 0.05. SPSS and Excel were used for running statistical tests.

Chapter 4

Results

Subject Characteristics

There were no significant differences among the characteristics of the study subjects, as shown in **Table 1**.

Table 1. Characteristics of the study population

Variable	Control (n=19)	0.32% DHA (n=25)	0.64% DHA (n=19)	0.96% DHA (n=28)	*P* value
Male*	8 (42%)	10 (40%)	6 (32%)	12 (43%)	0.88
Hispanic*	0 (0%)	2 (8%)	0 (0%)	3 (11%)	0.13
Caucasian*	2 (11%)	8 (32%)	7 (37%)	7 (25%)	0.13
Birth weight, g**	3375±384	3522±370	3364±317	3380±363	0.387
Birth length, cm**	49.7±1.82	50.5±1.86	50.1±1.84	50.2±1.56	0.516
Weight at 1 year, g*	9587±1090	10052±1132	9671±1200	10107±1192	0.331
Length at 1 year, cm**	75.0±2.93	75.7±3.30	75.9±3.46	75±2.25	0.787
Maternal education in years**	12.4±2.59	12.6±1.48	12.2±1.50	12.6±2.31	0.892
Paternal education in years**	11.8±1.81	12.3±1.44	12.3±1.67	11.7±1.55	0.560
Maternal allergies reported*	8 (42%)	8 (32%)	5 (26%)	12 (43%)	0.615
Yes to smoking by anyone in the household*	9 (47%)	9 (36%)	8 (42%)	8 (29%)	0.648
Pack years smoked by mother**	1.57±2.41	3.03±4.80	2.49±4.23	1.14±2.20	0.244

Greatest PPD smoked by household**	0.33±0.50	0.51±0.84	0.52±0.65	0.35±0.70	0.772
Yes to daycare in early life (between 3.5 to 5.5 yrs)*	11 (58%)	9 (36%)	11 (58%)	15 (54%)	0.809
Greatest number of children <13 years in home**	2.9±1.08	3.3±1.21	2.8±1.54	2.9±1.10	0.561
Yes to pets in the home*	6 (32%)	7 (28%)	8 (42%)	6 (21%)	0.493
Greatest number of furred pets**	0.47±0.800	0.53±0.717	0.81±1.109	0.30±0.559	0.282

*Reported as n (%); χ^2 test
**Reported as mean ± standard deviation; ANOVA

Incidence of Illness

When comparing the incidence of illness between the control group and the combined DHA and ARA treatment groups, significant differences were only found during the first year of life (**Table 2**). In the control group, there was a greater incidence of skin allergic illness ($p = 0.041$), combined wheeze/asthma/skin diagnoses ($p = 0.038$), and any allergy diagnoses ($p = 0.033$). Between 1 and 4 years of age, illnesses in children in the control group did not differ from illnesses in the supplemental groups (**Table 3**).

Table 2. Incidence of at least one diagnosis or illness during the first 12 months of life

Diagnosis/Combination of Diagnoses	Control (n=19)		0.32% DHA (n=25)		0.64% DHA (n=19)		0.96% DHA (n=28)		*P* value
Any Diagnosis	19	100%	25	100%	18	95%	28	100%	0.605
EENT Diagnosis	14	74%	21	84%	18	95%	22	79%	0.261
GI Diagnosis	13	68%	18	72%	11	58%	12	43%	0.365
GI Illness	5	26%	9	36%	6	32%	4	14%	0.995
RESP Diagnosis	18	95%	20	80%	16	84%	21	75%	0.113
Skin Diagnosis	17	90%	14	56%	16	84%	20	71%	0.078
Urogenital Diagnosis	2	11%	1	4%	2	11%	1	4%	0.437
URI	13	68%	18	72%	13	68%	17	61%	0.885
Nonallergic Respiratory Illnesses	16	84%	22	88%	15	79%	22	79%	0.817
Wheeze/Asthma	5	26%	5	20%	2	11%	2	7%	0.138
Skin Allergic Illness	9	47%	4	16%	6	32%	7	25%	0.041
Wheeze/Asthma/Skin	11	58%	9	36%	7	37%	7	25%	0.038
Any Allergy	12	63%	10	40%	8	42%	8	29%	0.033
OM	10	53%	13	52%	9	47%	11	39%	0.598
Respiratory Infectious Illness	15	79%	18	72%	13	68%	19	68%	0.415

P value calculated using Chi-squared test comparing control to the combined treatment groups.

Table 3. Incidence of at least one diagnosis or illness from 12 months to 4 years of age

Diagnosis/Combination of Diagnoses	Control (n=19)		0.32% DHA (n=25)		0.64% DHA (n=19)		0.96% DHA (n=28)		*P* value
Any Diagnosis	17	90%	24	96%	17	90%	26	93%	0.602
EENT Diagnosis	10	53%	15	60%	15	79%	17	61%	0.331
GI Diagnosis	7	37%	12	48%	8	42%	11	39%	0.625
GI Illness	3	16%	8	32%	4	21%	4	14%	0.539
RESP Diagnosis	14	74%	18	72%	15	79%	17	61%	0.719
Skin Diagnosis	14	74%	16	64%	14	74%	15	54%	0.364
Urogenital Diagnosis	0	0%	1	4%	2	11%	3	11%	0.193
URI	9	47%	14	56%	12	63%	13	46%	0.598
Nonallergic Respiratory Illnesses	13	68%	19	76%	15	79%	18	64%	0.744
Wheeze/Asthma	6	32%	7	28%	7	37%	5	18%	0.652
Skin Allergic Illness	8	42%	5	20%	7	37%	7	25%	0.182
Wheeze/Asthma/Skin	9	47%	11	44%	10	53%	11	39%	0.820
Any Allergy	10	53%	14	56%	11	58%	13	46%	0.991
OM	8	42%	12	48%	9	47%	10	36%	0.941
Respiratory Infectious Illness	12	63%	15	60%	14	74%	14	50%	0.785

P value calculated using Chi-squared test comparing control to the combined treatment groups.

Table 4. Incidence of at least one diagnosis or illness during the first 4 years of life									
Diagnosis/Combination of Diagnoses	Control (n=19)		0.32% DHA (n=25)		0.64% DHA (n=19)		0.96% DHA (n=28)		*P* value
Any Diagnosis	19	100%	25	100%	18	95%	28	100%	0.605
EENT Diagnosis	16	84%	22	88%	18	95%	27	96%	0.226
GI Diagnosis	16	84%	21	84%	13	68%	19	68%	0.337
GI Illness	8	42%	13	52%	9	47%	7	25%	0.885
RESP Diagnosis	19	100%	22	88%	16	84.2%	26	93%	0.128
Skin Diagnosis	19	100%	21	84%	18	95%	23	82%	0.085
Urogenital Diagnosis	2	11%	2	8%	4	21%	4	14%	0.700
URI	14	74%	21	84%	16	84%	23	82%	0.337
Nonallergic Respiratory Illnesses	18	95%	23	92%	17	89%	26	93%	0.655
Wheeze/Asthma	8	42%	8	32%	7	37%	6	21%	0.282
Skin Allergic Illness	12	63%	8	32%	10	53%	12	43%	0.095
Wheeze/Asthma/Skin	13	68%	14	56%	13	68%	14	50%	0.365
Any Allergy	14	74%	17	68%	14	74%	15	54%	0.423
OM	13	68%	18	72%	13	68%	16	57%	0.797
Respiratory Infectious Illness	17	89%	21	84%	16	84%	24	86%	0.599

P value calculated using Chi-squared test comparing control to the combined treatment groups.

Average Number of Illnesses per Child

Subjects receiving the formula that contained 0.96% DHA from fatty acids were significantly less likely to be diagnosed with any illness during the first year of life ($p = 0.01$) when compared to the control group. These subjects were also less likely to be diagnosed with respiratory diagnoses ($p = 0.05$), any allergy diagnoses ($p = 0.003$), combined wheeze/asthma/skin diagnoses ($p = 0.003$), and skin allergic illnesses ($p = 0.02$). Moreover, there was a trend toward less GI diagnoses ($p = 0.06$) and wheeze/asthma ($p = 0.06$) in the group receiving 0.96% DHA compared to the control group. Skin diagnoses did not reach significance, but trended lower in all three supplemental groups compared to controls ($p = 0.06$, 0.09, 0.11, respectively, for the groups receiving 0.32, 0.64, and 0.96% DHA). Skin allergic illness, which reached significance in infants fed the highest amount of DHA, trended lower (compared to controls) in both other supplement groups (0.32%, $p = 0.11$; 0.64%, $p = 0.13$). Similarly, any allergy and wheeze/asthma/skin trended lower in the 0.64% DHA group compared to the control group ($p = 0.08$ and $p = 0.10$, respectively). The 0.64% DHA group experienced more ears, eyes, nose, and throat diagnoses ($p = 0.05$) during the first year of life compared to the control group. These results are shown in **Tables 5 – 7**.

From 12 to 48 months of life, the only decrease in diagnoses was seen in the reduction of skin diagnoses among the 0.96% DHA group ($p = 0.03$).

During the first four years of life (**Table 7**), significantly fewer illnesses were seen in the treatment group receiving 0.96% DHA. The subjects in this group

experienced fewer episodes of skin diagnoses (p = 0.02), any allergy diagnoses (p = 0.02), combined wheeze/asthma/skin diagnoses (p = 0.01), skin allergic illness diagnoses (p = 0.03), as well as fewer illness diagnoses overall (Any Diagnosis, p = 0.03).

Table 5. Average number of illnesses per child (± standard deviation) during the first year of life

Diagnosis/Combination of Diagnoses	Control (n=19)	0.32% DHA (n=25)	*P* Value	0.64% DHA (n=19)	*P* Value	0.96% DHA (n=28)	*P* Value
Any Diagnosis	9.47	8.2 ±4.85	0.40	7.58 ±4.31	0.18	6.32 ±4.04	0.01
EENT Diagnosis	1.26	1.76 ±1.10	0.14	2.0 ±1.13	0.05	1.54 ±1.20	0.46
GI Diagnosis	1.21	1.20 ±1.08	0.98	1.05 ±1.15	0.68	0.64 ±1.02	0.06
RESP Diagnosis	2.21	2.4 ±2.02	0.76	1.68 ±1.36	0.24	1.46 ±1.28	0.05
Skin Diagnosis	2.53	1.32 ±2.12	0.06	1.47 ±1.91	0.09	1.57 ±1.98	0.11
Urogenital Diagnosis	0.11	0.04 ±0.25	0.41	0.16 ±0.41	0.70	0.04 ±0.24	0.35

URI	1.00	1.60 ±1.28	0.13	1.16 ±0.93	0.61	1.07 ±0.99	0.81
OM	0.63	0.60 ±0.65	0.88	0.47 ±0.59	0.43	0.43 ±0.61	0.28
Any Allergy	1.47	0.88 ±1.71	0.27	0.63 ±1.45	0.08	0.39 ±1.26	0.003
Wheeze/Asthma	0.32	0.36 ±0.85	0.87	0.16 ±0.53	0.38	0.07 ±0.43	0.06
Wheeze/Asthma/Skin	1.37	0.72 ±1.57	0.18	0.58 ±1.44	0.10	0.32 ±1.23	0.003
Skin Allergic Illness	1.05	0.36 ±1.41	0.11	0.42 ±1.27	0.13	0.25 ±1.13	0.02
Nonallergic Respiratory Illnesses	1.74	2.28 ±1.49	0.24	1.63 ±1.19	0.79	1.54 ±1.18	0.58
Respiratory Infectious Illness	1.37	1.68 ±1.42	0.48	1.16 ±1.07	0.56	1.18 ±1.10	0.57
GI Illness	0.32	0.48 ±0.65	0.42	0.42 ±0.62	0.62	0.14 ±0.46	0.21

P value calculated using t-test by comparing each treatment group to the control.

Table 6. Average number of illnesses per child (± standard deviation) from 12 to 48 months of life							
Diagnosis/Combination of Diagnoses	Control (n=19)	0.32% DHA (n=25)	P Value	0.64% DHA (n=19)	P Value	0.96% DHA (n=28)	P Value
Any Diagnosis	9.95	8.72 ±9.13	0.67	11.58 ±10.92	0.66	6.36 ±8.37	0.16
EENT Diagnosis	1.37	1.76 ±1.90	0.51	1.89 ±2.04	0.44	1.36 ±1.55	0.98
GI Diagnosis	0.95	0.84 ±1.56	0.83	0.84 ±1.70	0.85	0.61 ±1.49	0.45
RESP Diagnosis	2.74	2.60 ±4.04	0.91	3.11 ±3.98	0.78	1.39 ±3.22	0.17
Skin Diagnosis	2.37	1.52 ±1.93	0.16	2.63 ±2.53	0.76	1.11 ±1.90	0.03
Urogenital Diagnosis	0	0.04 ±0.15	0.39	0.21 ±0.50	0.21	0.18 ±0.47	0.21
URI	1.16	0.92 ±1.67	0.65	1.11 ±1.84	0.93	0.79 ±1.64	0.46
OM	0.53	1.04 ±1.17	0.16	0.95 ±1.23	0.30	0.79 ±1.13	0.45
Any Allergy	2.00	1.56 ±2.42	0.56	2.26 ±2.65	0.77	1.11 ±2.15	0.17

Wheeze/Asthma	0.63	0.68 ±1.48	0.92	0.63 ±1.22	1.0	0.25 ±0.96	0.19
Wheeze/Asthma/Skin	1.58	1.20 ±2.02	0.55	1.79 ±2.26	0.78	0.79 ±1.78	0.14
Skin Allergic Illness	0.95	0.52 ±1.22	0.26	1.16 ±1.76	0.72	0.54 ±1.24	0.27
Nonallergic Respiratory Illnesses	2.11	2.36 ±3.15	0.80	2.47 ±3.36	0.74	1.64 ±2.76	0.58
Respiratory Infectious Illness	1.53	1.44 ±2.27	0.90	1.80 ±2.40	0.74	0.89 ±1.95	0.28
GI Illness	0.32	0.40 ±0.71	0.70	0.26 ±0.68	0.82	0.14 ±0.58	0.33

P value calculated using t-test by comparing each treatment group to the control.

Table 7. Average number of illnesses per child (± standard deviation) during the first four years of life

Diagnosis/Combination of Diagnoses	Control (n=19)	0.32% DHA (n=25)	*P* Value	0.64% DHA (n=19)	*P* Value	0.96% DHA (n=28)	*P* Value
Any Diagnosis	19.42	16.92 ±10.90	0.46	19.16 ±12.57	0.95	12.68 ±10.22	0.03
EENT Diagnosis	2.63	3.52 ±2.42	0.24	3.89 ±2.46	0.12	2.89 ±1.93	0.66
GI Diagnosis	2.16	2.04 ±1.70	0.83	1.89 ±1.98	0.69	1.25 ±1.66	0.07
RESP Diagnosis	4.95	5.00 ±4.93	0.97	4.79 ±4.71	0.92	2.86 ±3.97	0.08
Skin Diagnosis	4.89	2.84 ±3.31	0.04	4.11 ±3.47	0.50	2.68 ±3.21	0.02
Urogenital Diagnosis	0.11	0.08 ±0.29	0.78	0.37 ±0.63	0.20	0.21 ±0.52	0.49
URI	2.16	2.52 ±2.33	0.62	2.26 ±2.36	0.89	1.86 ±2.11	0.64
OM	1.16	1.64 ±1.51	0.31	1.42 ±1.39	0.57	1.21 ±1.32	0.89
Any Allergy	3.47	2.44 ±3.21	0.30	2.89 ±3.10	0.58	1.50 ±2.77	0.02

Wheeze/Asthma	0.95	1.04 ±1.88	0.88	0.79 ±1.42	0.74	0.32 ±1.16	0.07
Wheeze/Asthma/Skin	2.95	1.92 ±2.89	0.25	2.37 ±2.82	0.54	1.11 ±2.45	0.01
Skin Allergic Illness	2.00	0.88 ±2.26	0.11	1.58 ±2.32	0.59	0.79 ±1.88	0.03
Nonallergic Respiratory Illnesses	3.84	4.64 ±3.91	0.51	4.11 ±3.92	0.84	3.18 ±3.24	0.50
Respiratory Infectious Illness	2.89	3.12 ±2.84	0.80	2.95 ±2.89	0.96	2.07 ±2.43	0.26
GI Illness	0.63	0.88 ±0.97	0.41	0.68 ±0.87	0.86	0.29 ±0.71	0.10

P value calculated using t-test by comparing each treatment group to the control.

Odds Ratio of having at Least One Diagnosis or Illness

The odds ratio of having at least one diagnosis or illness is shown in **Tables 8 – 10**. Significant values were found only in the first year of life. The 0.32% DHA group was less likely to have at least one skin diagnosis ($p = 0.02$) or skin allergic illness ($p = 0.04$). Additionally, the 0.96% DHA group was found to be less likely to experience at least one allergy diagnosis (Any Allergy, $p = 0.03$) or combined wheeze/asthma/skin diagnosis ($p = 0.03$). Overall, there appeared to be smaller odds of respiratory illness, skin illness, urogenital illness, any allergy, wheeze/asthma/skin, skin allergic illness, and respiratory infectious illness in all supplemental groups in the first year of life, but they did not reach statistical significance largely due to the low power of the study.

Table 8. OR (including 95% confidence interval) of having at least one diagnosis or illness during the first year of life compared to the unsupplemented group

Diagnosis/Combination of Diagnoses	0.32% DHA (n=25)	*P* Value	0.64% DHA (n=19)	*P* Value	0.96% DHA (n=28)	*P* Value
Any Diagnosis	*	1.00	*	1.00	*	1.00
EENT Diagnosis	1.88 (0.42-8.22)	0.47	6.43 (0.67-61.47)	0.18	1.31 (0.34-5.12)	0.74
GI Diagnosis	1.19 (0.32-4.37)	1.0	0.63 (0.17-2.40)	0.74	0.35 (0.10-1.18)	0.14
RESP Diagnosis	0.22 (0.02-2.09)	0.21	0.30 (0.03-3.14)	0.60	0.17 (0.02-1.49)	0.12
Skin Diagnosis	0.15 (0.03-0.79)	0.02	0.63 (0.09-4.26)	0.99	0.29 (0.05-1.58)	0.17
Urogenital Diagnosis	0.35 (0.03-4.23)	0.57	1.0 (0.13-7.94)	1.0	0.31 (0.03-3.74)	0.56
URI	1.19 (0.32-4.37)	1.0	1.0 (0.25-3.93)	1.0	0.71 (0.21-2.44)	0.76
OM	0.97 (0.30-3.22)	1.00	0.81 (0.23-2.89)	0.99	0.58 (0.18-1.89)	0.39
Any Allergy	0.39 (0.11-1.33)	0.22	0.42 (0.12-1.56)	0.33	0.23 (0.07-0.81)	0.03

Wheeze/Asthma	0.70 (0.17-2.88)	0.72	0.33 (0.06-1.97)	0.41	0.22 (0.04-1.26)	0.10
Wheeze/Asthma/Skin	0.41 (0.12-1.39)	0.22	0.42 (0.12-1.56)	0.33	0.24 (0.07-0.85)	0.03
Skin Allergic Illness	0.21 (0.05-0.86)	0.04	0.51 (0.14-1.92)	0.51	0.37 (0.11-1.28)	0.13
Nonallergic Respiratory Illnesses	1.38 (0.25-7.72)	1.00	0.70 (0.13-3.68)	0.99	0.69 (0.15-3.17)	0.72
Respiratory Infectious Illness	0.69 (0.17-2.80)	0.73	0.58 (0.13-2.51)	0.71	0.56 (0.14-2.19)	0.52
GI Illness	1.58 (0.43-5.82)	0.53	1.29 (0.32-5.28)	0.99	0.47 (0.11-2.03)	0.45

P value calculated using Fisher Exact Probability test; *denotes an infinite number or zero.

Table 9. OR (including 95% confidence interval) of having at least one diagnosis or illness from 12 to 48 months of life compared to the unsupplemented group						
Diagnosis/Combination of Diagnoses	0.32% DHA (n=25)	*P* Value	0.64% DHA (n=19)	*P* Value	0.96% DHA (n=28)	*P* Value
Any Diagnosis	2.82 (0.24-33.70)	0.57	1.00 (0.13-7.94)	1.00	1.53 (0.20-11.92)	1.00
EENT Diagnosis	1.35 (0.40-4.50)	0.76	3.38 (0.81-14.02)	0.17	1.39 (0.43-4.51)	0.76
GI Diagnosis	1.58 (0.47-5.35)	0.55	1.25 (0.34-4.59)	0.99	1.11 (0.33-3.69)	1.00
RESP Diagnosis	0.92 (0.24-3.52)	1.00	1.34 (0.30-6.02)	0.99	0.55 (0.15-1.97)	0.53
Skin Diagnosis	0.63 (0.17-2.35)	0.53	1.00 (0.24-4.24)	1.00	0.41 (0.12-1.46)	0.23
Urogenital Diagnosis	*	1.00	*	0.49	*	0.26
URI	1.41 (0.43-4.68)	0.76	1.90 (0.52-6.96)	0.52	0.96 (0.30-3.09)	1.00
OM	1.27 (0.38-4.22)	0.77	1.24 (0.34-4.45)	0.99	0.76 (0.23-2.52)	0.76
Any Allergy	1.15 (0.35-3.79)	1.00	1.24 (0.34-4.45)	0.99	0.78 (0.24-2.51)	0.77

Wheeze/Asthma	0.84 (0.23-3.10)	1.00	1.26 (0.33-4.84)	0.99	0.47 (0.12-1.85)	0.31
Wheeze/Asthma/Skin	0.87 (0.26-2.89)	1.00	1.23 (0.35-4.41)	0.99	0.72 (0.22-2.33)	0.76
Skin Allergic Illness	0.34 (0.09-1.31)	0.18	0.80 (0.22-2.95)	0.99	0.46 (0.13-1.60)	0.34
Nonallergic Respiratory Illnesses	1.46 (0.39-5.55)	0.74	1.73 (0.40-7.51)	0.71	0.83 (0.24-2.87)	1.00
Respiratory Infectious Illness	0.88 (0.26-2.99)	1.00	1.63 (0.41-6.51)	0.73	0.58 (0.18-1.92)	0.55
GI Illness	2.51 (0.56-11.16)	0.30	1.42 (0.27-7.44)	0.99	0.89 (0.18-4.51)	1.00

P value calculated using Fisher Exact Probability test; *denotes an infinite number or zero.

Table 10. OR (including 95% confidence interval) of having at least one diagnosis or illness during the first four years of life compared to the unsupplemented group						
Diagnosis/Combination of Diagnoses	0.32% DHA (n=25)	*P* Value	0.64% DHA (n=19)	*P* Value	0.96% DHA (n=28)	*P* Value
Any Diagnosis	*	1.00	0	1.00	0	1.00
EENT Diagnosis	1.38 (0.25-7.72)	1.00	3.38 (0.32-35.80)	0.60	5.06 (0.48-52.88)	0.29
GI Diagnosis	0.98 (0.19-5.04)	1.00	0.41 (0.08-1.95)	0.45	0.40 (0.09-1.72)	0.31
RESP Diagnosis	0	0.25	0	0.23	0	0.51
Skin Diagnosis	0	0.12	0	1.0	0	0.07
Urogenital Diagnosis	0.74 (0.09-5.79)	1.0	2.27 (0.36-14.19)	0.66	1.42 (0.23-8.64)	1.0
URI	1.88 (0.43-8.22)	0.47	1.90 (0.38-9.44)	0.69	1.64 (0.40-6.71)	0.72
OM	1.19 (0.32-4.37)	1.00	1.00 (0.25-3.93)	1.00	0.62 (0.18-2.09)	0.55
Any Allergy	0.76 (0.20-2.85)	0.75	1.00 (0.24-4.24)	1.00	0.41 (0.12-1.46)	0.23
Wheeze/Asthma	0.65 (0.19-2.23)	0.54	0.80 (0.22-2.95)	0.99	0.38 (0.10-1.35)	0.20

Wheeze/Asthma/Skin	0.59 (0.17-2.05)	0.54	1.00 (0.25-3.93)	1.00	0.46 (0.14-1.56)	0.24
Skin Allergic Illness	0.27 (0.08-0.96)	0.07	0.65 (0.18-2.37)	0.74	0.44 (0.13-1.45)	0.24
Nonallergic Respiratory Illnesses	0.64 (0.05-7.62)	1.00	0.47 (0.04-5.70)	1.00	0.72 (0.06-8.58)	1.00
Respiratory Infectious Illness	0.62 (0.10-3.79)	0.68	0.63 (0.09-4.26)	0.99	0.71 (0.12-4.30)	1.00
GI Illness	1.49 (0.45-4.96)	0.56	1.24 (0.34-4.45)	0.99	0.46 (0.13-1.60)	0.34

P value calculated using Fisher Exact Probability test; *denotes an infinite number or zero.

Chapter 5

Discussion

The results of this study show that children who were given DHA and ARA supplemented formula during the first year of life were significantly less likely to be diagnosed with illness during the first four years of life. Specifically, there was a reduced number of overall diagnoses, respiratory diagnoses, allergic diagnoses, skin diagnoses, combined wheeze/asthma/skin allergic diagnoses, as well as skin allergic illness diagnoses in the 0.96% DHA group. The 0.32% DHA group also experienced a reduction of skin diagnoses and skin allergic illnesses. There seemed to be a greater reduction of illness in the 0.96% DHA group compared to the other two treatment groups based on the fact that they were more likely to show a significant decrease in incidence. All supplemented groups trended lower on odds ratio data.

Previous studies have suggested that the supplementation of 0.32% DHA and 0.64% ARA during infancy leads to a significant reduction of upper respiratory infections, asthma, allergies, and atopic dermatitis (1-7). The results from this study align with previous studies that found a reduction in allergies, and atopic dermatitis. However, the results from this study did not find a significant reduction of upper respiratory infections or asthma during the first four years of life.

A DRI for DHA has yet to be set by the Food and Nutrition Board of the Institute of Medicine due to a lack of evidence on the topic (10,18). Since the results of this study found a significant decrease of illness in the group receiving 0.96% DHA from fatty acids, these results could contribute to a recommendation for DHA

supplementation for infants and children. However, more studies on this topic would be necessary to be able to determine a DRI for DHA during infancy. Future double-blind, randomized, controlled studies need to examine illness using a broader range of DHA supplementation in children.

Illnesses were collected during the first 12 months as part of adverse event monitoring. However, we did not hypothesize lower illness rates. We continued to obtain medical records during our continued follow-up of this cohort, because data were emerging to suggest benefits for less respiratory illness and allergy (1-7).

A limitation of this study is that illness was not a primary hypothesized outcome of the original study. The original double-blind, 2-phase, randomized, controlled, parallel-group, prospective trial was designed to measure visual acuity and cognitive development in children up to 6 years of age. Another limitation of this study is that parents did not report all hospitals or clinics to which they took their children for treatment. Additionally, parents may not have taken their children to the doctor for all illnesses. They might have been more likely to forget to report those occurrences of illness, particularly in years 1- 4 when they were seen in our follow-up at 6 month intervals (compared to about 2 month intervals in the first year of life). Another limitation is that during analysis, the skew was not checked. Outliers could possibly account for the increase in illness in the 0.64% DHA group. More accurate results would be achieved by accounting for any outliers.

In conclusion, the results of this study support the hypothesis that the supplementation of DHA and ARA from birth to 12 months reduces the occurrence of

illness, specifically respiratory diagnoses, skin diagnoses and skin allergic diagnoses. The results did not support the hypothesis that supplementation would decrease the incidence of upper respiratory infections or asthma. Subjects that were given 0.96% of fatty acids from DHA seemed to have a decreased incidence of illness as evidenced by the greater amount of significant differences compared to the control. The other two treatment groups did not yield as many significant differences when compared to the control as did the 0.96% DHA group.

Chapter 6

Summary

Dietary fats, like omega-3 and omega-6 fatty acids, play a role in regulating immune function and inflammation (8). It is these functions that suggest the importance of long-chain polyunsaturated fatty acids (LCPUFA) in preventing and reducing illness in children.

This study was a double-blind, 2-phase, randomized, controlled, parallel-group, prospective trial. The primary outcome of the study was to evaluate whether DHA and ARA supplemented infant formula affected visual evoked potential acuity in term infants. For the purposes of this thesis project, the outcome was illness as recorded through adverse events during the original study. The infants were randomized into 4 groups and were given milk-based infant formula containing no DHA (control formula), 0.32% DHA, 0.64% DHA, or 0.96% of total fatty acids as DHA. The study was controlled for ARA (0.64% of the total fatty acids) with a dose response to DHA – the control group received no DHA or ARA.

Children who were given formula supplemented with DHA and ARA had a reduced incidence of illness. In particular, children supplemented with 0.96% of fatty acids from DHA experienced fewer episodes of respiratory diagnoses, allergy diagnoses, and allergic skin illnesses (atopic dermatitis, contact dermatitis, eczema, and urticaria).

The results of this study could potentially aid the Food and Nutrition Board of the Institute of Medicine in setting a DRI for n-3 and n-6 fatty acids. However, more

studies on this topic would be necessary to be able to determine a DRI for DHA and ARA during infancy.

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Appendix A

Consent Forms

CONSENT FORM

The Effects of Infant Formula Supplemented with Long Chain Polyunsaturated Fatty Acids on Visual Development in Term Infants

Protocol #3370-4
Sponsor: Mead Johnson, Inc.

INTRODUCTION

As a woman who has delivered a term infant and who has specified that I plan to feed formula to my infant, I am being invited to enroll my child in a research study of infant formula. My baby and I are being asked to enroll at Truman Medical Center or the University of Kansas Medical Center because the investigators need to know the level of the nutrient studied in my blood and my baby's cord blood after my baby is born. The remainder of the study will be conducted at the University of Kansas Medical Center by Susan Carlson, Ph.D. Approximately 185 subjects will be enrolled in this study.

I do not have to allow my child to participate in this research study. It is important that before I make a decision for my child to participate, I read the rest of this form. I should ask as many questions as I need to understand what will happen if my baby and I participate in the study.

BACKGROUND

Two fats, docosahexaenoic acid (DHA) and arachidonic acid (ARA), are found in very large amounts in the brain. DHA and ARA are important for infant brain development and behavior, including how my baby sees and learning. My baby obtained DHA and ARA from me during the last three months of my pregnancy. Breast feeding is the preferred way to feed in terms of the best interests of the baby. Breast milk also contains DHA and ARA. Breast milk and formulas also contain fats that most babies can change to DHA and ARA.

Infants born early have been shown to have higher development when they consume formulas with DHA or DHA and ARA. This means that preterm infants do not make as much DHA and ARA as they need for best development from the nutrients in infant formula. Term babies (such as my baby) may or may not need DHA and ARA. Some studies indicate they do and others indicate they do not. Some formulas in the US contain DHA and ARA and some do not.

PURPOSE

The purpose of this study is to determine if term infants have higher development when they drink formulas with DHA and ARA. Another purpose is to determine if the amount of DHA and ARA in the formula is important. Human milk DHA can be as low as 0.05% and as high as 2.8%, depending upon a woman's diet. This study will test a range of formula DHA from 0.32% to 0.96% against a formula without DHA or ARA (marketed Enfamil). Infants will be tested for vision, attention (how babies look at faces, look and play with toys), learning, motor and language development.

PROCEDURES

If I choose to enroll my infant in this study after hearing about how the study will be conducted, and what I and my child will need to do, the investigators will record some

HSC Submission (HSC Address) Date: 4/27/05
HSC Approval (Rev CF CO!) Date: 6/17/04

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information from my medical record and my delivery including the weight I gained during pregnancy, my smoking history, and my baby's weight, length and head circumference. The investigators will also try to get cord blood for analysis of nutrients in my baby's blood and a sample of my blood when it is drawn after I deliver as part of routine blood work related to my pregnancy. The same nutrients will be analyzed in my blood at the University of Kansas Medical Center.

I will be sent home with an appointment to bring my baby to the University of Kansas Medical Center in about 1 week and given enough marketed Enfamil to feed him/her until that visit (visit 1).

Visit 1 (7-9 days of age) My baby will be weighed and measured. I will be asked what my baby has eaten in the past 24 hours. If I still plan to feed him/her formula, he/she will be assigned by chance (like pulling numbered pieces of paper out of a hat) to one of the following 4 formulas:

- Milk based infant formula containing 0.32% of the total fatty acids as DHA and 0.64% of the total fatty acids as ARA (same as marketed Enfamil Lipil)
- Milk based infant formula containing 0.64% of the total fatty acids as DHA and 0.64%of the total fatty acids as ARA
- Milk based infant formula containing 0.96% of the total fatty acids as DHA and 0.64% of the total fatty acids as ARA
- Milk based Infant Formula without DHA or ARA (same as marketed Enfamil)

I will receive 7 cases of ready-to-feed study formula at this visit. The visit should last about 30 minutes.

Visit 2 (6 weeks of age): The investigators will measure how my baby sees using a test that involves placing 3 electrodes directly on my baby's head. The process involves cleaning the area then placing a small amount of paste similar to toothpaste on the head. The electrodes are placed on top of the paste. The electrodes will be used to record my baby's brain waves while he/she is looking at pictures. My child's weight, height and head circumference will be measured again and I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 13 cases of ready-to-feed study formula at this visit. The visit should last about 40 minutes.

Visit 3 (4 months of age): The investigators will measure how my baby sees using the same test as before and another test. My baby will wear a pair of plastic glasses during the second test. My baby's height, weight and head circumference will be measured. In another test, my child will be given an object to look at several times. The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. My baby's heart rate will be measured during the test.

My baby will have a blood sample collected by either heel stick or drawn from a vein. The investigator may use a cream or spray that will numb the area before obtaining the sample. One-half teaspoon of blood will be drawn.

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I will be asked questions about how much formula my baby drank over the past 24 hours. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 10 cases of ready-to-feed study formula at this visit. The visit will take 60-90 minutes.

Visit 4 (6 months of age): The investigators will measure how my baby sees using the test that requires him/her to where a pair of plastic glasses. In another test, he/she will be given an object to look at several times (just like at 4 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object. My baby's heart rate will be measured during the test. My baby's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 16 cases of ready-to-feed study formula at this visit. The visit should take 40 -60 minutes.

Visit 5 (9 months of age): My baby will have both tests that measure how he/she sees. In another test, my child will be given an object to look at several times (just like at 4 and 6 months of age). The investigator will measure how long he/she looks at the object and how quickly he/she stops looking at the object and my baby's heart rate will be measured during the test. My baby's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my baby's bowel movements including color, number and consistency. I will be asked to report if my baby has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will receive 4 cases of ready-to-feed study formula at this visit. The visit should take about 40-60 minutes

Visit 6 (10 months of age): During this visit the baby will be placed on the parent or guardian's lap in front of a small table. A test will be completed with a small toy, foam block and 2 clothes that will be placed in front of the child. The investigator will describe this test to me in detail before it has been completed. I will also take a short language test. The small toy will be given to my child to keep. I should let the investigator know if my baby has been sick or not acting well since his/her last visit. I will be asked questions about what my baby eats. I will receive 10 cases of ready-to-feed study formula at this visit. I will be asked to bring any unopened cases of study formula to the next visit. The visit should take about 30 minutes.

Visit 7 (12 months of age): I will bring any unopened cases of study formula to this visit. I can feed any cans of formula that remain in an opened case before changing my baby's milk to whole cows' milk. The investigators will measure how my baby sees using both vision tests. My child will be video-recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. My child will have a blood sample collected by either heel stick or drawn from a vein. The investigator may use a

cream or spray that will numb the area before obtaining the sample. Approximately ½ teaspoon of blood will be drawn. My child's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will also be asked questions about my child's bowel movements including color, number and consistency. I will be asked to report if my child has been fussy, gassy or had constipation or diarrhea. I should let the investigator know if my child has been sick or not acting well since his/her last visit. The visit should take about 2 hours.

Visit 8 (18 months of age): The investigators will measure how my baby sees using the test that he/she had while wearing plastic glasses. My child will be video-recorded while playing with an interesting toy and the investigator will use the recording to measure some aspects of attention. My child will also be given a standardized test to measure mental and physical development. My child's height, weight and head circumference will be measured. I will be asked questions about what my baby eats. I will be asked questions about my child's language skills. I should let the investigator know if my child has been sick or not acting well since his/her last visit. The visit should take about 2 hours. It is important that my child be rested before the testing at this visit. If for some reason, he/she is not capable of completing all of the assessment, I may be offered the possibility to bring him/her on another day.

RISKS

It is possible that my child could be at risk by participating in this study. Risks of the study formulas may include: not being able to tolerate the formula, spitting up, vomiting, constipation, diarrhea, red itchy skin, rashes or other signs of food allergy and failure to thrive or temporary impairment of growth.

Enfamil Lipil, one of the formulas in this study has been available in stores for the past year. During that year, parent reports of formula problems have been recorded by Mead Johnson Nutritionals, the sponsor of this study. There have not been more problems with Enfamil Lipil than with Enfamil, another formula that will be fed in this study. Two of the formulas have higher DHA and ARA than Enfamil Lipil. None of the formulas fed in this study has more DHA and ARA than has been measured in some human milk, however, higher intakes of DHA and ARA may have some risks that have not yet been identified or unexpected side effects that have not been previously observed.

The importance of DHA and ARA for infants is controversial. Some experts think babies should consume formula with DHA and ARA, others do not. The American Academy of Pediatrics and the FDA have not given the opinion that formulas need to contain DHA and ARA. However, it is possible that my baby might benefit from DHA and ARA and not receive DHA and ARA if he/she is assigned to the formula without DHA and ARA.

Some redness, soreness, or bruising may occur at the site of blood sampling. There is also a very slight risk of infection.

NEW FINDINGS STATEMENT

Any problems of babies in the study will be recorded. I will be informed if any significant new findings develop during the course of the study that may affect my willingness to allow my child to participate in this study.

BENEFITS
My child may or may not benefit from participating in this study. It is hoped that additional information gained in this research study may be useful in the growth and development of infants. I will receive a video recording of my infant doing the 4, 6 and 8 month looking test when the 8 month visit is complete.

ALTERNATIVES
Formulas with no DHA and ARA and formulas with the lower level of DHA and ARA in this study are available in stores and from WIC (Women Infant Children Supplemental Feeding Program). Name brand formulas that contain DHA and ARA are Enfamil Lipil and Similac Advance. Name brands that do not contain DHA and ARA are Enfamil and Similac. As noted above, two of the formulas fed in this study are the same as Enfamil and Enfamil Lipil. The other formulas contain 2 and 3 times as much DHA as Enfamil Lipil and the same amount of ARA. Store brands of formula are also available locally (for example, Costco, Walmart) without DHA and ARA.

COSTS
Infant formula will be provided to me at no cost while my child is participating in this study. The investigators will work with WIC at Truman Medical Center to make sure that I receive baby foods other than formula until my baby is 12 months old. I will not incur any costs because of my child's participation.

PAYMENT TO SUBJECTS
I will receive a check for $50 at each visit to the University of Kansas Medical Center to cover the costs of transportation and to partially compensate me for my time required to participate in the study. There will be 8 regularly scheduled visits in 18 months. If an additional visit is required because my infant is unable to complete all of the testing at 18 months, I will receive an additional payment of $50 for another visit.

My name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

DISCLOSURE OF FINANCIAL INTERESTS
The principal investigator has been paid as a consultant and for program presentation on DHA for Mead Johnson Nutritionals (the sponsor). The University of Kansas Medical Center Conflict of Interests Committee monitors this research project to make it less likely that these financial interests inappropriately influence how the study is conducted. However, you should make your own decision about whether these financial interests affect your decision to participate. If you have any questions about this financial relationship, you may discuss them with the investigator or with the Research Compliance division at 913-588-5492.

IN THE EVENT OF INJURY
In the event my child experiences any serious health problem (hospitalization, life-threatening illness or death) for any reason while participating in this study, I should immediately seek treatment or help in the way I normally would as if my child were not in a study. I should let

Susan Carlson, Ph.D. know about any of these problems as soon as possible by calling her office (913-588-5359) between 8:30 and 5:30 Monday through Friday. If it is after 5:30 PM on a weekday, or it is a holiday or weekend, I should call Dr. Carlson at home (816 -960-1805). A message may be left at both numbers in the event that Dr. Carlson is not immediately available.

INSTITUTIONAL DISCLAIMER STATEMENT

Although the University of Kansas Medical Center does not provide free medical treatment or other forms of compensation to persons injured as a result of participating in research, such compensation may be provided under the terms of the Kansas Tort Claims Act. If I believe my child has been injured as a result of participating in research, I should contact the Office of Legal Counsel, University of Kansas Medical Center, Kansas City, KS 66160-7101. I do not give up any of my or my child's rights by signing this form.

It is not the policy of the University of Missouri nor Truman Medical Center to compensate human subjects in the even the research results in injury. The University of Missouri and Truman Medical Center, in fulfilling their public responsibilities, have individually and separately provided liability coverage for any physical injury in the event such injury is caused by the negligence of the University of Missouri, its faculty or staff or Truman Medical Center and its employees. The University of Missouri also will provide, within the limitations of the laws of the State of Missouri, facilities and medical attention to subjects who suffer injuries as a result of participating in the research projects of the University of Missouri. In the event I believe that I have suffered any physical injury as the result of my participation in the research program, I may contact Dr. Susan Carlson, 913-588-5359, or Sheila Anderman, Research Administrator of the University of Missouri-Kansas City Adult Health Sciences Institutional Review Board, telephone number 816-235-6150, who can review the matter with me and provide further information on how to proceed.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Study data will be recorded on the sponsor's forms and sent to the sponsor or their designee. Videotapes of my baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students and to make a videotape copy for me. The videotapes will be secured under lock and key like all of other information that could be linked directly to my child. The videotape of my child will not be shown without specific permission from me and even then would not identify my child by name. Efforts will be made to keep my and my child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies my baby will be removed.

The privacy of my and my child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If I choose to allow my child to participate in this study, I will be asked to give permission for researchers to use and disclose my and my baby's health information that is relevant to the study.

To perform this study, researchers will collect health information about me and my child from his/her medical record and from the study activities that are listed in the Procedures section of this consent form. My and my baby's study-related health information will be used at KU

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Medical Center by Dr. Carlson, members of the research team, Truman Medical Center, the KU Hospital Medical Record Department, the KUMC Research Institute and officials at KUMC and at Truman Medical Center that oversee research, including the KUMC Human Subjects Committee, the IRB that governs Truman Medical Center and other committees and offices that review and monitor research studies.

Dr. Carlson and her team may share information about me and my baby with representatives of Mead Johnson (the sponsor of the study), the monitoring company who verifies study data, the laboratory that processes study lab samples, other business partners of the sponsor who help with the study, Mead Johnson's Data Coordinating Center, Mead Johnson's designated Data and Safety Monitoring committee, the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues arise). My and my child's information will be shared in order to analyze and confirm the results of the study.

Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

Any research information that is placed in my and my child's medical record will be kept indefinitely.

QUESTIONS

I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

My and my child's participation in this study is voluntary and that the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study, by the sponsor of the study, or the FDA. My child's participation can be discontinued by the investigator or by the sponsor if it is felt to be in my child's best interest or if I do not follow the study requirements. If I choose to withdraw before my child is 18 months of age, I may be asked to answer questions about the study on the telephone.

If I want to cancel permission to use my or my child's health information, I should send a written request to Dr. Carlson. The mailing address is Susan Carlson, Ph.D., Dept. of Dietetics and Nutrition, 4019 Delp, University of Kansas Medical Center, 3901 Rainbow

Boulevard, Kansas City, KS 66160. If I cancel permission to use my child's health information, the research team will stop collecting any additional information about my child, unless they need information about a side effect of the milk-based formula. The information that was collected before my cancellation, and any information about side effects, will be sent to the study sponsor.

Should the study be terminated prior to the completion of my and my child's participation, neither the sponsor, the investigator, nor the University of Kansas Medical Center will be under any obligation to provide me with the milk-based formula used in the study. My child's physician will decide upon further treatment after study termination, if indicated.

CONSENT

Dr. Carlson or her associates have given me information about this research study. They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my and my child's health information to be used and disclosed for the purposes of this research study. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to allow my child and I to participate in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Witness

_____ _____
Signature of Witness Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

UMKC ADULT HEALTH SCIENCES
INSTITUTIONAL REVIEW BOARD
INIT APPRVD from: 4/11/06 to: 4/10/07

HSC #: 9198
Approval Date: 4-11-06 to 4-10-07

CONSENT FORM

The Effects of Infant Formula Supplemented with Long Chain Polyunsaturated Fatty Acids on Cognitive Development in Children

Protocol #10205
Sponsor: Mead Johnson, Inc.

INTRODUCTION

As a parent who enrolled my child in a study of infant formula between birth and 18 months, I am being asked if I will permit my child to be studied with more tests of infant development at 7 more ages (9 more times) ending when he/she reaches 6 years of age. The study will be conducted at the University of Kansas Medical Center by Susan Carlson, Ph.D. and other members of her study team. Up to 110 children will be studied.

I do not have to allow my child to participate in this research study. It is important that before I make a decision for my child to participate, I read the rest of this form. I should ask as many questions as I need to understand what will happen if my baby and I participate in the study.

BACKGROUND

Two fats, docosahexaenoic acid (DHA) and arachidonic acid (ARA), are found in very large amounts in the brain. DHA and ARA are important for infant brain development and behavior, including how my baby sees and learning. My baby was enrolled in a study that provided varying amounts of DHA and ARA when he/she was an infant. Until 18 months, my infant/toddler was followed for his/her development. Now the investigator (Dr. Carlson) has been given additional money to follow children from that study until they are 6 years of age.

PURPOSE

The purpose of the original study was to determine if term infants have higher development when they drink formulas with DHA and ARA. Another purpose was to determine if the amount of DHA and ARA in the formula is important. My baby had tests of, vision, attention (how babies look at faces, look and play with toys), learning, motor and language development. These are still the purposes of the study. This new consent would permit the investigators to continue studying my child's development until he/she was near school age. Child development experts believe that any benefits of formulas with DHA and ARA would become bigger as children became older.

PROCEDURES

If I choose to enroll my infant in this study after hearing about how the study will be conducted, and what I and my child will need to do, I will be given an appointment to bring my child in when he or she is 2 years old. At the 2-year appointment and all subsequent appointments, it is important that my child not be tired or sick so that he/she can do his/her best. The investigators will work with me to find a time of day that is a good one for his/her appointment.

Visit 1 (2 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. I will be asked to complete a questionnaire about my child's experiences and environment. During

this visit the child will sit in a toddler chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. My child will also be shown how to put together small toys and will be given a chance to do so. In addition, my child will play with interesting toys. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. I will also complete a survey about my child's everyday behavior. This visit will take approximately 1.5 to 2 hours.

Visit 2 (2.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. My child will also be shown how to put together small toys and will be given a chance to do so. In addition, my child will play with interesting toys. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 3 (3 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. In addition, my child will be shown cards and asked for a response to them or asked to sort them into piles. The investigator will describe these tests to me in detail before each is started. This visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 4 (3.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. During this visit the child will sit in a chair in front of a small table. A test will be completed with a small toy, in which the toy is hidden in one of two places in front of the child. In addition, my child will be shown cards and asked for a response to them or asked to sort them into piles... The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

Visit 5 (4 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit the child will sit in a chair in front of a small table. My child will be given a set of cards and asked to sort them into piles and will play a game in which monkeys will be placed in a tree according to a few rules. My child will be given a set of cards and asked to sort them into piles or give a certain response to a card. In addition, my child will be shown and set of pictures in a certain order and will be given a chance to put those pictures in order. The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. This visit will take approximately 1.5 hours.

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Visit 6 (4.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. I will be asked to complete a questionnaire about my child's experiences and environment. My child's blood pressure will be taken. During this visit, 24 sensors will be placed on my child's head and 2 additional sensors will be placed on my child's chest. This procedure involves using a cotton swab to gently clean the area where each sensor will be placed and then sticking the sensor in place using a paste that washes out with water. After the sensors are in place, my child will be shown how to play a computer game in which buttons are pressed when certain pictures come up on a television screen or will be asked simply to watch pictures on the television. My child's brain activity and heart rate will be recorded during the computer games. The investigator will describe these tests to me in detail before each is started. I will be with my child the whole time. You will be asked some questions about your child's health and home life. This visit will take approximately 1.5 hours.

Visit 7 (5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit the child will sit in a chair in front of a small table. My child will be given a set of cards and asked to give a certain response to a card and will play a game in which monkeys will be placed in a tree according to a few rules. In addition, my child will be shown and set of pictures in a certain order and will be given a chance to put those pictures in order. The investigator will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. In addition, my child will be given a test of language abilities. You will be asked some questions about events in your child's life and his or her behavior. This visit will take approximately 1.5 hours.

Visit 8 (5.5 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. My child's blood pressure will be taken. During this visit, 34 electrical sensors will be placed on my child's head and 2 additional sensors will be place on my child's chest. This procedure involves using a cotton swab to gently clean the area where each sensor will be placed and then sticking the sensor in place using a paste that washes out with water. After the sensors are in place, my child will be shown how to play a computer game in which buttons are pressed when certain pictures come up on a television screen or will be asked simply to watch pictures on the television. My child's brain activity **and heart rate** will be recorded during the computer games. The investigator will describe these tests to me in detail before each is started. I will be with my child the whole time. You will be asked some questions about your child's health and home life. This visit will take approximately 1.5 hours.

Visit 9 (6 years of age): My child will be weighed and measured. I will be asked what my child has eaten in the past 24 hours and questions about my child's general health. In addition, my child's blood pressure will be taken. During this visit, my child will sit in a chair in front of a small table. My child will be play a game in which monkey's will be placed in trees according to a few rules. In addition, my child will also be asked to play with blocks, put puzzles together, and be asked questions to test their general knowledge, comprehension, and vocabulary. My child will also be presented with different patterns or shapes and be asked to fill in the missing piece. In addition, my child will be shown a series of pictures and

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be asked which two go together. My child will also be shown a series of symbols and be asked to find the two that match. My child will play a game and try to figure out what the investigator is thinking of based on the clues given. My child will also be shown a series of pictures and be asked what is missing. The investigators will describe these tests to me in detail before each is started. The visit will be videotaped and I will be with my child the whole time. I will be asked some questions about events in my child's life. This visit will take approximately 2 hours.

RISKS
There are no known risks from any of the tasks that my child will be asked to do. Some of the tasks may be tiring and my child may not like wearing the cap, but the investigators will not continue with a test if the child is not performing at his/her best because he/she is tired or excessively bothered by wearing the cap.

NEW FINDINGS STATEMENT
The study will continue to follow the development of my child between 2 and 6 years of age. I will be informed if any significant new findings develop during the course of the study that may affect my willingness to participate or to allow my child to participate in this study.
I may request to know results when the study is complete.

BENEFITS
My child will not benefit from participating in this study. It is hoped that additional information gained in this research study may be useful in the growth and development of infants.

ALTERNATIVES
My child does not have to participate in this research study.

COSTS
I will not incur any costs because of my child's participation.

PAYMENT TO SUBJECTS
I will receive a check for $100 at each visit to the University of Kansas Medical Center to cover the costs of transportation and to partially compensate me for my time required to participate in the study. If I do not have enough money to come for the visit, I may ask the investigators to pay for a cab to and from the appointment andI will be given the $100 check, however, the investigators will have to deduct the cost of the cab from my next check. There will be 8 regularly scheduled visits in 4 years. If an additional visit is required because my infant is unable to complete all of the testing at 6 years of age, I will receive an additional payment of $50 for another visit.

My name, address, social security number, and the title of this study will be given to the KUMC Research Institute. This is done so that the Research Institute can write a check for study payments. Payments are taxable income.

DISCLOSURE OF FINANCIAL INTERESTS
The principal investigator has been paid as a consultant and for program presentation on DHA for Mead Johnson Nutritionals (the sponsor). The University of Kansas Medical Center

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Conflict of Interest Committee monitors this research project to make it less likely that these financial interests inappropriately influence how the study is conducted. However, you should make your own decision about whether these financial interests affect your decision to participate. If you have any questions about this financial relationship, you may discuss them with the investigator or with the Research Compliance division at 913-588-5492.

INSTITUTIONAL DISCLAIMER STATEMENT

If you believe you have been injured as a result of participating in research at Kansas University Medical Center (KUMC), you should contact the Director, Human Research Protection Program, Mail Stop #1032, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160. Compensation to persons who are injured as a result of participating in research at KUMC may be available, under certain conditions, as determined by state law or the Kansas Tort Claims Act.

CONFIDENTIALITY AND PRIVACY AUTHORIZATION

Names of subjects or information identifying subjects will not be released without written permission unless required by law. Study data will be shared with the sponsor, but I will not be identified. Videotapes of my baby when he/she is looking at pictures and playing with toys will be used only by the investigators and their students. The videotapes will be secured under lock and key like all of other information that could be linked directly to my child. The videotape of my child will not be shown without specific permission from me and even then would not identify my child by name. The videotapes will be destroyed after all of the study data are collected and analyzed. Because study will continue for 4 more years and enrollment occurred during 2 years, the investigators may keep a copy of my child's videotape for as long as 8 years. Efforts will be made to keep my and my child's personal information confidential. Researchers cannot guarantee absolute confidentiality. If the results of this study are published or presented in public, information that identifies my baby will be removed.

The privacy of my and my child's health information is protected by a federal law known as the Health Insurance Portability and Accountability Act (HIPAA). If I choose to allow my child to participate in this study, I will be asked to give permission for researchers to use and disclose my and my baby's health information that is relevant to the study.

Because this is a continuation of an existing study, researchers already have some health information about my child from his/her medical record with consent. They will not obtain any other information except the information that they conduct as shared in the Procedures section. My baby's study-related health information will be used at KU Medical Center by Dr. Carlson, members of the research team, the KU Hospital Medical Record Department, the KUMC Research Institute and officials at KUMC that oversee research, including the KUMC Human Subjects Committee, and other committees and offices that review and monitor research studies.

Dr. Carlson and her team may share information about me and my baby with representatives of Mead Johnson (the sponsor of the study), the U.S. Food and Drug Administration (FDA), and U.S. agencies that govern human research (if and when regulatory compliance issues

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arise). My and my child's information may be shared in order to analyze and confirm the results of the study.

Some of the persons or groups that receive my and my baby's study information may not be required to comply with HIPAA privacy laws. My and my child's information may lose its federal protection if those persons or groups disclose it.

Permission granted on this date to use and disclose my health information remains in effect indefinitely. By signing this form I give permission for the use and disclosure of my and my child's information for purposes of the study at any time in the future.

QUESTIONS

I have read the information in this form. Dr. Carlson or her associates have answered my question(s) to my satisfaction. I know if I have any more questions after signing this I may contact Dr. Carlson or one of her associates at (913) 588-5359. If I have any questions about my child's rights as a research subject, I may call (913) 588-1240 or write the Human Subjects Committee, University of Kansas Medical Center, 3901 Rainbow Blvd. MSN 1032, Kansas City, KS 66160.

SUBJECT RIGHTS AND WITHDRAWAL FROM THE STUDY

My and my child's participation in this study is voluntary and that the choice not to participate or to quit at any time can be made without penalty or loss of benefits. Not participating or quitting will have no effect upon the medical care of treatment my child receives now or in the future at the University of Kansas Medical center. The entire study may be discontinued for any reason without my consent by the investigator conducting the study or by the sponsor of the study. My child's participation can be discontinued by the investigator if I do not come for scheduled visits.

You have a right to change your mind about allowing the research team to have access to your healthy information. To cancel your permission you must send a written request to Dr. Carlson at the University of Kansas Medical Center, Dept. of Dietetics and Nutrition, Mail Stop 4013, 3901 Rainbow Boulevard, Kansas City, KS 66160. If you cancel permission to use your health information, you will be withdrawn from the study and the researchers will stop collecting information about you. The researchers and the sponsor may continue to use and share information that was gathered before your cancellation.

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CONSENT

Dr. Carlson or her associates have given me information about this research study. They have explained what will be done and how long it will take. They explained the inconvenience, discomfort and risks that may be experienced during this study.

By signing this form, I give my permission for my child to continue with followup for an additional 8 visits (at 6 ages) between 2 and 6 years of age. If I choose not to sign this form, my child and I will not be able to participate in the study.

I voluntarily consent to allow my child and I to participate in this research study. I have read the information in this form and have had an opportunity to ask questions and have them answered. ***I will be given a copy of the signed form to keep for my records.***

_____
Type/Print Subject's Name

_____ _____ _____
Signature of Subject Time Date

_____
Type/Print Name of Witness

_____ _____
Signature of Witness Date

_____
Type/Print Name of Person Obtaining Consent

_____ _____
Signature of Person Obtaining Consent Date

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Appendix B

Consent for Release of Medical Records

THE UNIVERSITY OF KANSAS HOSPITAL
CONSENT FOR THE RELEASE OF CONFIDENTIAL INFORMATION

I, _____ born on _____, hereby

authorize _____ to disclose to:

The University of Kansas Medical Center

3901 Rainbow Boulevard MS 4013

Kansas City, Kansas 66160-7200

Attention: Infant/Toddler Nutrition Research Clinic Phone: (913) 588-5743; **Fax: (913) 945-6621**

the following information:_____

_____

_____

_____

_____

I understand that my medical records (including any alcohol or drug abuse information) may be protected by Federal Regulations. I also understand that I may revoke this consent at any time except to the extent that action has been taken in reliance on it (e.g., probation, parole, etc.) and that in any event this consent expires automatically as described below.

SPECIFICATION OF THE DATE, EVENT, OR CONDITION UPON WHICH THIS CONSENT EXPIRES (if left blank this consent expires in one year)

_____

EXECUTED THIS _____ DAY OF _____, 20_____

_____ (Witness)

_____ (Signature of Patient)

_____ (Signature of patient, guardian, or authorized representative)

_____ (Nature of relationship)

PROHIBITION ON REDISCLOSURE: THIS INFORMATION HAS BEEN DISCLOSED TO YOU FROM RECORDS WHOSE CONFIDENTIALITY IS PROTECTED BY FEDERAL LAW. FEDERAL REGULATIONS (42O FR PART 2) PROHIBIT YOU FROM MAKING ANY FURTHER DISCLOSURE OF THIS INFORMATION EXCEPT WITH THE SPECIFIC WRITTEN CONSENT OF THE PERSON TO WHOM IT PERTAINS. A GENERAL AUTHORIZATION FOR THE RELEASE OF MEDICAL OR OTHER INFORMATION IF HELD BY ANOTHER PARTY IS NOT SUFFICIENT FOR THIS PURPOSE. FEDERAL REGULATIONS STATE THAT ANY PERSON WHO VIOLATES ANY PROVISION OF THIS LAW SHALL BE FINED NOT MORE THAN $500, IN THE CASE OF A FIRST OFFENSE, AND NOT MORE THAN $5,000, IN THE CASE OF EACH SUBSEQUENT OFFENSE.

Drug Abuse Office and Treatment Act of 1972 (21 USC 1175) Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment, and Rehabilitation Act of 1970 (42 USC 4582)

Appendix C

Adverse Event Log Sheet

Adverse Event Log Sheet

Subject ID _____ Subject DOB _____/_____/_____

AGE AT EVENT: _____ months

Adverse Event _____ Start Date: _____/_____/_____

Code _____ Stop Date: _____/_____/_____

Ongoing: _____/_____/_____

Serious: Yes No

Action taken (circle none or list as applicable):

None

Medications

Other, specify_____

Hospital/Clinic seen at _____

Reported at visit _____

Coded ☐ Entered ☐ Checked ☐

AGE AT EVENT: _____ months

Adverse Event _____ Start Date: _____/_____/_____

Code _____ Stop Date: _____/_____/_____

Ongoing: _____/_____/_____

Serious: Yes No

Actions taken (circle none or list as applicable):

None

Medications

Other, specify_____

Hospital/Clinic seen at _____

Reported at visit _____

Coded ☐ Entered ☐ Checked ☐

Appendix D

Demographics Form

INVESTIGATOR	PROTOCOL	RANDOM CODE	DATE
CARLSON	HSC #10205		

DEMOGRAPHICS

Maternal Education ☐

Paternal Education ☐

Does anyone living in the child's home smoke?

☐ No ☐ Yes If yes, how many people smoke & how many ppd? _____

List any maternal allergies: _____

Including the child enrolled in this study, how many children 13 years of age or younger live in your house?

☐1 ☐2 ☐3 ☐4 ☐5 ☐6 or more

Do any pets live in the child's home?

☐ No ☐ Yes If yes, how many pets? _____

What kind? _____

Do you take your child to a daycare (facility or homecare) with other infants and children?

☐ No

☐ Yes, with 1 to 5 children

☐ Yes, with 6 to 10 children

☐ Yes, with more than 10 children

Appendix E

Grouped Adverse Event List

Diagnosis	Adverse Events Included
Any Diagnosis	• All diagnoses relating to illness (no accident or trauma adverse event codes included)
Eyes, Ears, Nose, Throat (EENT) Diagnosis	• Conjunctivitis • Ear Wax Excessive • Eye Movement Disorder • Infectious Conjunctivitis • Infectious Rhinitis • Infectious Sinusitis • Nasal Congestion • Nasal/Tear Duct Obstruction • Otitis Media • Purulent Rhinitis • Retinal Detachment/Hemorrhage • Rhinorrhea • Thrush • Watery Eye
Gastrointestinal (GI) Diagnosis	• Acute Gastroenteritis • Anal Fissure • Bloody Stool • Colic • Constipation • Diarrhea • Emesis • G.E. Reflux • Rotavirus • Stomatitis • Umbilical Hernia
Respiratory (RESP) Diagnosis	• Bronchiolitis • Bronchitis • Cough • Infectious Cough (antibiotic given) • Influenza • Pharyngitis • Pneumonia • Respiratory Distress Syndrome • Respiratory Syncytial Virus • Strep Throat • Tachypnea • Tonsillitis • URI

Skin (SK) Diagnosis	• Bacterial Skin Infection • Chicken Pox • Diaper Rash • Dry Skin • Erythema • Fungal Skin Infection • Hemangioma • Impetigo • Inclusion Cyst • Infection • Neonatal Acne • Nevus • Other Rash • Parasitic Skin Infection • Seborrhea • Staph Infection • Viral Skin Infection • Wart • Yeast Infection
Urogenital (UG) Diagnosis	• Abnormal Urine • Inguinal Hernia • Labial Adhesions • Penile Adhesions • Undescended Testes • Urinary Tract Infection • Vaginal Discharge • Vulvitis/Vaginitis
Upper Respiratory Infections (URI)	• URI alone
Otitis Media (OM)	• OM alone
Any Allergy	• Allergic Conjunctivitis • Allergic Cough (allergy medication given) • Allergic Rhinitis • Allergic Sinusitis • Allergy • Asthma • Atopic Dermatitis • Contact Dermatitis • Croup • Drug Allergy • Eczema • Food Allergy • Urticaria • Wheezing
Wheeze/Asthma	• Asthma • Wheezing

Wheeze/Asthma/Skin	• Asthma • Atopic Dermatitis • Contact Dermatitis • Eczema • Urticaria • Wheezing
Skin Allergic Illness	• Atopic Dermatitis • Contact Dermatitis • Eczema • Urticaria
Nonallergic Respiratory Illness	• Bronchiolitis • Bronchitis • OM • Pneumonia • Sinusitis • URI
Respiratory Infectious Illness	• Bronchiolitis • Bronchitis • Influenza • Pneumonia • Respiratory Syncytial Virus • Strep Throat • URI
Gastrointestinal Illness	• Acute Gastroenteritis • G.E. Reflux • Infectious Gastroenteritis

Appendix F

Adverse Event Codes

Listing of AE Codes in the Format Library

Body System	Code	Event
BODY AS A WHOLE	BODY024	ABNORMAL UMBILICUS
	BODY028	ACCIDENT
	BODY014	ALLERGY
	BODY036	ANAPHYLACTIC SHOCK
	BODY025	ANAPHYLAXIS
	BODY010	ANEMIA
	BODY007	APPARENT LIFE-THREATENING EVENT (ALTE)
	BODY017	ASPHYXIA
	BODY029	ASYMETRICAL FAT FOLD
	BODY015	BENIGN MASS
	BODY030	BREAST ENLARGEMENT
	BODY032	CARRIER BIOTIN DEFICIENCY
	BODY002	DEHYDRATION
	BODY016	DEVELOPMENTAL DELAY
	BODY021	DIAGNOSTIC PROCEDURE
	BODY034	DRUG ALLERGY
	BODY004	EXCESSIVE CRYING
	BODY003	FAILURE TO THRIVE
	BODY022	FEVER
	BODY038	FEVER OF UNKNOWN ORIGIN
	BODY033	FOOD ALLERGY
	BODY012	FUSSINESS
	BODY005	INFECTION
	BODY035	INSECT STING ALLERGY
	BODY001	IRRITABILITY
	BODY023	MICROCEPHALY
	BODY018	PAIN
	BODY027	PECTUS EXCAVATION
	BODY026	PLAGIOCEPHALY
	BODY019	REACTION TO VACCINE
	BODY008	RULE OUT SEPSIS
	BODY009	SEPSIS
	BODY020	SHORT STATURE
	BODY039	SPHEROCYTOSIS
	BODY006	SUDDEN INFANT DEATH SYNDROME (SIDS)
	BODY013	SURGERY
	BODY037	SYSTEMIC FUNGAL INFECTION
	BODY031	TEETHING
	BODY011	WEAKNESS

Listing of AE Codes in the Format Library

Body System	Code	Event
CARDIOVASCULAR	CARD007	ARRHYTHMIA
	CARD001	BRADYCARDIA
	CARD010	CARDIAC DEFECT
	CARD006	CARDIOMYOPATHY
	CARD002	CONGENITAL HEART DISEASE
	CARD003	COR PULMONALE
	CARD004	HEART MURMUR
	CARD005	HYPERTENSION
	CARD008	PERICARDIAL EFFUSION
	CARD009	TACHYCARDIA

Listing of AE Codes in the Format Library

Body System	Code	Event
EYES, EARS, NOSE AND THROAT	EENT020	ABNORMAL REFRACTION
	EENT039	ALLERGIC CONJUNCTIVITIS
	EENT037	ALLERGIC RHINITIS
	EENT038	ALLERGIC RHINO-CONJUNCTIVITIS
	EENT041	ALLERGIC SINUSITIS
	EENT031	BACTERIAL EYE INFECTION
	EENT026	BLIND
	EENT022	CATARACTS
	EENT036	CHOANAL STENOSIS
	EENT006	CONJUNCTIVITIS
	EENT008	CORNEAL ABRASION
	EENT029	EAR DRAINAGE
	EENT024	EAR WAX EXCESSIVE
	EENT018	EYE MOVEMENT DISORDER
	EENT023	EYELID INFECTION
	EENT013	HEARING DEFICIT
	EENT032	HEMANGIOMA
	EENT040	INFECTIOUS CONJUNCTIVITIS
	EENT043	INFECTIOUS RHINITIS
	EENT015	INFECTIOUS RHINITIS/SINUSITIS
	EENT042	INFECTIOUS SINUSITIS
	EENT012	LARYNGEAL EDEMA
	EENT034	LARYNGOMALACIA
	EENT025	LASER SURGERY/CRYOTHERAPY
	EENT021	MYRINGOTOMY/TM TUBES
	EENT002	NASAL CONGESTION
	EENT003	NASAL/TEAR DUCT OBSTRUCTIONS
	EENT030	OTITIS EXTERNA
	EENT001	OTITIS MEDIA
	EENT004	PURULENT RHINITIS
	EENT027	RETINAL DETACHMENT/HEMORRHAGE
	EENT014	RETINOPATHY OF PREMATURITY
	EENT011	RHINORRHEA
	EENT028	SEPTAL DEVIATION
	EENT010	SNEEZING/ITCHING
	EENT007	STAPH INFECTION IN EYE
	EENT019	SWALLOWING DISORDER
	EENT009	THRUSH
	EENT017	TONSILLECTOMY/ADENOIDECTOMY
	EENT016	TRAUMA

Listing of AE Codes in the Format Library

Body System	Code	Event
EYES, EARS, NOSE AND THROAT	EENT033	TUGGING AT EAR
	EENT035	VARIX
	EENT005	WATERY EYE

Listing of AE Codes in the Format Library

Body System	Code	Event
ENDOCRINE	END003	ABNORMAL LABORATORY RESULT
	END004	ADRENAL HYPERPLASIA
	END001	ADRENAL INSUFFICIENCY
	END002	HYPOTHYROIDISM

Listing of AE Codes in the Format Library

Body System	Code	Event
GASTROINTESTINAL	GI018	ABDOMINAL CRAMPING
	GI036	ABDOMINAL DISTENTION
	GI008	ACUTE GASTROENTERITIS
	GI043	ALLERGIC COLITIS
	GI045	ALLERGIC ENTEROCOLITIS
	GI046	ALLERGIC ESOPHAGITIS
	GI044	ALLERGIC GASTROENTERITIS
	GI010	ANAL FISSURE
	GI039	ANAL IRRITATION
	GI048	ANAL SWELLING
	GI023	BLOATING
	GI009	BLOODY STOOL
	GI025	BURPING
	GI007	COLIC
	GI014	COLITIS
	GI006	CONSTIPATION
	GI020	COW'S MILK INTOLERANCE
	GI040	DEFECATION PROBLEM
	GI005	DIARRHEA
	GI022	DYSPEPSIA (INDIGESTION/HEARTBURN)
	GI001	EMESIS
	GI002	EXCESSIVE SPITTING
	GI030	FEEDING PROBLEMS
	GI037	FEEDINGS WITHHELD DUE TO INTOLERANCE
	GI024	FLATULENCE
	GI017	FREQUENT STOOLS
	GI003	G.E. REFLUX
	GI004	GAS
	GI035	GASTRIC RESIDUALS
	GI031	GASTRIC TUBE PLACEMENT
	GI015	GI INFECTION
	GI038	HARD STOOLS
	GI051	HEMORRHOID
	GI032	HEPATIC CALCIFICATION
	GI041	HUNGER
	GI021	ILEUS
	GI047	INFECTIOUS GASTROENTERITIS
	GI049	LOOSE STOOLS
	GI050	MUCOUS IN STOOL
	GI027	NAUSEA

Listing of AE Codes in the Format Library

Body System	Code	Event
GASTROINTESTINAL	GI028	NECROTIZING ENTEROCOLITIS
	GI033	PERIANAL FISTULA
	GI011	PERIRECTAL ABSCESS
	GI042	PERSISTENCE OF UMBILICAL CORD
	GI016	PYLORIC STENOSIS
	GI034	RECTAL STENOSIS
	GI012	SALMONELLA IN STOOL
	GI013	SPITTING UP
	GI029	STOMATITIS
	GI026	STRAINING
	GI019	UMBILICAL HERNIA

Listing of AE Codes in the Format Library

Body System	Code	Event
METABOLIC AND NUTRITION	MAN009	ABNORMAL LABORATORY RESULT
	MAN005	ELECTROLYTE INBALANCE
	MAN008	FAILURE TO THRIVE
	MAN003	FETAL MALNUTRITION
	MAN011	FORMULA REJECTION
	MAN010	GLUTARIC ACIDEMIA TYPE 1
	MAN002	LACK OF APPETITE
	MAN006	MALNUTRITION
	MAN004	OSTEOPENIA/RICKETS
	MAN007	POOR WEIGHT GAIN
	MAN001	WEIGHT LOSS

Listing of AE Codes in the Format Library

Body System	Code	Event
MUSCULOSKELETAL	MS002	CRANIOSYNOSTOSIS
	MS004	DEFORMITY
	MS001	FRACTURE
	MS006	HIP CLICK
	MS007	HIP TIGHTNESS
	MS008	KNEE CLICK
	MS005	TORTICOLLIS
	MS003	TRAUMA

Listing of AE Codes in the Format Library

Body System	Code	Event
NERVOUS	NER007	ABNORMAL EEG
	NER010	ABNORMAL TONE
	NER021	BEHAVORIAL ISSUE
	NER012	CEREBRAL PALSY
	NER013	CONCUSSION
	NER011	CYST
	NER003	DIZZINESS
	NER015	ENCEPHALOPATHY
	NER004	FAINTING
	NER006	HEADACHE
	NER005	IMPAIRED CONGNITION
	NER009	INTRACRANIAL HEMORRHAGE
	NER014	MACROCEPHALY
	NER002	MENINGITIS
	NER020	MICROCEPHALY
	NER016	PARESIS
	NER017	PERIVENTRICULAR LEUKOMALACIA
	NER001	SEIZURE
	NER018	SHUNT REVISION
	NER019	TETHERED SPINAL CORD
	NER008	VENTRICULOMEGALY/HYDROCEPHALUS

Listing of AE Codes in the Format Library

Body System	Code	Event
RESPIRATORY	RESP025	ABNORMAL X-RAY FINDING
	RESP013	APNEA
	RESP008	ASTHMA
	RESP009	BRONCHIOLITIS
	RESP005	BRONCHITIS
	RESP017	BRONCHOPULMONARY DYSPLASIA
	RESP002	COUGH
	RESP004	CROUP
	RESP022	CYANOSIS
	RESP018	LARYNGITIS
	RESP012	PHARYNGITIS
	RESP007	PNEUMONIA
	RESP014	POSITIVE PRESSURE VENTILATION
	RESP020	PULMONARY EDEMA
	RESP021	PULMONARY HYPERTENSION
	RESP019	PULMONARY INSUFFICIENCY
	RESP016	REACTIVE AIRWAY DISEASE
	RESP015	REINTUBATION
	RESP010	RESPIRATORY DISTRESS SYNDROME
	RESP011	RESPIRATORY SYNCYTIAL VIRUS (RSV)
	RESP003	STREP THROAT
	RESP023	TACHYPNEA
	RESP024	TONSILLITIS
	RESP001	UPPER RESPIRATORY INFECTION (URI)
	RESP006	WHEEZING

Listing of AE Codes in the Format Library

Body System	Code	Event
SKIN	SK014	ANGIOEDEMA
	SK021	ATOPIC DERMATITIS
	SK027	BACTERIAL SKIN INFECTION
	SK012	CHICKEN POX
	SK030	CONTACT DERMATITIS
	SK001	DIAPER RASH
	SK003	DRY SKIN
	SK004	ECZEMA
	SK015	ECZEMA/SEBORRHEA
	SK018	EDEMA
	SK023	ERYTHEMA
	SK028	FUNGAL SKIN INFECTION
	SK017	HEMANGIOMA
	SK006	IMPETIGO
	SK024	INCLUSION CYST
	SK022	INFECTION
	SK031	INSECT BITE
	SK026	INTERTRIGO
	SK019	IV INFILTRATE
	SK008	JAUNDICE
	SK011	NEONATAL ACNE
	SK025	NEVUS
	SK010	OTHER RASH
	SK013	PRURITIS
	SK005	SEBORRHEA
	SK007	STAPH INFECTION
	SK016	TRAUMA
	SK009	URTICARIA
	SK029	VIRAL SKIN INFECTION
	SK020	WART
	SK002	YEAST INFECTION

Listing of AE Codes in the Format Library

Body System	Code	Event
UROGENITAL	UG012	ABNORMAL GENITALIA
	UG016	ABNORMAL URINE
	UG014	CIRCUMCISION
	UG003	FETAL MALNUTRITION
	UG007	HYPOSPADIAS
	UG005	INGUINAL HERNIA
	UG009	LABIAL ADHESIONS
	UG002	LACK OF APPETITE
	UG013	PENILE ADHESION
	UG010	PENILE LESION
	UG011	RENAL CALCULUS
	UG008	UNDESCENDED TESTES
	UG004	URINARY TRACT INFECTION
	UG001	VAGINAL DISCHARGE
	UG006	VESICO-URETERAL REFLUX
	UG015	VULVITIS/VAGINITIS