

INFANT FORMULA DOCOSAHEXAENOIC ACID AND
BODY MASS INDEX TO FOUR YEARS OF AGE

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B.S., Iowa State University, 2006

Submitted to the Department of
Dietetics and Nutrition and to the
Graduate Faculty of the University of
Kansas in partial fulfillment of the
Requirements for the degree of Master
of Science.

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ABSTRACT

Docosahexaenoic acid (DHA) is a long-chain fatty acid essential for brain growth and cognitive development in infancy. There is some evidence that DHA can also influence growth in infancy and early childhood. Several clinical trials with infants and young children have found lower normalized growth following DHA increased exposure through maternal supplementation during pregnancy and/or lactation or infant formula supplementation. The aim of this study was to evaluate the impact of feeding one of four concentrations of DHA in infant formula on weight-for-length percentile (<2 years) and body mass index (BMI-age) percentile (≥ 2 years) at twelve study visits from birth to four years of age. Healthy formula-fed infants were randomized to one of four infant formulas containing 0, 0.32, 0.64, or 0.96% of total fatty acid from DHA. Weight, length, and head circumference were measured at 6 weeks, 4 months, 6 months, 9 months, 12 months, 18 months, 2 years, 2.5 years, 3 years, 3.5 years, and 4 years of age. Subjects were normalized to the Center for Disease Control weight-for-length (<2 years) and BMI-age growth charts (≥ 2 years) by calculating percentiles. The relationship between weight-for-length and BMI-age percentiles across study visits and DHA concentration in the study formula were evaluated with a two way repeated measures ANOVA using a p-value of 0.05 as statistically significant. The concentration of DHA consumed through infant formula during the first year of life did not impact weight-for-length or BMI-age percentile from birth to 4 years of age ($P = 0.683$). When grouped by DHA or no DHA there was no statistical significance ($P = 0.416$). There is no observable difference in weight-for-length or BMI-age in infants supplemented DHA through infant formula in the first four years of life.

ACKNOWLEDGEMENTS

First I would like to thank my mentor Dr. Susan Carlson for all of your wisdom, guidance, and support in developing and writing my thesis. You have such a wealth of knowledge and creative innovation that I hope to emulate even after this process is complete. I cannot thank you enough for all of the time and effort you have invested into this project; I could not have done it without you. I would especially like to thank the Infant Nutrition Clinic Team: Beth Kerling, Laura Minns, and Jocelynn Jacoby for answering my questions and pointing me in the right direction. I would also like to thank Dr. Kingsley and Jill Shaddy for your instruction and direction with SPSS and the statistical analysis. I learned a lot and appreciate your help. I would also like to thank my thesis committee members; Dr. Griffith for helping to develop my scientific writing and to Dr. Goetz for all of your assistance with the 24-hour recalls and NDSR. Additionally, thanks to my entire committee for your encouragement and commitment to this thesis. I would like to thank my family and friends for your support and encouragement. Thank you to Matt Knecht for your positive words and technical support through this all.

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Glossary

Acanthosis nigricans: a brown to black, velvety hyperpigmentation of the skin associated with insulin resistance and obesity

Acceptable Macronutrient Distribution Range (AMDR): the National Academy of Science's recommended intake ranges for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids developed in 2002

Adequate Intake (AI): the amount of a nutrient that appears to sustain good health, developed by the Food and Nutrition Board for nutrients that have not yet received enough scientific study to merit setting of an official Recommended Dietary Allowance (RDA)

Adipocyte: also known as lipocytes and fat cells, the cells that primarily compose adipose tissue and specialize in storing energy as fat

Aminotransferase: an enzyme that catalyzes the transfer of an amino group from a donor molecule to a recipient molecule

Dietary Reference Intake (DRI): a system of nutrition recommendations to assess groups from the Institute of Medicine (IOM) of the US National Academy of Sciences intended for the general public and health professionals

Gestational age: the age of an embryo or fetus

Lipolysis: the breakdown of fat stored in fat cells

Metabolic syndrome: a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes

Chapter 1: Introduction

Docosahexaenoic acid (DHA) is an omega-3 fatty acid essential for brain growth and cognitive development in infancy and is fortified in most commercial infant formula. There is conflicting evidence on the impact of DHA on weight-for-length in infancy and BMI-age in early childhood. Animal research indicates that DHA plays a role in pre-adipocyte proliferation and reduces adiposity (1, 2). Reduction in adipose tissue could translate into lower body weight with supplementation of omega-3 polyunsaturated fatty acids (n-3 PUFA). Randomized controlled trials with preterm and term infants have shown lower growth indices with DHA supplementation. Additionally, clinical trials with maternal supplementation of DHA during pregnancy and/or lactation have shown associations with growth during early childhood.

A 2001 review of 13 randomized studies in preterm infants and 19 randomized studies in term infants determined that n-3 PUFA supplementation may lead to lower normalized growth in preterm and term infants under some experimental conditions. The difference appears to be minimal with limited physiological and clinical relevance (3). There is some evidence that DHA in infant formula may contribute to lower normalized growth in VLBW preterm infants but may be dependent on the concentration of DHA and other constituents in the formulas.

In term infants few studies demonstrate a difference in growth when supplemented with a formula source of DHA. One study found significantly lower mean weight at 4 months of age ($P = 0.055$) with a high alpha-linolenic acid (ALA) supplemented formula (3.2% of fatty acids). Infants who received the high ALA formula had higher plasma concentrations of DHA but lower concentrations of arachidonic acid at

21, 40 and 120 days (4 months) of age. The study did not find differences in growth before or after the 4 month assessment (4).

Morris et al. (5) reported lower subscapular skinfold thickness in infants consuming formula supplemented with 0.2% DHA and 0.4% ARA compared to a standard unsupplemented formula. Small but statistically significant differences were found between the two groups at 6 weeks (7.3 mm in supplement group, 7.8 mm in control group; $P < 0.046$) and at 3 months (7.6 mm in supplement group, 8.3 mm in control group; $P < 0.012$) but these differences were not evident at 6 or 12 months.

Three clinical trials investigated the relationship between early DHA supplementation and BMI in young children. In all three clinical trials mothers consumed DHA supplements during pregnancy and/or lactation and investigators measured BMI in the offspring. Only one study showed a negative association between maternal DHA supplementation and weight and BMI (6). Another showed no association between maternal DHA supplementation and BMI (7, 8). A third study showed a positive association between maternal DHA supplementation and BMI and head circumference (9).

Research on infant supplementation of DHA and weight-for-length in infancy and BMI-age in early childhood is inconsistent. Some studies suggest that supplementing DHA in infancy may lower weight-for-length and BMI-age at one or more points during infancy and early childhood.

Justification for Further Investigation

Currently there are gaps in the literature. Although there is a good amount of research on DHA supplementation through infant formula on growth in infancy and there is growing research on maternal supplementation and BMI in early childhood there is no present research on DHA supplementation with infant formula that contains both DHA and arachidonic acid (ARA) and BMI in childhood. The research in preterm and term infants is mixed with some indicating lower weight, weight-for-length, or fat mass with DHA supplementation through infant formula compared to unsupplemented formula. Maternal DHA supplementation during pregnancy and/or lactation has resulted in a positive, negative, or no association to BMI in different studies at various ages during childhood.

The amount of DHA supplemented through infant formula and maternal supplementation varies among studies. Therefore it is desirable to examine the effects of different doses of intake. Utilizing a sample of children supplemented with one of four concentrations of DHA as infants, we had an opportunity to evaluate the growth from birth to four years of age. One formula contained no DHA or ARA. The other formulas provided 0.32, 0.64, and 0.96% of intake of total fatty acids as DHA and 0.64% ARA.

Statement of Purpose

The objectives of this study were to 1) determine if the concentration of DHA intake consumed throughout infancy in infant formula impacts growth, particularly weight-for-length percentiles (< 2 years) and body mass index percentiles (\geq 2 years) during the first four years of life and 2) to determine if DHA supplementation during

infancy lowers weight-for-length (< 2 years) or BMI percentiles (\geq 2 years) from birth to 4 years of age for infants receiving DHA through formula.

Research Questions

Primary Questions:

1. Is there a relationship between the concentration of DHA supplemented in infant formula consumed from birth to one year of age and weight-for-length percentiles (< 2 years) or body mass index percentiles (\geq 2 years) during the first four years of life?
2. Does DHA and ARA supplementation during infancy lower body mass index at four year of age?

Secondary Questions:

1. Is energy intake as determined by analysis of 24-hour dietary recalls related to growth in the first four years of life?
2. Are there other maternal or infant characteristics that impact weight-for-length/BMI-age in the first four years of life?

Chapter 2: Review of Literature

Normal Infant Growth

Infants grow exponentially in the first year of life. Although individual growth varies, the rate and proportion of weight gain typically follow a pattern. From birth to 6 months of age, an infant may grow 1/2 to 1 inch (about 1.5 to 2.5 centimeters) a month and gain 5 to 7 ounces (about 140 to 200 grams) a week. Infants typically double their birth weight by 5 to 6 months of age. From 6 to 12 months of age, an infant may grow 3/8 inch (about 1 centimeter) a month and gain 3 to 5 ounces (about 85 to 140 grams) a week (10). As a result infants will double their birth height and triple their birth weight by 12 months of age. During the second year, toddlers grow about 1 inch and 2 pounds about every 3 months. Children's growth slows considerably after age 2 years (10).

The 2000 Center for Disease Control (CDC) Growth Charts illustrate the distribution of normal growth in United States infants and children. They are used by health professionals such as pediatricians, nurses, dietitians, and parents to track the growth of infants, children, and adolescents. The 2000 revision of the growth curves were developed with data collected in five cross-sectional, nationally representative health examination surveys: the NHES II (1963–65) and III (1966–70), and NHANES I (1971–74), II (1976–80), and III (1988–94) (11).

The most recent CDC Growth Charts consist of a set of charts for infants at birth to 36 months of age and a set of charts for children and adolescents from 2 to 20 years of age. They are gender-specific and include smoothed percentiles for weight-for-age, length-for-age, head circumference-for-age, and weight-for-length for infants. Boys and

girls have slightly different growth patterns with boys tending to gain weight and height in infancy more rapidly than girls (12).

The growth charts for children and adolescents include weight-for-age, stature-for-age, and body mass index-for-age (BMI-age) curves. The BMI-age charts were added in 2000 and can be used to identify children who are overweight and obese during childhood. Although BMI is calculated the same way for children and adults, the criteria used to interpret the meaning of BMI for children and adolescents are different. For children and adolescents, BMI, age, and gender-specific percentiles are used primarily because the amount of body fat changes during growth. The amount of body fat also differs between girls and boys. The CDC BMI-age growth charts take into account these differences and allow professionals to translate BMI into a percentile for a child's sex and age (11).

Table 1 illustrates the categories used to indicate child weight status. The main BMI categories are the same for children and adults: underweight, normal or healthy weight, overweight, and obese.

TABLE 1 BMI-age categories for children 2 to 20 years

Weight status category	Percentile range
Underweight	Less than the 5th percentile
Healthy weight	5th percentile to less than the 85th percentile
Overweight	85th to less than the 95th percentile
Obese	Equal to or greater than the 95th percentile

Factors that Affect Infant Growth

There are many pre- and postnatal factors that affect birth weight and growth. Among these are genetic factors such as mother's height and ethnicity; environmental factors such as exposure to cigarette smoke and socioeconomic status. A study completed at the University of Kansas Medical Center that included a number of mixed race children compared risk factors among three distinct growth trajectories: 1) early onset overweight (10.9%), defined as overweight before 2 years of age; 2) late onset overweight (5.2%), defined as overweight occurring between 2 and 4 years of age; and 3) never overweight (83.9%) (13).

Investigators found there were several risk factors associated with early onset overweight status, i.e. overweight occurring before 2 years of age. Maternal overweight [BMI ≥ 25 and < 30 kg/m², odds ratio (OR), 2.2; 95% confidence interval (CI), 1.3 to 3.7] or obesity (≥ 30 kg/m², OR, 5.1; 95% CI, 2.9 to 9.1), maternal weight gain during pregnancy (≥ 20.43 kg, OR, 1.7; 95% CI, 1.0 to 2.9), birth weight (≥ 4000 g, OR, 2.0; 95% CI, 1.2 to 3.4), male gender (OR, 1.5; 95% CI, 1.0 to 2.2), and black ethnicity (OR, 1.7; 95% CI, 1.1 to 2.6) were associated with an increased risk of early onset overweight. These risk factors, except maternal weight gain, were also related to late onset overweight (13).

In that study, maternal smoking (OR, 1.6; 95% CI, 0.8 to 3.1) was associated with an increased risk of late onset overweight only (4). As noted above, the mean maternal pre-pregnancy BMI was also a predictor of overweight. In addition, mothers of children in the late onset overweight group were slightly older and had a lower level of education ($P < 0.001$) and lower family net income compared with those in the normal weight

trajectory. The mean birth weight in the early onset overweight group was greater than in the normal weight group ($P < 0.01$) (13).

Differences in size and growth are observed among the major racial-ethnic groups in the United States related most likely to environmental factors. Children of all major racial-ethnic groups have similar growth potential. Inherited effects on growth are small compared with the effects of the environment, nutrition, and health. Regardless of racial-ethnic status, children given good nutrition, access to health care, and good social and overall living conditions have similar growth patterns (14-17) .

Breast-feeding is considered the gold standard of nutrition for full-term infants during the first 6 months after birth and should be continued with the addition of solid foods through the first 12 months. The association between breast-feeding and infant growth has been carefully investigated. Breast-fed infants tend to gain weight more rapidly in the first 2–3 months than formula-fed infants; however, from 6 to 12 months breast-fed infants tend to weigh less than formula-fed infants (18). The growth pattern associated with breastfeeding compared to formula feeding is likely due to physiological differences in feeding with the breast compared to bottle and feeding patterns rather than to the nutrients in the diets (8). Most studies that examined the effects of breastfeeding versus formula feeding on body mass index after infancy have not found differences (19, 20).

Low Birth Weight and Infant Growth

A significant predictor of infant growth is weight at birth. Infants born with a low birth weight tend to grow differently than normal weight infants. Birth weight can be

categorized by either weight or size. The CDC Pregnancy Nutritional Surveillance System (PNSS) classifies birth weight in five categories (Table 2).

TABLE 2 Classifications of birth weight

PNSS indicator	Birth weight
Very Low (VLBW)	<1500 g
Moderately Low (MLBW)	1500 to <2500 g
Low (LBW)	<2500 g
Normal (NBW)	2500-<4000 g
High (HBW)	>4000 g

Within birth weight categories infants are also classified in relation to size for gestational age. These classifications include small-for-gestational age, appropriate-for-gestational age, and large-for-gestational age. Small-for-gestational age (SGA) infants' weight, length, and/or head circumference fall below the 10th percentile for gestational age. Appropriate-for-gestational age (AGA) infants' weight, length, and/or head circumference fall between the 10th and 90th percentiles for gestational age. Large-for-gestational age (LGA) infants' weight, length, and/or head circumference are above the 90th percentile for gestational age.

Infants born small for gestational age (SGA) or born with very low birth weight (VLBW) tend to grow more rapidly after birth and can catch up in weight to infants born at a normal birth weight. Even though these children experience catch up growth, they may achieve a height >2 standard deviations (SD) below the mean. Catch up growth is usually completed by the time they are 2 years of age (21).

Catch up growth during infancy and toddlerhood is very important because if it does not occur VLBW may lead to stunting. Investigators found that children who remained short at 2 years of age had a high risk of short stature later in life. The risk of having a short final height (<-2 SD) was five times higher for children with a low birth weight and seven times higher for those with a low birth length in comparison with children with a normal birth size. About 20% of all children of short stature were born SGA (22).

Results of one study of children showed that catch up growth in weight, height, and BMI occurred between 8 and 20 years among VLBW females but not among VLBW males who remained significantly smaller than their controls at 20 years old (23). Among the VLBW males, mean weight-for-age z scores at birth, 40 weeks, and 8 years were -0.7, -1.8, and -0.5; and height-for-age z scores were -1.2, -2.6, and -0.5, respectively.

For VLBW females at the same respective ages, mean weight-for-age z scores were -1.1, -2.0, and -0.2 and height-for-age z scores were -1.2, -2.4, and -0.2, respectively. At 8 years of age, VLBW males had a significantly lower mean weight, height, and BMI than normal birth weight (NBW) controls, whereas VLBW females differed significantly from their NBW controls in mean weight and BMI but not in height (23).

Catch up growth may lead to normalized stature in children, particularly females. Weight and BMI may take longer to reach the normal distribution (50th percentile) during childhood, but they may continue to increase after linear growth slows and lead to overweight status in adolescence and/or adulthood. Rapid changes in weight status

during infancy may substantially increase individuals' risk of obesity later in life. A 2006 review concluded that rapid prolonged catch up growth may lead to excessive weight gain in subsequent years (24). In both preterm and term LBW infants rapid growth compared to normal growth increases the risk of later obesity. A systematic review found that the odds ratio (OR) for obesity with rapid growth ranged from 1.17 to 5.70 (25).

Rapid weight gain is associated with high body mass index z scores in later years even when an infant is born with a low weight-for-length. Ong et al. (26) found children who showed catch up in weight or length between birth and two years were heavier and taller than other children at five years and were also taller in relation to their mothers' heights (0.15 ± 1.01 , paired t test: $P < 0.0005$) and fathers' heights (0.05 ± 0.92 , $P < 0.0005$). These children had greater body mass index, total fat mass, percentage body fat, and central fat distribution (26).

Hui et al. (27) found rapid catch up growth from birth to 3 months in infants born with low birth weight (mean birth weight of 2.8 kg) which had a larger effect on the BMI z scores in boys (mean difference, 0.88; 95% CI, 0.69-1.07) than in girls (mean difference, 0.52; 95% CI, 0.33-0.71). With each unit increase in the weight z score at ages 0 to 3 and 3 to 12 months increased the BMI-age z score by 0.52 and 0.33, respectively (27).

A New York State cohort of parents/guardians of children participating in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) found that ethnicity and rapid gains in weight-for-length in the first 6 months of life were associated with sharply increased risk of later obesity. The study sample was 32%

Hispanic, 19% black, and 49% white. The odds of being overweight at 4 years of age for Hispanic children were twice that of non-Hispanic children (odds ratio, 2.2; 95% confidence interval, 1.5 to 3.3). The risk of overweight at 4 years of age was 19% for children in the highest quintile of infant weight gain in this WIC population (28).

High Birth Weight and Infant Growth

High birth weight is also important to recognize because it is associated with obesity in later years. More than two dozen studies have addressed the association between high birth weight and later obesity [for review see (25)]. Almost all found that higher birth weight was associated with higher attained BMI in childhood and adulthood. In the 2005 metaanalysis, 18 studies assessed the relationship between infant size and obesity later in life. Most studies showed that infants who were at the highest end of the distribution for weight or weight for length had an increased risk of obesity. When compared with non-obese infants, those who had been obese had a relative risks for subsequent obesity of 1.35 to 9.38 (25).

In a 2009 study of 559 children, infants in the highest quartile of birth weight-for-length z scores compared with those in the lowest quartile had higher BMI-age z scores at 3 years of age ($\beta = 0.51$; 95% confidence interval, 0.28–0.75) (29). This recent study confirmed that infants with higher relative weight-for-length at birth have a greater risk of obesity in older years. In Hong Kong a study of 6,075 term births (77.5% successful follow-up) found an association between high birth weight, growth rate, and BMI at 7 years of age. Children in the highest birth weight and growth rate percentiles had the highest BMI-age z scores at 7 years of age (27).

Childhood Overweight Status

Childhood overweight status is a growing problem in the United States with 11-17% of children and adolescents ages 2 through 19 years at or above the 95th percentile (obese) and 32% at or above the 85th for weight (overweight) (30, 31). Children who are overweight are more likely to be overweight as adults. Krassas et al. (32) observed that over 80% of obese children reported being obese adults.

Research indicates that a child's body mass index is predictive of overweight status later in life. Nader et al. (33) showed that 2 in 5 children whose BMIs were $\geq 50^{\text{th}}$ percentile during the preschool period (2, 3, and 4.5 years of age) were overweight at 12 years of age. Additionally, children who were overweight ($< 85^{\text{th}}$ percentile) during the preschool period were > 5 times more likely to be overweight at 12 as those who were not overweight at any of the three preschool ages.

Overweight status in children is important to monitor because it is associated with significant health problems. Disease risk factors can develop during childhood and persist throughout adolescents and adulthood. Bell et al. (34) reported a positive relationship between increasing BMI-age z scores and components of metabolic syndrome including blood pressure, fasting insulin levels, presence of acanthosis nigricans, and elevated aminotransferase levels. Investigators also found an association with high density lipoprotein (HDL) cholesterol and triglyceride levels indicating that high BMI-age z scores have an impact on unfavorable lipid profiles (34).

There are many contributing factors to childhood overweight status. Some lifestyle and environmental factors in children are defined include inactivity, high intake of energy-dense low-micronutrient foods, high intake of sugar-sweetened beverages,

heavy marketing of energy-dense foods and fast food restaurants, adverse socioeconomic conditions, and large portion sizes (35). Due to the growing childhood obesity epidemic and large number of contributing factors, there is a need to decrease obeseogenic factors and increase factors that inversely affect weight.

Docosahexaenoic Acid

Docosahexaenoic acid (DHA) is a long-chain omega-3 polyunsaturated fatty acid (n-3 LC-PUFA) essential for brain growth and cognitive development. DHA is essential for optimal visual and neurological development during infancy and toddler years. It has been studied both pre- and post-natally in many clinical trials around the world [for reviews see (36) and (37)]. Eicosapentaenoic acid (EPA) is an omega-3 fatty acid precursor for DHA.

Human brain and retinal tissue are naturally concentrated with DHA. It makes up 40% of the PUFAs in the brain and 60% of the PUFAs in the retina (38). Some of the functions of DHA include structure, cellular signaling, and gene regulation. DHA plays a role in cellular signaling due to its presence in cellular membranes (39). PUFAs are important components of the phospholipids in membranes and reflect dietary fatty acid intake. A diet rich in DHA results in a higher concentration in membranes and influences physiological functions. Many effects of LC-PUFAs depend on the formation of their active metabolites, eicosanoids, and other lipid mediators (40). Eicosanoids are signaling molecules that have an anti-inflammatory effect when derived from omega-3 fatty acids (41).

DHA plays a role in fatty acid oxidation and metabolism. In experiments using cell cultures, DHA inhibited adipocyte differentiation and induced cellular death in

preadipocytes. DHA also increased rate of lipolysis compared with the control group (42, 43). Recent research indicated that DHA plays a role in inhibition of fat cell proliferation and reduces adiposity in mice (1, 44). A reduction in adipose tissue could translate into lower body weight and lower BMI with high omega-3 fatty acid intake.

Dietary DHA Intake

Fat is a significant macronutrient in the diets of infants and young children. The Institute of Medicine recommends an Adequate Intake (AI) of 31 grams of fat per day in infants from birth to 6 months and 30 grams per day from 6 to 12 months. The Dietary Reference Intake (DRI) for children 1 to 3 years of age is 30% to 40% of total energy from dietary fat and <10% of energy from saturated fat.

Recommended AIs for omega-3 fatty acids are 0.5 g for infants, 0.7 g for children 1-3 years, and 0.9 g for children 4-6 years. This amount is based on an intake that “supports normal growth and neural development and results in no nutrient deficiency” (Food and Nutrition Board, 2004). Although there is no DRI for EPA and DHA, the National Academies have recommended that approximately 10% of the Acceptable Macronutrient Distribution Range (AMDR) for essential fatty acids can be consumed as EPA and/or DHA (45). The current mean intake of EPA and DHA by adults in the United States is 80-100 mg/day, which is much lower than what many groups worldwide are currently recommending (46).

The Technical Committee on Dietary Lipids of the International Life Sciences Institute North America sponsored a workshop June 4-5, 2008 to reconsider the DRI for EPA and DHA based on evidence specific to the major chronic diseases in the United States including coronary heart disease (CHD), cancer, and cognitive decline. The

workshop participants concluded that research demonstrates a clear, inverse relation between EPA and DHA intake and risk of major chronic diseases. Current evidence supports a DRI for EPA and DHA between 250 and 500 mg per day for adults (46).

Most United States infants receive DHA from either mother's milk or DHA supplemented infant formula during the first year of life. The amount of DHA in human milk is dependent on the mother's dietary and supplemental intake of DHA and is highly variable (47). Data from NHANES (1999-2000) indicate US children less than six years of age typically consume only about 20 mg/day of DHA (48).

A 2008 review of LC-PUFAs in pregnancy, lactation, and infancy by an international expert committee concluded that pregnant and lactating women should aim to achieve an average daily intake of at least 200 mg DHA (49). For healthy term infants breastfeeding is the preferred source of nutrition; however, if breastfeeding is not possible infants' should consume a formula that contains DHA at levels between 0.2 and 0.5 % weight of total fatty acids, and with the minimum amount of arachidonic acid (ARA) equivalent to the contents of DHA (49, 50). Arachidonic acid (ARA) is important in infant growth because ARA conditional deficiency, usually from low omega-6 intake, can retard growth in preterm infants (51). Linoleic acid (LA) is an essential omega-6 fatty acid and precursor for ARA.

DHA and Infant Growth

Randomized clinical trials with preterm and term infants have shown lower growth indices with DHA supplementation through infant formula. A 2001 review of 13 randomized studies in preterm infants and 19 randomized studies in term infants determined that n-3 PUFA supplementation may lead to lower normalized growth in

infants, particularly preterm, under some experimental conditions (3). A Fleith and Clandinin (52) review in 2005 suggests the body of literature on PUFAs for preterm and term infants shows improved growth and development of infants; however, growth indices should include composition changes such as lean versus fat mass. The following studies looked at differences in infants' weight, weight-for-length, and/or body composition with supplementation of PUFAs compared to unsupplemented formula.

One of the earliest studies with omega-3 PUFA supplementation for very low birth weight (VLBW) preterm infants showed lower head circumference and weight at all measurement times (2,4,6,5,9 and 12 months past expected term) with fish oil (0.2% DHA, 0.3% EPA) supplementation for 11 months (53). Another VLBW preterm study with a low-EPA fish oil (0.25% DHA, 0.06% EPA) formula found lower weight-for-length indices at 2, 6, 9, and 12 months past expected term in the group fed fish oil compared to a typical formula that contained alpha-linolenic acid (54). However, in these studies lower growth indices were possibly due to a reduction in arachidonic acid (ARA) levels that occurs when long chain omega-3 fatty acid supplements are used without an arachidonic acid supplement.

Birch et al. (55) found no difference in growth parameters between VLBW preterm infants fed a fish-oil formula (0.35% DHA, 0.65% EPA) compared to two other formulas (corn-oil or soy-oil based). Woltil et al. (56) supplemented LBW infants with gamma-linolenic acid (GLA), an essential omega-6 fatty acid, from evening primrose oil and either a high or low dose of high-EPA fish oil (0.43% DHA and 0.34% EPA or 0.20% DHA and 0.17% EPA). Investigators analyzed weight, length, and head circumference growth rate and brain weight in the first 6 weeks of life. No change in

growth rate was associated with fatty acid composition of the formula. Brain-weight gain was greater in appropriate for gestational age (AGA) preterm infants fed LC-PUFAs.

Another study with 3 groups of preterm infants fed formula with low-EPA fish oil and blackcurrant-seed oil (1.2 % ALA, 0.6% DHA, 0.1% EPA, and 0.1% ARA), a standard formula (1.3% ALA), or human milk found a positive association between LC-PUFA supplementation and growth. The infants had an average birth weight of 1750 grams which is considered moderately low birth weight. The group consuming the supplemented formula had greater weight gain and had a higher formula intake (57).

Fewtrell et al. (58) compared the growth of infants receiving supplemented formula containing 0.17% DHA, 0.31% ARA, and 0.04% EPA to a control without DHA, ARA, or EPA. Measurements were taken at 9 months and 18 months of age. At 9 months infants who had received LC-PUFA supplemented formula weighed 310 g less (95% CI: -6 to 620 g) and were shorter by 0.86 cm (-0.06 to 1.78) than control infants. At 18 months, infants fed the LC-PUFA supplemented formula weighed 370 g less (95% CI, 12 to 735; $P = .04$) and were shorter by 1.5 cm (95% CI, 0.52; $P = .004$) than control infants. The difference in weight and length between formula groups was present in both males and females. In males the weight difference was -211 g (95% CI, -337 to 759) and the length difference was -1.05 cm (95% CI, -0.55 to 2.64); while in females the weight difference was -438 g (95% CI, -31 to 907) and the length difference was -1.70 cm (95% CI, 0.48 to 2.91).

To adjust for gender differences between groups, investigators reported weight and length as standard deviation (SD) scores. They showed mild reductions in length (difference in length standard deviation score, 0.44; 95% confidence interval, 0.08-0.8)

and weight z scores at 18 months with LC-PUFA supplemented formula. Investigators observed no other significant differences in growth across visits using SD scores.

In a 2005 study of body composition in VLBW premature infants, subjects were fed one of two formulas with different sources of DHA and ARA or a control formula with no added DHA or ARA to 12 months. Both experimental formulas contained 0.26% DHA and 0.42% ARA. One derived PUFAs from fungal oil and fish oil and the other formula was made from egg-derived triglyceride and fish oil. There were no significant differences among the three study groups in weight, length, or head circumference at any of the age points; however, there were differences in body composition at 12 months following consumption of DHA and ARA supplemented formulas compared with the infants who were fed the unsupplemented control formulas. The mean lean body mass was greater for infants in the DHA and ARA groups (6.83 ± 0.13 and 7.00 ± 0.14) compared to the control group (6.53 ± 0.15). Average fat mass was less for infants in the DHA and ARA groups (2.60 ± 0.12 and 2.60 ± 0.13) than in the control group (3.07 ± 0.14 ; $P < 0.05$) (59).

There is some evidence that DHA in infant formula may contribute to growth in LBW preterm infants but may be dependent on the concentration of DHA, amount of formula intake, and other constituents in the formulas. The latest Cochrane review indicated that formula supplemented with LC-PUFAs in preterm infants has no clear benefit or harm to infant growth, established by the pooled results of 13 random controlled trials. However, the 2008 review found that a “meta-analysis of five studies (Uauy 1990; Carlson 1996; Hansen 1997; Vanderhoof 1999; Innis 2002) showed increased weight and length at two months post-term in supplemented infants. A meta-

analysis of four studies at 12 months ($n = 271$) and two studies at 18 months ($n = 396$) post-term showed no significant effect of supplementation on weight, length or head circumference” (60).

In term infants fewer studies demonstrate differences in growth when given formula supplemented with DHA compared to unsupplemented formula. Lapillonne and Carlson (3) reviewed 7 of 19 studies with sufficient statistical power and concluded no difference in weight or length for term infants no matter the source of supplemented DHA.

One study; however, reported lower head circumference at 4 months with formula containing 0.45% DHA and low-EPA fish oil compared to control formula without DHA. The head circumference in the supplemented group was similar to the reference breastfeeding group (61). Another study found significantly lower mean weight at 4 months of age ($P = 0.055$) with a high alpha-linolenic acid (ALA) supplemented formula (3.2% of fatty acids). Infants who received the high ALA formula had higher plasma concentrations of DHA but lower concentrations of arachidonic acid at 21, 40 and 120 days (4 months) of age. The study did not find differences in growth before or after the 4 month assessment (4).

A large study ($n = 294$) found greater weight gain for males from enrollment to 4 months in the group fed formula containing fish oil and fungal oil (0.13% DHA and ≤ 0.04 EPA) compared to the control group (31.4 ± 4.6 g/d and 27.8 ± 4.2 , respectively; $P < 0.05$). No other differences in weight, length, or head circumference were found at 1, 2, 6, 9, or 12 months (62). Morris et al. (5) reported lower subscapular skinfold thickness in infants ($n = 109$) consuming formula supplemented with 0.2% DHA and 0.4% ARA

compared to a standard unsupplemented formula. Small but statistically significant differences were found between the two groups at 6 weeks (7.3 mm in supplement group, 7.8 mm in control group; $P < 0.046$) and at 3 months (7.6 mm in supplement group, 8.3 mm in control group; $P < 0.012$) but these differences were not evident at 6 or 12 months.

The 2008 Cochrane review of PUFA supplementation in term infants concluded that there is no identifiable physical growth benefit or harm during infancy with PUFA supplementation no matter the growth measure, specific type, or amount of PUFAs in formula (63). Research on infant supplementation of DHA and growth in infancy and early childhood is inconsistent. Few studies suggest that supplementing DHA in infancy may lower weight indices at one or more points during infancy.

DHA and BMI in Children

There are a small number of clinical trials that evaluated the relationship between DHA intake through maternal supplementation during pregnancy and/or lactation and BMI during childhood and results are inconsistent. Helland et al. (7, 8, 64) supplemented pregnant and lactating women with 10 ml of either cold liver oil or corn oil from 18 weeks of pregnancy until 3 months after delivery. The cod liver oil contained approximately 1200 mg DHA and 800 mg EPA per daily serving. Investigators found no significant effect of omega-3 supplementation on infant growth or on BMI at 4 and 7 year follow-ups. However, ALA content in breast milk correlated positively with BMI. This is contrary to what investigators report in animal research (1, 44). The study was conducted in Norway and was the first published report of children subjected to higher DHA exposure in utero who were followed long term (>18 months). A total of 341 subjects began participation in the trial and 143 completed follow-up through 7 years.

A randomized controlled trial done in Germany compared longitudinal growth in infants whose mothers received supplementation during pregnancy and lactation. There were three different supplement groups including vitamins and minerals only (basic supplementation), basic supplementation and probiotics, and basic supplementation, probiotics, and 200 mg DHA from fish oil. Investigators found that maternal DHA supplementation during pregnancy lowered weight and BMI at 21 months of age. There were no differences in length. The mean weight of the DHA group was lower by 601 g (95% CI, - 171; - 1030g) and BMI was lower by 0.76 kg/m² (95% CI, -0.07; -1.46) compared to children at 21 months of age whose mothers did not receive DHA. Only 69 of 144 subjects were available at 21 months (6).

Lauritzen et al. (9) studying children in Denmark reported omega-3 intake of lactating mothers in a fish oil supplement group compared with a placebo group given olive oil resulted in higher BMI (0.06 kg/m²; $P = 0.022$) and larger head circumference (0.5±0.2 cm) at 2.5 years of age in the offspring. Weight and length did not differ between groups. BMI, waist circumference, and percentage of body fat were positively associated with maternal red blood cell DHA content at the end of the 4 month intervention ($r=0.238$, $P = 0.021$; $r=0.301$, $P = 0.007$; and $r=0.264$, $P = 0.035$, respectively).

The women received 4500 mg/day of a fish oil that provided 800 mg DHA, 600 mg EPA or an equivalent amount of fat from olive oil in a daily supplement for 4 months of lactation. Subjects were required to have had a fish intake below the median (<0.4g/day n-3 LC-PUFAs) in Denmark, where the trial was conducted. There was a

higher ratio of males to females in the fish oil group (37:25) compared to the olive oil group (28:32). At the 2.5 year follow-up 105 out of 150 completed the BMI assessment.

Research on maternal supplementation of DHA and BMI in late infancy and early childhood is sparse. Only one study showed a negative association between maternal DHA supplementation and weight and BMI at 21 months of age. Another showed no association between maternal DHA supplementation and BMI at 4 and 7 years of age. A third study showed a positive association between maternal DHA supplementation and BMI and head circumference at 2.5 years of age.

There are significant differences in supplementation source and amount, study design, timing, and baseline seafood intake in each geographic location among studies. In addition, ARA intake varies. The effect of direct infant supplementation of DHA through infant formula on BMI during childhood remains unclear. Preliminary research shows that supplementing DHA in infancy may affect BMI. Analyzing the impact of DHA from infant formula in four different concentrations will help to determine the optional amount of DHA and its affect on BMI in children.

Chapter 3: Methods

This is an observational study of infants who participated in a double-blinded, randomized, controlled, parallel-group trial, known as the DIAMOND Study (DHA Intake And Measurement Of Neural Development). The primary objective of the DIAMOND Study was to determine the effect of infant formula with four different concentrations of DHA supplementation on visual acuity of formula-fed infants measured at one year of age. The formula concentrations studied were 0.0% fatty acids from DHA, 0.32% fatty acids from DHA (17 mg/100 kcal), 0.64% DHA (34 mg/100 kcal), and 0.96% DHA (51 mg/100kcal). The DHA containing formulas all provided 0.64% arachidonic acid (ARA).

The DIAMOND Study was conducted at two clinical sites. It was designed to a) determine if DHA supplementation in formula during infancy impacts the development of body mass index from birth to 4 years of age b) to determine the amount of DHA supplementation required to effect on body mass index. All subjects reported to the University of Kansas Medical Center Infant Nutrition Clinic for parent-study appointments at 6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years, and at 4 years of age. Anthropometric data, descriptive data, and 24-hour dietary recalls were obtained at each of these 11 study appointments.

Ethics

This study was approved from the Human Subjects Committee as part of the parent trial entitled “The DIAMOND Study: A Double Masked, Randomized Controlled Clinical Trial of the Maturation of Infant Visual Acuity as a Function of the Dietary

Level of Docosahexaenoic Acid” (HSC#9198 and 10205). Infants’ parents or guardians were provided written informed consent prior to study participation.

Subject Selection

Subject selection was according to inclusion and exclusion criteria of the parent study. The infants included healthy, term (37-42 weeks gestation; 2490-4200 grams birth weight), formula-fed, singleton-birth infants born between September 3, 2003 and September 25, 2005 in the Kansas City metropolitan area. Exclusion criteria were infants receiving human milk within 24 hours of randomization and diseases or congenital abnormalities likely to interfere with growth, development, vision maturation or cognitive function, poor formula intake, or intolerance to cow’s milk infant formula. Infants born to mothers with chronic illnesses such as HIV, renal or hepatic disease, diabetes, or substance abuse were also excluded.

Randomization to Infant Formula

All of the formulas were cow’s milk-based formulas with the same micronutrients and macronutrient levels and ingredients except for the long-chain polyunsaturated fatty acids (LCPUFAs). The DHA and ARA sources were single-cell algal and fungal oils from Martek Biosciences in Columbia, MD. All DHA supplemented formulas contained 0.64% (34 mg/100 kcal) from arachidonic acid (ARA). The control formula did not contain DHA or ARA, the first level of DHA supplementation contained 0.32% fatty acids from DHA (17 mg/100 kcal), the second level contained 0.64% DHA (34 mg/100 kcal), and the third level contained 0.96% DHA (51 mg/100kcal). Other major fatty acids were present in similar concentrations in all four formulas including the essential

fatty acids linoleic acid (16.9-17.5% fatty acids) and alpha-linolenic acid (1.61-1.98% fatty acids).

Subject randomization was generated using random-number generator function with separation between males and females. Randomization was performed by the study sponsor, Mead Johnson & Co, Evansville, IN. Study formulas were designated by codes, each formula with two different codes (separating male and female) for a total of 8 codes. Using the randomized lists, envelopes were labeled with consecutive numbers for subjects containing the code of the study formula that was to be assigned. Once enrolled in the study, the next sequential numbered envelope of the appropriate gender was opened at the study site to designate which formula the infant would consume. Formula (packaged by the study sponsor and only identifiable by its code) was directly provided for the infant at the study site. All study personnel (directly or indirectly involved) were masked to which formula the infants received.

Infants were fed the designated formula for the 12 months of life. The study formula was to be the sole source of nutrition until about 4 months of age at which time additional foods could be introduced as instructed by the infants' physicians. DHA supplemented or enriched foods were to be avoided until 12 months of age.

Data Collection

Anthropometric Data

Anthropometric data were obtained in the first nine days of life including birth weight, length and head circumference. Longitudinal anthropometric data were collected at each of the infant clinic visits at 6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years and at 4 years of age (See Appendices A and

B for sample data collection forms). The technique used for the collection of each anthropometric data is as follows:

- a. Body weight – Subjects were weighed one time on a standard calibrated pediatric scale to the nearest gram by standard methods with dry diapers.
- b. Body length/height – Until they were 2 years of age, subjects were measured recumbent on a length board after positioning the head at the top of the board and legs held straight measuring to the nearest tenth of a centimeter. From 2.5 to 4 years of age, height was measured with a stadiometer.
- c. Head circumference – Flexible, non-stretchable measuring tape was wrapped snugly around each subject’s forehead measuring the full circumference of the head to the nearest tenth of a centimeter.
- d. Center for Disease Control percentiles were calculated for weight-age, length-age, head circumference-age, and weight-for-length (<2 years) and BMI-age (≥ 2 years) using Growth BP Software for all visits.

Formula Intake Data

Formula intake and tolerance were assessed and collected from the parent(s) or caregiver(s) at each study visit. Descriptions of the infants’ stools were obtained by the parent(s) or caregivers(s) including number, color, and consistency for one day. Also any occurrence of constipation or diarrhea, signs of excess gas, or unusual fussiness were recorded.

Dietary Intake Data

Twenty-four dietary hour recalls were collected from the parent(s) or caregiver(s) of study subjects by trained registered dietitians during study visits at 6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years, and at 4 years of age. All food and beverages consumed the day prior to coming in were captured including times, portion sizes, brand names and ingredients added in preparation. A multiple pass method was used with the following three steps: 1) quick list, 2) detailed description including portion sizes and amount consumed, and 3) review of the record probing for missing foods and beverages. The serving sizes were estimated using descriptions and a kit containing measuring tools. Tools included measuring cups and spoons, pre-portioned bean bags of differing amounts, and labeled utensils with reference charts. See Appendix C for the 24-hour dietary recall form.

The dietary information from each visit was entered into the Nutrition Data System for Research (NDS-R) software program (v4.06_34 University of Minnesota, Minneapolis, MN) by trained research staff. Dietary intakes were uploaded into the 2008 version of NCS-R, calorie intakes were outputted, and transferred to an Excel spreadsheet to be entered into Statistical Package for the Social Sciences (SPSS). Twenty-four-hour dietary recalls with missing meals and total calorie intakes greater than 200 kcals/kg or less than 40 kcals/kg were excluded. See Appendix D for a list of excluded dietary recalls. The remaining calorie intakes for subjects grouped by formula were averaged for each visit (6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years, and at 4 years of age).

Collection and Analysis of Data

Data collection included each subject's formula code, weight, length, head circumference, and calorie intake for each of the visits: 6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years and at 4 years of age. The mother's height and smoking status were obtained by interview.

The data were entered into an Excel spreadsheet and transferred into SPSS. The relationship between weight-for-length percentiles for birth to 18 months and BMI-age percentiles for 2 to 4 years and the DHA concentration in the subject's formula were evaluated with repeated measures analysis of variance (ANOVA) with correction for potentially influential variables observed in the study. The repeated measures ANOVA enhances the power of the study because it's within subjects design allows the subjects to function as their own control. Potential covariance included mother's smoking status during pregnancy, mother's weight, and ethnicity. Additionally, the average calorie intake for each formula group at each visit was calculated.

Chapter 4: Results

The objective of the study was to 1) determine the concentration of DHA intake during infancy consumed through infant formula that impacts weight-for-length percentiles (< 2 years) and body mass index percentiles (\geq 2 years) during the first four years of life and 2) to determine if DHA supplementation during infancy lowers weight-for-length (< 2 years) or BMI percentiles (\geq 2 years) from birth to 4 years of age for infants receiving DHA through formula.

The total number of subjects enrolled in the original study was 159. A subset of 77 subjects was seen through 4 years of age and was used to generate summary statistics and simple correlations. There were 3 subjects that missed 2 or more consecutive visits and 8 subjects that missed 1 visit from 6 weeks to 4 years of age. Subjects that missed one visit were kept in the analysis. Means were calculated for their weight and length from visit measures before and after the missing age. There were 74 complete data sets included in the final analyses.

Subjects were randomized to one of four formula groups. Formula Group 1 contained no DHA or ARA, Formula Group 2 had 0.32% DHA, Formula Group 3 had 0.64% DHA, and Formula Group 4 had 0.96% of total fatty acids in the infant formula from DHA. Formula Groups 2, 3 and 4 had 0.64% of total fatty acids in the infant formula from ARA. There were only slight differences in the number of subjects per formula group at 4 years ($n = 16, 19, 17, 22$). The smallest number of subjects was in the control group and the largest number of subjects was in the formula group with the highest concentration of DHA (0.96%).

Characteristics were distributed evenly across formula groups. See Table 3 for characteristics of the subset seen through 4 years in comparison to the original study population. Table 4 shows the mean calorie intakes and Table 5 shows calories per kilogram across ages separated by group. Calories and calories per kilogram were generated from 24-hour dietary recalls and weights taken at study visits. Calories were consumed evenly between groups.

TABLE 3 Characteristics of the subset in comparison with the original study population

Characteristics	Subset study population (<i>n</i> = 77)	Original study population (<i>n</i> = 169)
Maternal		
Weight at delivery (kg)	73.5 ± 39.4	69.7 ± 18.4
Height (cm)	163.5 ± 7.4	163.8 ± 7.0
Age at delivery (y)	23.4 ± 4.4	23.7 ± 5.7
Education level (y)	12.0 ± 1.4	12.0 ± 1.6
Smoking before pregnancy [<i>n</i> (%)]	35 (46.7%)	63 (42.9%)
Pack Years (PPD*years smoked)	2.1 ± 4.0	1.9 ± 3.8
Ethnicity		
African American [<i>n</i> (%)]	51 (66%)	98 (61.6%)
Caucasian [<i>n</i> (%)]	19 (24%)	50 (31.4%)
Hispanic [<i>n</i> (%)]	5 (7%)	9 (5.7%)
Other [<i>n</i> (%)]	2 (3%)	2 (1.3%)
Infant		
Female [<i>n</i> (%)]	49 (64%)	84 (52.8%)
Male [<i>n</i> (%)]	28 (36%)	75 (47.2%)
Weight at birth (g)	3401.8 ± 350.4	3392.2 ± 377.2
Length at birth (cm)	50.0 ± 1.6	50.2 ± 1.9
Head circumference at birth (cm)	34.2 ± 1.3	34.2 ± 1.3

PPD = Packs per day

TABLE 4 Mean calorie intakes at each age by formula group

Group	6 weeks	4 months	6 months	9 months	12 months	18 months	2 years	3 years	4 years
1	634	757	867	1051	1181	1415	1524	1510	1673
2	660	773	845	905	1056	1488	1634	1748	1730
3	605	703	817	994	1170	1295	1512	1586	1528
4	632	701	853	915	1057	1192	1421	1442	1529
Mean Kcal	633	734	845	966	1116	1347	1523	1572	1615

TABLE 5 Mean calories per kilogram body weight at each age by formula group

Group	6 weeks	4 months	6 months	9 months	12 months	18 months	2 years	3 years	4 years
1	133	116	107	116	125	130	128	107	104
2	139	113	108	100	106	128	124	116	96
3	125	106	109	110	121	110	116	102	91
4	129	101	109	101	105	104	112	96	91
Mean Kcal/kg	132	109	108	107	114	118	120	105	96

Two-tailed Pearson correlations for maternal weight and age at delivery as well as maternal height, pack years prior to pregnancy, highest attained education level, and infant weight-for-length/BMI-age percentiles at 12 ages (birth, 6 weeks, 4 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years and at 4 years) were obtained by using SPSS. Maternal smoking in pack years was negatively related to birth weight-for-length percentile ($P < 0.05$). Maternal age at delivery was positively related to birth ($P < 0.01$) and 6 week ($P < 0.05$) weight-for-length percentile. Younger mothers had smaller infants than older mothers in this study population.

Maternal weight in pounds at delivery was positively related to weight-for-length percentile at 18 months and BMI-age percentile at 3, 3.5, and 4 years of age ($P < 0.05$). See Table 6 for correlation values. Mother's weight is often positively related to infant birth weight; although we did not find this in our study population we did find that heavier mothers raised heavier children, observed at 18 months of age and at 3 years and older.

There were no significant correlations between maternal height or education level and weight-for-length/BMI-age percentile at any age.

TABLE 6 Correlations for maternal characteristics and infant weight-for-length/BMI-age percentile across age

Weight-for-length/BMI-age percentile:								
Age:								
	Birth	6 weeks	6 months	12 months	18 months	3 years	3.5 years	4 years
Maternal pack years	-.251*	-.220	-.175	-.129	-.002	-.071	.042	.074
Maternal delivery weight	.070	.126	.095	.179	.265*	.292*	.271*	.243*
Maternal age at delivery	.300**	.268*	.067	-.086	.215	.033	.055	.084

* $P < 0.05$ level

** $P < 0.01$ level

DHA and Weight-for-Length/BMI-age Percentile

Weight-for-length/BMI-age percentile was entered into age (12) and formula group (4) repeated measures analysis of variance (ANOVA) with maternal age and weight at delivery and pack years as covariates. We chose covariates based on Pearson correlations and controlled for those that had significant associations with weight-for-length or BMI-age percentiles. There were no significant main effects for age and formula group ($P = 0.683$). The observed power was 0.147 with 16 subjects in Formula Group 1, 19 subjects in Formula Group 2, 17 subjects in Formula Group 3 and 22 subjects in Formula Group 4. There was no significant interaction between formula groups. Mean weight-for-length/BMI-age percentiles for each formula group followed a similar trajectory across age with no apparent effect from DHA concentration consumed through infant formula. Figure 1 illustrates weight-for-length/BMI-age percentiles across age separated by formula group.

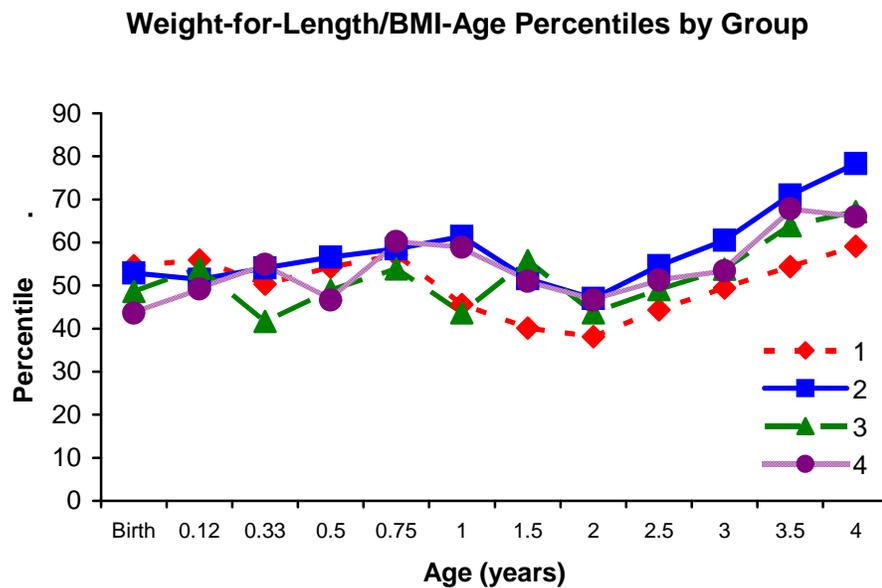


FIGURE 1 Weight-for-length/BMI-age percentiles from birth to 4 years by formula group. Formula Group 1 contained no DHA, Formula Group 2 contained 0.32% DHA, Formula Group 3 contained 0.64% DHA, and Formula Group 4 contained 0.96% DHA.

After finding no significance between the 4 formula groups separated by concentration of DHA and weight-for-length/BMI-age percentiles, we explored the absence versus the presence of DHA and weight-for-length/BMI-age percentiles. New DHA groups were separated by no DHA or ARA ($n = 16$) and exposure to DHA ($n = 58$; 0.32, 0.64, or 0.96% DHA) and ARA (0.64%) in infant formula. Similar repeated measures ANOVA with weight-for-length/BMI-age percentiles entered into age (12) and DHA group (2) with maternal age and weight at delivery and pack years as covariates and had no significant main effects for age and DHA group ($P = 0.416$) and interaction between these terms was not significant. The observed power was 0.127. Results are illustrated in Figure 2. There were no apparent differences in growth percentiles from

birth to 4 years of age in infants who consumed DHA and ARA in infant formula and those who did not.

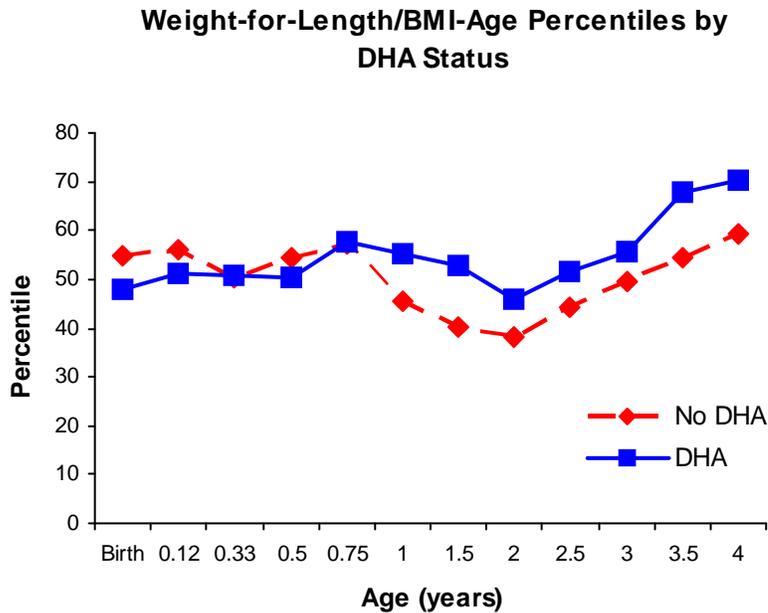


FIGURE 2 Growth percentiles from birth to 4 years with or without DHA

supplementation. DHA Group 1 ($n = 16$) consumed formula with no DHA compared to DHA Group 2 ($n = 58$) which were supplemented with DHA (0.34, 0.64, or 0.96% DHA).

The mean BMI-age percentile for the study population at 4 years of age was at the 70th percentile. Thirty-nine percent of the children were overweight or obese ($\leq 85^{\text{th}}$ percentile) at 4 years of age.

Maternal Smoking and Weight-for-Length/BMI-age Percentile

As a result of finding a significant negative association between maternal pack years and birth weight-for-length percentile and having a population with a high percentage of mothers who smoked and growth data of offspring to 4 years of age we explored maternal smoking status and growth. Weight-for-length/BMI-age percentiles

entered into age (12) and maternal smoking status (2) repeated measures ANOVA with maternal pack years as a covariate had significant main effects for weight-for-length/BMI-age percentiles and maternal smoking status ($P = 0.035$, $f = 4.601$).

Maternal smoking status was a self-report of whether the mother smoked before pregnancy (yes=33, no=39). Some of the smokers reported not smoking during pregnancy (11 of 33). Maternal pack years was an indicator of the amount and duration of smoking with a mean of 2.12 (SD=3.871) and range of 0 to 20 in the study population. Pack years were calculated by the number of packs per day (PPD) multiplied by the number of years smoked. The observed power was 0.562. Interaction between these terms was not significant. Figure 3 shows weight-for-length/BMI-age percentiles across age broken out by maternal smoking status adjusted for maternal pack years. It is evident that women who smoked, even those who stated they did not smoke during pregnancy, had infants/children with higher weight-for-length/BMI-age percentiles than women who did not smoke.

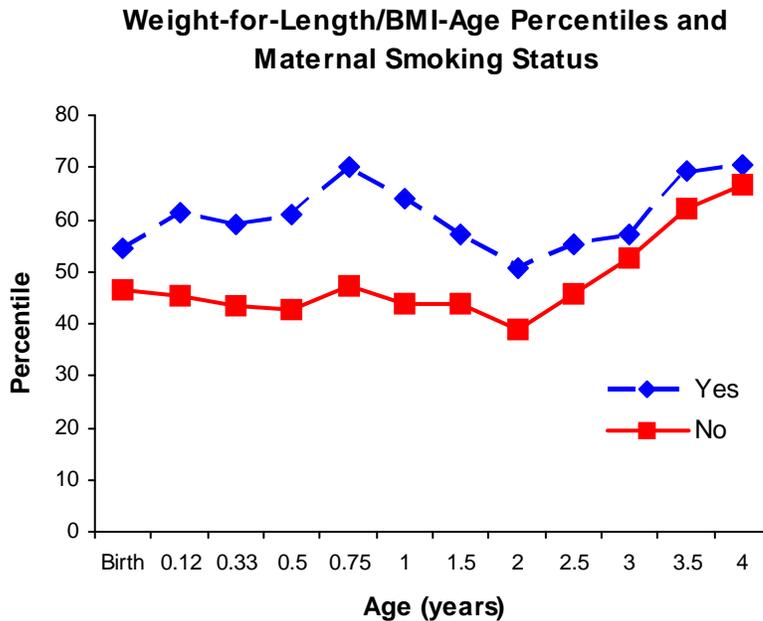


FIGURE 3 Weight-for-length/BMI-age percentiles across age were separated by maternal smoking status prior to pregnancy adjusted for pack years ($\mu=2.12$).

We subsequently ran weight-for-age (weight-age) and length-for-age/height-for-age (length/height-age) percentiles into age and maternal smoking status repeated measures ANOVAs adjusted for pack years. Weight-age percentiles and maternal smoking status had marginal interaction ($P = 0.092$) but no difference in main effects ($P = 0.831$). The observed power was 0.55. Maternal smoking status did not have an effect on weight-age percentiles.

Length/height-age percentiles and maternal smoking status had significant main effects ($P = 0.043$) with no interaction across ages. The observed power was 0.53. Mothers that smoked had children who were shorter.

Also given that maternal smoking status was not associated with weight-age percentile but was associated with length/height-age percentile and weight-for-

length/BMI-age percentile, it appears offspring of mothers that smoked were shorter but had similar weight-age percentiles when compared to children whose mothers did not smoke and put them in a higher weight-for-length/BMI-age percentile. Illustrations of maternal smoking status and weight-age and length/height-age percentiles across age are found in Figures 4 and 5.

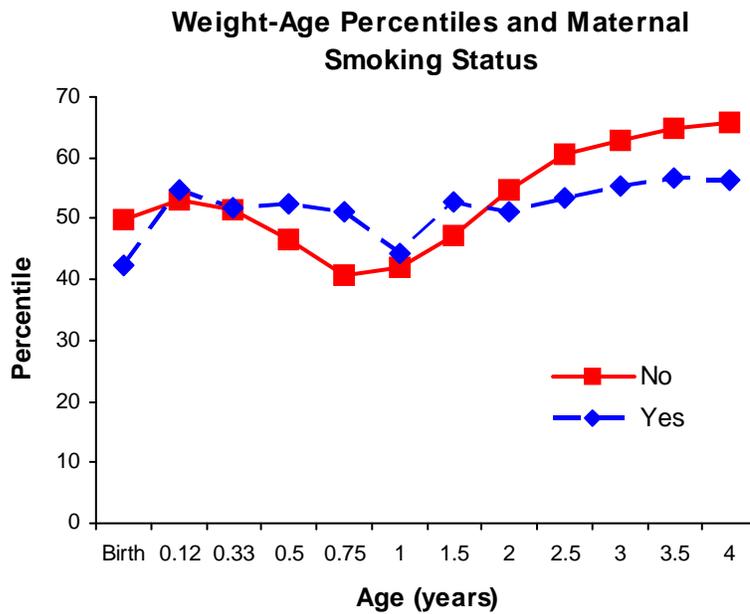


FIGURE 4 Weight-age percentiles across age were separated by maternal smoking status prior to pregnancy adjusted for pack years ($\mu=2.12$).

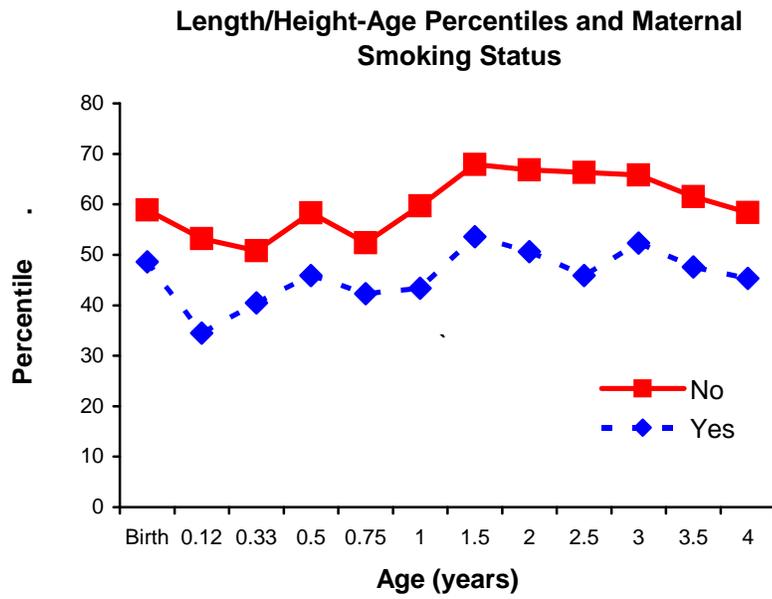


FIGURE 5 Length/height-age percentiles across age were separated by maternal smoking status prior to pregnancy adjusted for pack years ($\mu=2.12$).

Chapter 5: Discussion

Currently research is sparse looking at the potential benefits of DHA supplementation through infant formula in relationship to BMI to four years of age. Although there is some evidence that DHA supplementation may affect growth in infancy and early childhood, there was no significant effect in our study population. The number of subjects per formula group by 4 years of age was small ($n = 16, 19, 17, 22$). The effect size of DHA on growth percentiles may have been too small to detect a difference with the number of subjects per group and power of 0.147. After separating the formulas by DHA or no DHA (DHA group) there was still insufficient power (0.127) and it created uneven groups ($n = 16, 58$).

Most studies of term infants that supplemented DHA through infant formula did not find a significant difference on weight-for-length during infancy compared to no supplementation (3, 63). There was one study that found lower head circumference at 4 months with formula containing 0.45% DHA and low-EPA fish oil compared to control formula without DHA (61). Another study found significantly lower mean weight at 4 months of age ($P = 0.055$) with a high alpha-linolenic acid (ALA) supplemented formula (3.2% of fatty acids) (4). There are no consistent physical growth differences during infancy with DHA supplementation compared to no DHA exposure though infant formula.

Studies of BMI in young children show mixed results with maternal supplementation during pregnancy and/or lactation. One study ($n = 143$) also found no significant effect of omega-3 supplementation on infant growth or on BMI at 4 years (7) and still no difference at 7 years (8). Another study ($n = 105$) found higher BMI and

larger head circumference at 2.5 years (9), and a third study ($n = 69$) found lower BMI at 21 months. Lauritzen et al. (9) supplemented mothers during lactation alone while the other trials supplemented women during pregnancy and lactation. Exclusive post-natal supplementation is most similar to our study design; however, we fed infant formula not human milk. Breast-fed infants grow differently than formula-fed infants but growth differences are not likely the result of any single nutrient (18, 19).

The amount of DHA given to mothers varied between studies: 1200, 800, and 200 mg DHA per day. The study with the highest dose (1200 mg/day) had no effect, while the group supplementing only 200 mg per day found lower BMI in toddlers. The fish consumption in the regions where the studies were conducted (Germany, Norway, and Denmark) varies considerably, so baseline dietary DHA was inconsistent between studies. In the study from Norway researchers excluded mothers with high fish intake and used a larger dose of DHA (1200 mg) compared to the other trials and still found no effect on growth to 7 years of age. Our study was conducted in the United States where DHA intake is relatively low (48) and did not find a difference in growth percentiles between groups supplemented with varying levels of DHA in infant formula.

Arachidonic acid (ARA) is an omega-6 fatty acid that has shown lower growth indices when absent from infant formula (51). There was no ARA in the control formula while there was consistent ARA in the three formulas that contained DHA. The effect of ARA on growth is not fully understood and could have counteracted the effect of DHA in this study, but is highly speculative.

Limitations

There are certainly many contributing factors to growth in infancy and early childhood that affect weight-for-length and BMI. It is possible that other factors overpowered the effect of DHA consumed during the first 12 months of age. Some factors include the introduction of various foods and the quality of the diet during and after weaning from formula. Additionally, the amount of formula and food consumed has an impact on weight.

We obtained very careful dietary recalls; however, the calorie intakes obtained were estimates from parent(s) or caregiver(s) and may have contained some overestimation of calories. There is evidence that when obtaining 24-hour recalls for infant and toddler energy intake, parents and caregivers often overestimate due to portion size error (65). Our calorie intake data were fairly consistent between formula groups as shown in Table 4. The energy intakes were all significantly higher than the Dietary Reference Intakes (DRIs) for energy in infants and children; however, we cannot say if or to what degree there was overestimation.

The Center for Disease Control (CDC) growth percentiles changed from weight-for-length to BMI-age at two years of age. The BMI-age percentiles are lower than weight-for-length percentiles and caused a dip in the growth trajectories for all formula groups. The World Health Organization (WHO) has growth percentiles that include BMI-age starting at birth and continuing through adulthood. Although there would be consistency from birth to four years, the WHO percentiles are not representative of our population. The WHO percentiles were developed from the Multicenter Growth Reference Study (MGRS) with growth data from six countries (Brazil, Ghana, India,

Norway, Oman, and the USA). The data represents breast-fed infants with broadly different ethnic backgrounds. Breast-fed infants grow differently than formula-fed infants and the diverse ethnicity of the WHO percentiles is not representative of our study population.

Exploratory Data

This study found that maternal age is positively related to the size of infant at birth and 6 weeks of age. In our population younger mothers gave birth to smaller infants while older mothers birthed larger infants. The mean age of mothers at delivery was 23.4 with a range of 16 to 35 years of age. Research suggests that socioeconomic factors, poverty, limited education, underutilization of prenatal care, and race/ethnicity may explain the effect of young maternal age on pregnancy outcomes such as low birth weight (66). Although our study did not include low birth weight infants, younger mothers had smaller babies than older mothers.

Our study supports other research findings that mother's weight is related to child's BMI (67, 68). Although a large body of research has assessed direct genetic links between parent and child weight status, relatively little research has assessed the extent to which parents, particularly those who are overweight, select environments that promote overweight among their child/children; more research is needed to develop and test theoretical models describing how a wide range of environmental and behavioral factors impact childhood obesity (69). Our study showed a correlation between maternal weight and child weight-for-length/BMI-age percentile starting at 18 months of age and continuing from 3 to 4 years of age.

This study also has large implications concerning the rise of overweight and obesity in children as young as two years of age. Our population had an alarming increase in BMI-age percentile from 2 to 4 years of age with 39% overweight or obese ($\geq 85^{\text{th}}$ percentile) at 4 years of age and of those 14.3% were obese ($\geq 95^{\text{th}}$ percentile). The obesity rate is similar to a 2008 report of 14.6% prevalence of childhood obesity in low-income preschool aged children from CDC's Pediatric Nutrition Surveillance System (PedNSS) (70).

Further observation of these children's BMI-age percentiles would be valuable to monitor whether the rapid increase in BMI-age percentile continues or if it plateaus during older years. Additionally, further research on the prevalence of overweight and obesity starting in toddlerhood is appropriate given these findings and with the recent rise in overweight status in children in the United States (31, 70).

Maternal Smoking and Infant Growth

Smoking during pregnancy is associated with a higher rate of small-for-gestational-age (SGA) and low birth weight (LBW) infants (71, 72). Additionally, high smoke exposure has a modest association with low birth weight (72). One mechanism behind intrauterine exposure to tobacco smoke and small-for-gestational-age (SGA) and low birth weight (LBW) is accumulation of toxins such as cadmium in the maternal placenta. The build-up of cadmium reduces the transport of micronutrients such as zinc to the growing fetus (73).

Although our infants' mean birth weight was near the 50th percentile, mothers who smoked before or during pregnancy had infants that were shorter and had higher weight-for-length measurements than infants born to nonsmokers. Catch-up growth may

explain higher weight-for-length in infants and BMI in children whose mothers smoked and birthed smaller infants. Figure 4 illustrates that the mean weight percentile of infants born to smokers was lower at birth than the mean weight percentile of infants born to nonsmokers but they come together by 6 weeks of age; however, this difference is not statistically significant. It appears that infants experienced ample catch-up in weight but not in height as illustrated in Figure 5.

As discussed in Chapter 2, rapid weight gain in infancy leads to an increased risk of overweight status in early childhood. Our study population has an alarming number of children at or above the 85th percentiles by 4 years of age and maternal smoking may be a contributing factor to shorter stature and higher BMI.

Another study conducted at the University of Kansas Medical Center found maternal smoking was associated with an increased risk of late onset overweight but not early onset overweight (occurring before 2 years of age). In our study population, it appears infants whose mothers smoked were more likely to be overweight early (6 weeks and older) but do not become more overweight with time as illustrated in Figure 4. Infants whose mothers did not smoke had a lower BMI than those whose mothers did smoke in the first two years of life, however, their BMIs eventually caught up to the BMI of children born to smokers. Further studies are warranted to understand the impact of maternal smoking and/or smoke exposure during pregnancy and the long term implications on stature and weight in offspring.

Chapter 6: Summary

Docosahexaenoic acid (DHA) is an omega-3 fatty acid essential for brain growth and cognitive development in infancy. It is currently supplemented in infant formulas in the United States. There is some evidence that DHA can also influence growth in infancy and early childhood. Several clinical trials with infants and young children have found lower normalized growth following DHA increased exposure through maternal supplementation during pregnancy and/or lactation or infant formula supplementation.

The aim of this study was to evaluate the impact of feeding one of four concentrations of DHA in infant formula on weight-for-length percentile (<2 years) and body mass index percentile (≥ 2 years) at twelve ages from birth to four years of age. Healthy formula-fed infants were randomized to one of four infant formulas containing 0, 0.32, 0.64, or 0.96% of total fatty acid from DHA. Maternal physical and demographic characteristics such as age, height, weight at delivery, highest level of education, and smoking status before and during pregnancy were recorded at the beginning of the study. Weight, length, and head circumference of the infant were measured at 6 weeks, 4 months, 6 months, 9 months, 12 months, 18 months, 2 years, 2.5 years, 3 years, 3.5 years, and 4 years of age. Subjects were normalized to the Center for Disease Control weight-for-length (<2 years) and BMI-age growth charts (≥ 2 years) by calculating percentiles. Trained study personnel obtained 24-hour dietary recalls of the infant or child intake at each of the study visits and they were analyzed in NDSR.

Two-tailed Pearson correlations for maternal weight and age at delivery, height, as well as maternal pack years prior to pregnancy, highest attained education level, and infant weight-for-length/BMI-age percentiles at 12 ages (birth, 6 weeks, 4 months, 6

months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 3.5 years and at 4 years) were obtained by using SPSS. There were no significant correlations between maternal height or education level and weight-for-length/BMI-age percentile at any age. Maternal smoking in pack years was negatively associated with birth weight-for-length percentile ($P < 0.05$). Maternal age at delivery was positively associated with birth ($P < 0.01$) and 6 week ($P < 0.05$) weight-for-length percentile. Maternal weight in pounds at delivery was positively associated with weight-for-length percentile at 18 months and BMI-age percentile at 3, 3.5, and 4 years of age ($P < 0.05$).

The relationship between weight-for-length/BMI-age and concentrations in the study formula was evaluated with a two way repeated measures ANOVA across age using a p-value of 0.05 as statistically significant. Then infants were separated by DHA or no DHA and we ran a similar repeated measures ANOVA for weight-for-length and BMI-age percentiles across study visits and DHA status (DHA supplementation versus no DHA supplementation) using a p-value of 0.05.

The concentration of DHA consumed through infant formula during the first year of life did not impact weight-for-length or BMI-age percentile from birth to 4 years of age ($P = 0.683$). The number of subjects per group was 16, 19, 17, and 22; the observed power was low (.147). When grouped by DHA or no DHA ($n = 16, 58$) there was no statistical significance ($P = 0.416$) and low observed power (0.127). There is no observable difference in weight-for-length or BMI-age in infants supplemented DHA through infant formula in the first four years of life.

As a result of finding a significant negative association between maternal pack years and birth weight-for-length percentile and having a population with a high

percentage of mothers who smoked and growth data of offspring to 4 years of age we explored maternal smoking status and growth. A repeated measures ANOVA with weight-for-length/BMI-age percentiles entered into age (12) and maternal smoking status (2) with maternal pack years as a covariate had significant main effects for age and maternal smoking status ($P = 0.035$, $f = 4.601$).

We subsequently ran weight-age and length/height-age percentiles into age and maternal smoking status repeated measures ANOVAs adjusted for pack years. Weight-age percentiles and maternal smoking status had marginal interaction ($P = 0.092$) but no difference in main effects ($P = 0.831$). The observed power was 0.55. Maternal smoking status did not have an effect on weight-age percentiles. Length/height-age percentiles and maternal smoking status had significant main effects ($P = 0.043$) with no interaction across ages. The observed power was 0.53. Mothers that smoked had infants/children who were shorter and had higher weight-for-length/BMI-age percentiles compared to infants birthed from nonsmoking mothers. Further studies are warranted to understand the impact of maternal smoking and/or smoke exposure during pregnancy and the long term implications on stature and weight in the offspring.

Our study population had an alarming increase in BMI-age percentile from 2 to 4 years of age with 39% overweight or obese ($\geq 85^{\text{th}}$ percentile) at 4 years of age and of those 14.3% were obese ($\geq 95^{\text{th}}$ percentile). Further observation of these children's BMI-age percentiles would be valuable to monitor whether the rapid increase in BMI-age percentile continues or if it plateaus during older years. Further research on the prevalence of obesity starting in the toddler years is appropriate given these findings and with the recent rise in obesity in children in the United States.

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Appendix A

Subjects Demographic Data Collection Form

INVESTIGATOR
CARLSON

PROTOCOL
HSC
#10205

RANDOM CODE

DATE

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 B

Anthropometric Data Collection Form

ANTHROPOMETRICS

2 Year Visit

--	--	--

MO DA YEAR

Weight

 g

Length

 cm

Head circumference

 cm

2.5 Year Visit

--	--	--

MO DA YEAR

Weight

 g

Length

 cm

Head circumference

 cm

3 Year Visit

--	--	--

MO DA YEAR

Weight

 g

Length

 cm

Head circumference

 cm

3.5 Year Visit

--	--	--

MO DA YEAR

Weight

 g

Length

 cm

Head circumference

 cm

4 Year Visit

--	--	--

MO DA YEAR

Weight

 g

Length

 cm

Head circumference

 cm

INVESTIGATOR

CARLSON

PROTOCOL

HSC #10205

INITIALS

RANDOM CODE

DOB

ANTHROPOMETRICS PAGE 2

4.5 Year Visit

MO	DA	YEAR

Weight

 g

Length

 cm

Head circumference

 cm

5 Year Visit

MO	DA	YEAR

Weight

 g

Length

 cm

Head circumference

 cm

5.5 Year Visit

MO	DA	YEAR

Weight

 g

Length

 cm

Head circumference

 cm

6 Year Visit

MO	DA	YEAR

Weight

 g

Length

 cm

Head circumference

 cm

Appendix C

24 Hour Dietary Recall Collection Form

Appendix D

List of Omitted 24-Hour Dietary Recalls

Unable to recall one or more meals: 21 total

Subject 5 at 2 years (at school)
Subject 9 at 2.5, 3, and 4 years (primary caregiver at work)
Subject 24 at 2 (daycare) and 2.5 years (mom at work, dad brought child to appointment)
Subject 34 at 2 years (with grandma)
Subject 35 at 2 years (daycare)
Subject 39 at 6 months (with grandma) and 2.5 years (unable to recall dinner, not at home)
Subject 46 at 3.5 years (daycare)
Subject 50 at 6 months (with babysitter)
Subject 62 at 2 years (with dad for dinner)
Subject 84 at 3.5 years (daycare)
Subject 89 at 18 months (no amounts specified)
Subject 97 at 4 years (daycare)
Subject 102 at 18 months, 2 years, and 4 years (missing meals, daycare)
Subject 113 at 3.5 years (snacked all day, couldn't recall all eating occasions)
Subject 154 at 4 years (daycare)

Over reporting (>200 kcals/kg): 23 total

Subject 4 at 18 months
Subject 9 at 6 weeks
Subject 17 at 9 months
Subject 19 at 18 months
Subject 30 at 2 years
Subject 59 at 12 months
Subject 85 at 6 weeks
Subject 86 at 6 weeks
Subject 88 at 2 years
Subject 95 at 12 months
Subject 97 at 2.5 years
Subject 98 at 18 months
Subject 101 at 6 weeks and 9 months
Subject 103 at 4 years
Subject 108 at 2.5 years
Subject 114 at 6 weeks
Subject 120 at 18 months and 2 years
Subject 121 at 18 months
Subject 125 at 6 weeks
Subject 140 at 6 weeks
Subject 149 at 6 months

Under reporting (<40 kcals/kg): 6 total

Subject 46 at 4 years
Subject 54 at 9 months
Subject 62 at 18 months
Subject 82 at 6 weeks
Subject 87 at 18 months
Subject 118 at 6,9,12 months

Appendix E

Parent Study Consent Form Birth to 18 Months of Age

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 COI) 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

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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.

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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

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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

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Appendix F

Parent Study Consent Form 2 to 6 Years of Age

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

HSC Submission Amend#3 Date: 8/21/2009
HSC Approval Date: 11/15/07

HSC #: 10205 Approval Date: 9/2/09 to 7/12/10 Assurance #: FWA00003411
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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 and I 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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