Effect of maternal DHA supplementation
on childhood allergies and asthma

By

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ABSTRACT

Some evidence shows that fetal development may be a critical for the development of allergies and asthma later in life, and that DHA may play a key role in the development of the immune system. This study aimed to investigate the effects of DHA supplementation during this time period on the incidence of allergies and asthma up to six years of age. Women in the KUDOS cohort were given 600 mg of DHA or a placebo during their second and third trimesters of pregnancy, and their offspring were followed up to six years of age. Two-hundred thirty infants were included. Their medical records were collected until six years of age or until their records could no longer be acquired if the infant dropped out before six years of age. All records were coded and allergic diagnoses were examined for this study. These diagnoses were grouped into three categories, skin allergies, wheeze/asthma, and other allergies. Associations and relative risks were calculated for each type of allergy diagnosis and no significant associations were found. DHA supplementation during the prenatal period did not provide protection against asthma or allergies during the first year of life, the second through sixth years of life, or from birth to six years of age for any allergy, skin allergies, other allergies, or wheeze/asthma.

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Chapter 1

INTRODUCTION

In the past two decades, evidence has emerged that shows omega-3 polyunsaturated fatty acids (n-3 PUFAs) may protect against childhood asthma and allergies. Studies have been conducted using both prenatal and postnatal supplementation of marine n-3 PUFAs. Some of these studies supplemented during infancy, but this may not be the ideal time frame for allergy and asthma prevention. The prenatal period may be more critical, as discussed later in the review of literature. The cohort of the Kansas University DHA Outcomes Study (KUDOS) at the University of Kansas Medical Center was supplemented with an n-3 PUFA prenatally, making it a sample of convenience for studying childhood allergy and asthma development.

Research on the use of prenatal and postnatal n-3 PUFA supplementation to prevent allergies and asthma has grown considerably in recent years, and this year a meta-analysis of randomized controlled trials was published (1). The analysis included trials of prenatal (five trials), postnatal (two trials), and both prenatal and postnatal supplementation (one trial). The analysis concluded there was a significantly reduced likelihood of any IgE-mediated allergy or eczema from one to three years of age, but not beyond this age. There was no significant effect on wheezing or asthma development. A major weakness acknowledged in the meta-analysis, however, was that only two of the studies had a low risk of bias. Thus, the results of the meta-analysis could not be conclusive, and this warrants further investigation using sound methods with low risk of bias.

Allergies and asthma are growing problems in the United States (2, 3). If these conditions are preventable with adequate n-3 PUFA consumption during pregnancy and infancy, this could be the focus of future dietary guidelines and primary prevention efforts in the United States.

The purpose of the proposed research project is to explore the effect of prenatal DHA supplementation on development of childhood allergies and asthma.

My research question is: Does prenatal supplementation with 600 mg of docosahexaenoic acid (DHA) compared to a placebo vegetable oil prevent the development of allergies or asthma in young children?

My hypothesis is that prenatal DHA supplementation will reduce the development of both allergies and asthma in children.

Chapter 2

REVIEW OF LITERATURE

Childhood asthma and allergy prevalence

Approximately 29.5 million Americans have asthma (2), including nearly one in ten children (2). Asthma is one of the most common diseases in childhood, and it accounts for 10.5 million missed school days each year (4). Asthma prevalence has been increasing in the United states for over thirty years across all age, socioeconomic, and racial groups (2). Allergic conditions, which are related to asthma, are also increasing among children (3).

Allergies are hypersensitivities that cause the immune system to react to things in the environment that should not provoke a reaction (3). Respiratory allergies are the most common type of allergies among children, affecting 17% of children in the United States in 2009-2011 (3). In addition, 9.5 million American children have allergic skin conditions such as eczema (3). The prevalence of these is increasing. Approximately 12.5% of children had skin allergies in the United States in 2009-2011, compared to 7.4% less than 15 years earlier (3). Food allergies are the third most common allergies in childhood, affecting around 5% of children in 2009-2011 (3). Respiratory allergies are the only type that did not increase from 1997-1999 to 2009-2011. The increases in allergies and asthma among children are alarming because both have potentially dangerous consequences.

Consequences of childhood asthma and allergies

One of the most dangerous consequences of allergies is anaphylaxis, a severe whole-body reaction that occurs rapidly and can be deadly (3). Severe respiratory distress occurs in 60-70% of children with anaphylaxis (5). Food allergies are the most common triggers for anaphylaxis (3). Asthma can also have dangerous consequences, and it is the third leading reason for

hospitalization of children (2). Asthma is highly hereditary (4), but researchers have discovered many environmental predictors of asthma and allergy development (6) which may explain their increasing prevalence.

Importance of the prenatal and postnatal periods in development of asthma and allergies

Some researchers believe that asthma and allergy development may be triggered in the intrauterine environment (6). One reason for this is that early life is the critical period for the establishment of the delicate balance of Th1 and Th2 cells required for proper immune function (6). Intrauterine factors associated with asthma or allergy development include preterm delivery, antibiotic use during pregnancy, inappropriate vitamin A intake during pregnancy (6), maternal smoking during pregnancy (7), and maternal asthma or allergies (8-10). In addition to these factors that may increase the risk of asthma and allergies, there are dietary factors during pregnancy and infancy that may decrease the risk of these conditions.

Protective dietary factors during pregnancy and infancy

Several nutritional factors during pregnancy and infancy may protect against childhood development of allergies and asthma. Erkkola et al. (11) found an inverse association between intake of vitamin D from food during pregnancy and asthma in offspring. Some studies have found that breastfeeding may protect against offspring asthma, eczema, and allergic rhinitis, but not food allergies (12). A case-control study showed that maternal intake of apples during pregnancy may be inversely associated with asthma development (13). Fish intake during pregnancy is another dietary factor that may have an inverse association with asthma and allergies.

Epidemiological studies of fish intake in pregnancy and development of asthma or allergies

Epidemiological studies of asthma

Several epidemiological studies have found that intake of oily fish during pregnancy may be inversely associated with the development of asthma and various allergic conditions (13-18). Oily fish is rich in omega-3 polyunsaturated fatty acids (n-3 PUFAs). Pike et al. (15) and Romieu et al. (15, 18) found an inverse association between plasma levels of n-3 PUFAs during pregnancy and offspring wheeze. Salam et al. (16) found an inverse association between maternal intake of oily fish during pregnancy and asthma in offspring, but this association was only found in offspring of asthmatic mothers.

Epidemiological studies of allergies

One epidemiological study showed an inverse association between fish intake during pregnancy and positive skin prick results for food allergens, but only in offspring of non-allergic mothers (14). Three studies showed an inverse association between fish intake during pregnancy and offspring eczema (13, 17, 18). Only one cohort showed no association between maternal serum levels of omega-3 fatty acids during pregnancy, which are markers of fish intake, and allergy or asthma development in offspring (19). Most observational studies show an inverse association between fish intake during pregnancy, indicative of n-3 PUFA intake, and development of asthma and allergies in offspring.

DHA in early development

Alpha-linolenic acid is the main n-3 PUFA consumed from food, and it can be converted to eicosapentaenoic acid (EPA), which can then be converted to docosahexaenoic acid (DHA) (20). EPA and DHA are longer and more complex n-3 PUFAs than ✔ linolenic acid, and they are easier for the body to use. Preformed EPA and DHA can also be consumed from food. One of the main n-3 PUFAs under exploration is DHA. DHA is essential throughout the lifespan, and

it may be especially important during the third trimester of pregnancy and the first several months of after birth when infants organs are developing and growing rapidly (20).

During pregnancy, adequate intake of DHA is essential for central nervous system development (20). DHA and arachidonic acid (AA) are the two most common long-chain PUFAs that comprise neural tissues (21), so DHA is critical for brain development. Fetal organs such as the liver and brain use large amounts of DHA as they grow (20), so it is important for women to consume adequate DHA during pregnancy.

DHA sources and recommended intake

Some fish are a food source with a high concentration of DHA per serving, particularly cold water fatty fish such as salmon, tuna, and sardines (20). Consensus recommendations state that women should consume at least 200-300 milligrams of DHA during pregnancy and lactation (21, 22). This can be met by consuming one or two servings of fatty fish per week (22). Nochera et al. found that most low-income women are not meeting this recommendation (23). In this study, United States citizens consumed only 2.45 g/month of EPA and DHA combined (23)—well short of recommendations. Another study found that the median DHA intake of mothers in Mexico was 55 mg/day (24). This is not surprising given the change in intake of n-3 and n-6 fatty acids over the past several decades (25).

Intake of omega-3 and omega-6 fatty acids

Over the last 20 to 30 years, intake of n-3 PUFAs has decreased, while intake of omega-6 fatty acids (n-6 PUFAs) has increased (25). This change in intake of PUFAs correlates with the increase of allergies and asthma, and researchers have proposed that this intake pattern may be responsible for the rise in allergies and asthma (26). Researchers have proposed a mechanism for the correlation between PUFA intake and development of asthma and allergies.

Proposed mechanism of PUFAs in allergy and asthma development

Aggravating role of arachidonic acid

Linoleic acid is the main n-6 PUFA consumed, and it is found in vegetable oils and other foods derived from them (27). Arachidonic acid (AA), an n-6 PUFA is readily synthesized from linoleic acid, is a precursor to mediators that play a role in the development of allergies and asthma (27). Eicosanoids are mediators that can be produced from AA, and one key eicosanoid in the development of allergies and asthma is prostaglandin E_2 (PGE_2) (27). PGE_2 enhances production of immunoglobulin E (IgE), which encourages the development of allergies and asthma (26, 27). Thus, a high intake of n-6 PUFAs has been proposed to contribute to the development of these conditions (26, 27). Conversely, n-3 PUFAs may oppose the action of AA in this process.

Protective role of omega-3 fatty acids, particularly EPA and DHA

Long chain n-3 PUFAs (EPA and DHA) occupy the same space as AA in inflammatory cell membrane phospholipids, so increasing consumption of DHA and EPA reduces AA in these membranes (28). Since AA is needed to produce PGE_2, this decreases the amount of PGE_2 that can be produced (26). In addition, EPA and DHA in particular inhibit cyclo-oxygenase, an enzyme necessary for forming PGE_2 from AA (26). Lower levels of PGE_2 result in lower IgE levels and potentially lower chances of developing asthma or allergies (26). This finding laid the foundation for experimental research on the use of EPA and DHA for the prevention of allergies and asthma.

Purpose of the review

The discovery of a potential protective mechanism of DHA and EPA,and the inverse associations between fish intake and asthma or allergies in epidemiological studies, led to

randomized controlled trials of DHA supplementation during pregnancy, lactation, and infancy designed to determine the effect of n-3 fatty acids on childhood asthma and allergies. These studies address the following question: does DHA supplementation reduce childhood development of allergies or asthma? The purpose of this review is to collate and interpret literature that is relevant to the hypothesis that DHA supplementation during pregnancy, lactation, and infancy reduces childhood development of asthma and allergies.

Randomized controlled trials of DHA supplementation

Prenatal supplementation

Three studies were found that investigated the effects of prenatal DHA supplementation on asthma symptoms. Olsen et al. found a 63% lower hazard rate of asthma with marine n-3 PUFA supplementation during the last trimester of pregnancy (6). The hazard rate of allergic asthma was reduced by 87%, a larger decrease than for total asthma. This study followed the offspring out to 16 years of age, the longest follow-up of all studies retrieved. Women in this study received 2.7 grams/day of EPA and DHA, with 1.2 g from DHA. Another study did not find a significant effect of supplementation on asthma symptoms in offspring of women with allergies who were given 400 mg/day of DHA from 18-22 weeks of gestation until delivery (24). A third study found no significant difference in wheeze at six months in offspring of women who received two portions of salmon per week from 20 weeks of gestation until delivery (29).

Four studies of the effect of prenatal DHA supplementation on allergies were retrieved. Three of these studies showed protective effects (24, 25, 30). In these three studies, supplementation took place from ≤22 weeks of gestation through delivery. The level of DHA supplementation in these studies was 400 (24), 800 (24), and >2000 mg/day (25).

Dunstan et al. and Palmer et al. found that offspring of women who received supplementation had lower rates of sensitization to egg at 12 months of age (0.34 odds ratio, 0.11 to 1.02 confidence interval (25); adjusted relative risk 0.62, 0.41 to 0.93 confidence interval (30), but only the second result was significant. Dunstan et al. found that severity, but not incidence, of atopic dermatitis (a form of eczema), was lower at one year of age in the intervention group (25). Palmer et al. (30) found that incidence of atopic eczema was lower (adjusted relative risk 0.64, 0.40 to 1.02 confidence interval). A follow-up study at six years of age, however, showed no remaining protective effects against allergies (31). Escamilla-Nuñez et al. (24) found that offspring of atopic mothers in the intervention group were less likely to have nasal discharge, which may be associated with allergic rhinitis, at 18 months of age.

Only one study retrieved for this review found no positive effect of n-3 fatty acids on allergies in the offspring. In this study, pregnant women in the intervention group consumed two 150-gram portions of salmon per week (29). Each portion contained 1.16 grams of DHA on average. This amounted to an average of 330 mg/day of DHA, lower than the amount in any of the three studies above. Women in the intervention group were given salmon from 20 weeks of gestation until delivery, and women in the control group were asked to continue consuming their usual diet. Follow-up occurred at six months of age, earlier than in the studies previously discussed. The results of this study were not clinically significant.

Offspring of women in the intervention group did not have a lower incidence or decreased severity of atopic dermatitis compared to the control group at six months of age (29). Results of skin prick tests for common food and inhalant allergies did not significantly differ between groups, and neither were serum IgE levels. These results may have been influenced by

the comparatively low dose of DHA and the short follow-up period. The authors noted that a later follow-up period would be necessary to determine long-term clinical outcomes (29).

Only one study of prenatal DHA supplementation and allergies continued supplementation during lactation (32, 33). Participants in this study had allergies or a family history of them. Women in the intervention group consumed capsules containing 1100 mg/day of DHA from 25 weeks of gestation through three to four months of breastfeeding. The control group received soybean oil capsules during the same period. The prevalence of both food allergy and atopic eczema was lower in the intervention group than the control group at one year of age (2% compared to 15%, $p < 0.05$; 8% compared to 24%, $p < 0.05$) (33). The incidence of all IgE-related diseases in the intervention group was lower than in the control group at two years of age (13% compared to 30%, $p = 0.01$) (32), but a lower rate of allergy symptoms was not observed in the intervention group (32).

Supplementation in infancy

Studies of the effect of DHA supplementation in infancy were also retrieved. Three studies was retrieved that investigated DHA supplementation in infancy (34-36). In the first study, infants were supplemented with 500 mg/day of tuna oil (DHA amount not specified) (34). Parents were also given canola oil, rich in n-3 PUFAs, to use in cooking. The control group received placebo supplements and cooking oil rich in n-6 PUFAs. Follow-up was at eighteen months of age. The results showed that supplementation reduced the development of symptoms of asthma (34). The rate of asthma was not lower in the intervention group, but the occurrence of several symptoms and indicators of asthma severity were lower. These symptoms and indicators included wheezing, doctor visits for wheezing, and bronchodilator use. The above study also investigated allergy occurrence (34). Results showed no effect of the intervention on allergy

development. The group with the highest plasma levels of n-3 PUFAs had insignificantly reduced IgE levels.

Foiles et al found that infant formula supplementation with varying levels of DHA (0.32%, 0.64%, and 0.92%) was protective against wheeze/asthma and allergies (35). Time to first diagnosis of allergic illness was delayed in the DHA supplemented groups. The effects of supplementation differed by whether maternal allergies were also present. In non-allergic mothers, DHA reduced the incidence of any allergy and skin allergies. If the mother reported allergies, DHA reduced the incidence of wheeze/asthma in the offspring.

Birch et al also conducted a study of DHA supplementation during infancy (36). In this study, exclusively formula-fed infants were given either formula supplemented with DHA and arachidonic acid (ARA) or a control formula without supplemental DHA or ARA for the first year of life. At three years of age, the children who had received the supplemented formula had significantly lower odds for developing wheezing/asthma (odds ratio (OR), 0.32; 95% CI, 0.11-0.97) or any allergy (OR, 0.28; 95% CI, 0.10-0.72). These children also had lower odds for having an upper respiratory infection (OR, 0.22; 95% CI, 0.08-0.58).

Conclusions

Overall, most randomized controlled trials show a protective effect of maternal DHA supplementation on childhood development of allergies and asthma. Due to the relatively small number of studies and the discrepancies among them, more research is needed in this area to confirm the effects of pre- and post-natal DHA supplementation on childhood development of allergies and asthma.

The studies used different forms of supplementation—from portions of salmon to fish oil capsules. The salmon study was useful for determining whether simply consuming the

commonly recommended amount of fatty fish is adequate to prevent allergies and asthma in offspring. Other studies used a variety of formulations of fish oil, containing different amounts of EPA and DHA. Research is needed to determine the optimal amount of DHA for allergy and asthma prevention.

Only one study in this review investigated DHA supplementation during both the prenatal and postnatal period (32, 33), and this leaves room for further exploration of whether there are greater benefits from continuing supplementation after delivery. Early life seems to be a critical time period for allergy and asthma development, but research has not pinpointed when this crucial period ends. Thus, it may be beneficial to further investigate DHA supplementation during both pregnancy and lactation or infancy.

The follow-up period in most of the studies was relatively short. Seven of the eight studies followed the offspring for two years or less. Olsen et al., the only study with a longer follow-up period, found that the hazard ratio for asthma were reduced at sixteen years of age in offspring of women who were given prenatal DHA supplementation (6). This finding should be investigated in more studies with a similar follow-up period.

Given the growing prevalence of childhood asthma and allergies, as well as the potential adverse outcomes and costs associated with these conditions, research on asthma and allergy prevention has the potential for significant impact. Widespread DHA supplementation during pregnancy and early life may help reverse the trend of rising allergy and asthma prevalence. This could save millions of dollars in healthcare costs per year in the United States alone. It could also improve quality of life for millions of children. Future research should determine the optimal dosage and period of DHA supplementation for asthma and allergy prevention and clarify long-term effects of supplementation on these conditions.

Chapter 3

METHODS

Overview

The research project involved analyzing data previously collected from the KUDOS cohort at the University of Kansas Medical Center. This data included incidence of medically documented allergies, asthma, and associated symptoms of children in the cohort up to six years of age. This will allow the researcher to investigate the hypothesis that DHA supplementation during the second and third trimesters of pregnancy reduces childhood development of asthma and allergies. The following sections will provide details about the sample, setting, ethics, procedures, materials, analysis of data, and schedule of activities.

Setting

All data collection took place at the University of Kansas Medical Center in Kansas City, Kansas. The data analysis for this research project will also take place at the University of Kansas Medical Center.

Ethics

The Human Subjects Committee approved data collection for the KUDOS cohort. Data analysis for this project did not require further ethical review.

Subjects

Women were enrolled in the study if they met the following inclusion criteria: 16.0-35.0 years of age, 8-20 weeks of gestation (determined by ultrasound), agreed to consume study capsules until delivery, agreed to return to the study center for delivery, BMI less than 40, no serious illnesses (cancer, diabetes, lupus, hepatitis, sexually transmitted diseases, HIV), and availability by telephone. They were excluded for any of the following: < 16 or > 35 years of

age, BMI > 40, serious illness, expecting multiple infants, diabetes or gestational diabetes at baseline, elevated blood pressure, not planning to return to the study center for delivery, gestational age at baseline < 8 weeks or > 20 weeks, unable or unwilling to consume capsules until delivery, or unable to provide informed consent in English.

A total of 231 infants were included in the original cohort, whose mothers had consumed the capsules from a mean of 14 weeks of gestation until delivery. Of these, one participant was excluded from data analysis for this study due to a serious genetic disorder. For the survival analysis, subjects were grouped by the last year of life for which complete medical records had been obtained. All subjects were included for the rest of the analysis, regardless of whether their records were obtained up to six years of age. As seen in **Figure 1**, there were 231 participants whose mothers were enrolled in the original study. Of these, the mothers of 106 were assigned to the control group and the mothers of 125 were assigned to the DHA intervention. One subject in the DHA group was excluded from all analysis due to a genetic disorder. There were no significant differences between the baseline characteristics of the treatment and control groups, as shown in **Table 1**. Baseline data for certain risk factors, including paternal allergy, was missing for some subjects.

Figure 1: Consort Diagram

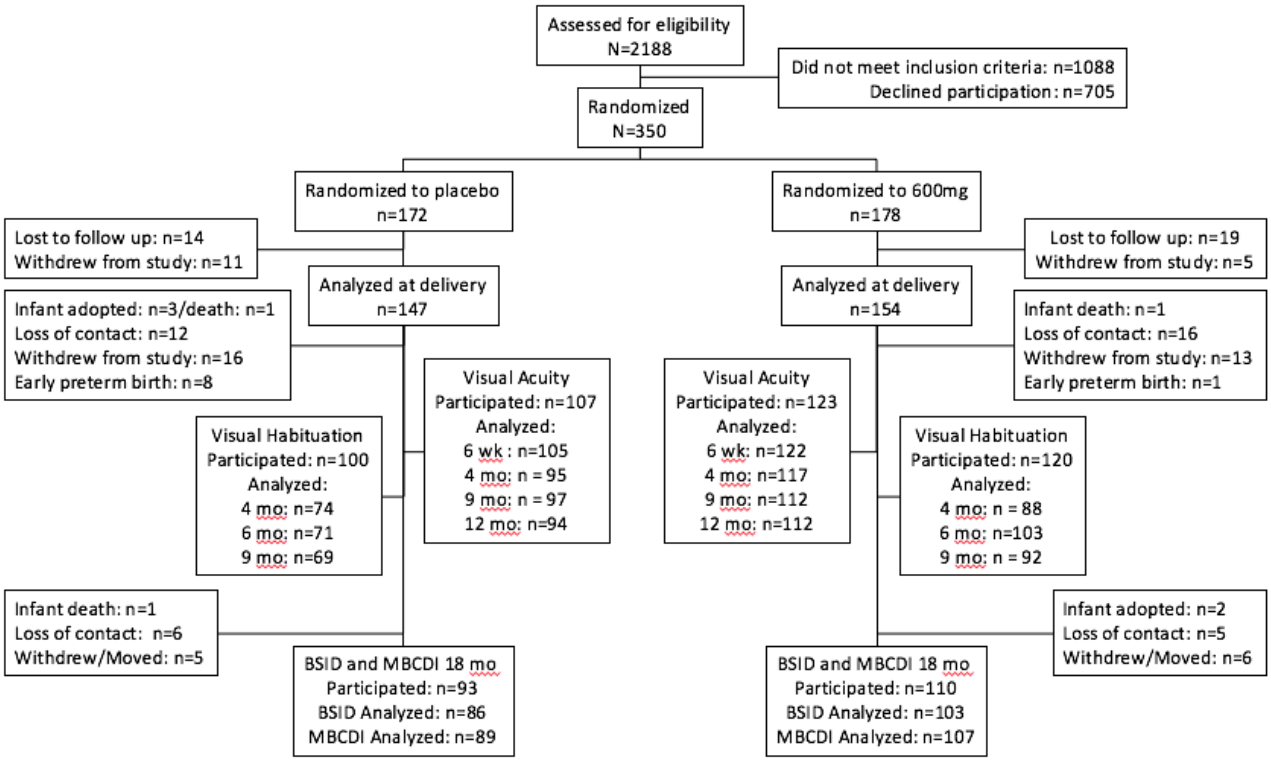

Table 1: Baseline characteristics of the study population			
Variable	Control	DHA	P Value
Male*	57 (53.8%)	59 (47.6%)	0.349
Non-Black*	70 (67.3%)	89 (73.6%)	0.305
Birthweight (grams)**	3302 ± 422	3417 ± 506	0.064
Breastfed Days**	166 ± 208	181 ± 231	0.621
Gestational Weight Gain (pounds)**	35.8 ± 15.6	39.0 ± 15.9	0.160
Maternal Education (Years)**	14.0 ± 2.8	14.5 ± 2.8	0.136
Maternal Allergies Ever Reported*	62 (62.6%)	66 (55.9%)	0.318
Paternal Allergies Ever Reported*	42 (57.5%)	39 (47.6%)	0.215
Yes to Maternal Ever Smoke*	49 (46.2%)	47 (37.9%)	0.202
Yes to Furred Pets in the Home*	54 (54.0%)	58 (48.7%)	0.438
Yes to Daycare Attendance by 12 Months of age*	38 (48.7%)	41 (44.6%)	0.589

*Reported as n (%), χ^2 test; **Reported as mean $\pm$ standard deviation, T-test

Data Collection

For the KUDOS cohort, women were randomized to either receive 600 mg total supplemental DHA in two capsules per day during the second and third trimesters of pregnancy or similar supplements with a mixture of corn and soybean oils. The DHA capsules contained a marine algal source of the nutrient (DHASCO, Martek Biosciences, Columbia, MD). The soybean oil capsules contained 120 mg of alpha-linolenic acid (ALA), a precursor of DHA. This amount of ALA is about 10% of the average amount consumed by US adults. Maternal levels of DHA were measured by collecting and analyzing blood drawn at baseline and delivery. All medical records from primary and specialty providers were collected and used as the records for incidence of allergies and asthma. Only medical diagnoses were coded and included in the analysis.

The following diagnoses were included in the analysis of wheeze/asthma (WA): wheezing, asthma, reactive airway disease, and allergic cough. Atopic dermatitis, eczema, and contact dermatitis were coded as skin allergies (SA). The following diagnoses were coded as other allergies (OA): allergic rhinitis, allergic sinusitis, allergic conjunctivitis, food allergy, drug allergy, allergic rhino-conjunctivitis, vaccine allergy, insect bite allergy, non-specified allergy, and anaphylaxis. All of the above diagnoses were included in the analysis for any allergy (AA).

The researcher or another member of the research team received faxed copies of all medical records for study participants through six years of age. Medical diagnoses in these records were then coded as one of four types of adverse events based on standard coding criteria. These types included infectious, allergic, unknown, and not applicable. Diagnoses were coded as allergies if the diagnosis included “allergy” or an allergic etiology, there was an allergy medication prescribed by the physician at the time of service, or the diagnosis was an evidence-

based allergic condition (asthma, eczema, atopic dermatitis, reactive airway disease (RAD), and wheezing). All diagnoses were recorded in the KUDOS cohort ACCESS database.

Materials

The intervention group was provided with capsules containing 600 mg per two capsules of DHA from marine algal sources during the second and third trimesters of pregnancy. The control group received capsules that were identical but contained a 50-50 mixture of corn and soybean oils and no DHA, as described above. All capsules were donated by DSM (formerly Martek Biosciences), Columbia, MD. For the proposed research project, the only material acquired was SAS University Edition 9.4.

Data Analysis

Paired equal variance t-tests and Chi-squared tests were used to analyze similarity of baseline characteristics of the control and intervention groups. Time to first illness was analyzed using Wilcoxon tests and Kaplan-Meier survival curves. Chi-squared tests were used to examine associations and relative risks of allergic illness across treatment groups. Z-tests comparing two population proportions were used to calculate differences in wheeze/asthma when both treatment group and maternal allergies were included in the analysis. Exploratory data analysis was conducted using Chi-squared tests and binary logistic regression for categorical and continuous variables, respectively. A p value < 0.05 was considered significant for all analyses. The student researcher used SAS University Edition version 9.4 for all analysis except z-test calculations, which were conducted using cell counts from Microsoft Excel and an online statistical calculator (http://www.socscistatistics.com/tests/ztest/). Only the student researcher, statistician, and principal investigator of the study (the thesis chairperson) were aware of the treatment group assigned to each subject.

Schedule of activities

Most data collection had occurred prior to the beginning of the study, and collection of adverse events data up to six years of age was completed in June 2016. The student investigator began statistical analysis in February 2016 and completed this analysis in June 2016. The student investigator submitted the final paper for review on June 28, 2016. Oral defense of the thesis took place on July 12, 2016.

Chapter 4

RESULTS

Incidence of at least One Allergic Illness

No significant difference was found in the incidence of at least one allergic illness by six years of age between the treatment and control group (**Table 2**). There were also no significant differences found in the incidence of allergy either during the first year or the first to the 6th year of age (**Tables 3-4)**.

Table 2. Incidence of at least one allergy diagnosis by six years of age.

Diagnosis Type	Control (n = 106)		DHA (n = 124)		P Value
Any Allergy	60	56.6%	75	60.5%	0.551
Wheeze/Asthma	26	24.5%	23	18.6%	0.270
Skin Allergy	37	34.9%	44	35.5%	0.927
Other Allergy	42	39.6%	40	32.3%	0.245

P values calculated using Chi-squared analysis.

Table 3. Incidence of at least one allergy diagnosis in first year of life.

Diagnosis Type	Control (n = 106)		DHA (n = 124)		P Value
Any Allergy	28	26.4%	39	31.5%	0.402
Wheeze/Asthma	3	2.8%	5	4.0%	0.620
Skin Allergy	21	19.8%	28	22.6%	0.609
Other Allergy	9	8.5%	9	7.3%	0.729

P values calculated using Chi-squared analysis.

Table 4. Incidence of at least one allergy diagnosis from 12 to 71.99 months of age.					
Diagnosis Type	Control (n = 106)		DHA (n = 124)		P Value
Any Allergy	54	50.9%	64	51.6%	0.919
Wheeze/Asthma	26	24.5%	22	17.7%	0.207
Skin Allergy	29	27.4%	31	25.0%	0.685
Other Allergy	38	35.9%	36	29.0%	0.270

P values calculated using Chi-squared analysis.

Relative Risk of having at Least One Allergic Illness

No significant difference was found between the relative risk of allergic illness in the control versus treatment group for the first year of life, the first six years of life, or between twelve and 71.99 months, as shown in **Tables 5-7**.

Table 5. RR (including 95% confidence interval) of at least one allergy diagnosis by six years of age compared to the DHA group.		
Diagnosis Type	Relative Risk	95% Confidence Interval
Any Allergy	0.94	0.75-1.17
Wheeze/Asthma	1.32	0.80-2.17
Skin Allergy	0.98	0.69-1.40
Other Allergy	1.23	0.87-1.74

Table 6. RR (including 95% confidence interval) of at least one allergy diagnosis in first year of life compared to the DHA group.		
Diagnosis Type	Relative Risk	95% Confidence Interval
Any Allergy	0.84	0.55-1.27
Wheeze/Asthma	0.70	0.17-2.87
Skin Allergy	0.88	0.53-1.45
Other Allergy	1.17	0.48-2.84

Table 7. RR (including 95% confidence interval) of at least one allergy diagnosis from 12 to 71.99 months compared to the DHA group.		
Diagnosis Type	Relative Risk	95% Confidence Interval
Any Allergy	0.99	0.77-1.27
Wheeze/Asthma	1.38	0.83-2.29
Skin Allergy	1.09	0.70-1.69
Other Allergy	1.23	0.84-1.80

Incidence of Wheeze/Asthma Based on Treatment Group and Maternal Allergies

Of all allergy types, the rate of wheeze/asthma appeared to differ the most between treatment groups, so further analysis was conducted that included maternal allergies (**Tables 8 and 9**). This was to determine whether there was a stronger protective effect in offspring of women who reported allergies. The protective effect did appear to be stronger, with a 6.2% decreased incidence of wheeze/asthma by six years of age in offspring of allergic mothers who received DHA, compared to a 3.5% decreased incidence in offspring of non-allergic mothers receiving DHA (**Table 9**).

Table 8. Wheeze/Asthma Diagnosis from 12-71.99 mo based on Tx Group + Maternal Allergy*				
Control + Yes to Maternal Allergy Ever		DHA + Yes to Maternal Allergy Ever		P-Value
17 out of 62	27.4%	13 out of 66	19.7%	0.303
Control + No to Maternal Allergy Ever		DHA + No to Maternal Allergy Ever		P-Value
7 out of 37	18.9%	8 out of 52	15.4%	0.660

P-value calculated using Z-test for two independent population proportions.
*Missing maternal allergy data for 13 participants (not included in this analysis).

Table 9. Wheeze/Asthma Diagnosis in first six years based on Tx Group + Maternal Allergy*				
Control + Yes to Maternal Allergy Ever		DHA + Yes to Maternal Allergy Ever		P-Value
17 out of 62	27.4%	14 out of 66	21.2%	0.412
Control + No to Maternal Allergy Ever		DHA + No to Maternal Allergy Ever		P-Value
7 out of 37	18.9%	8 out of 52	15.4%	0.660

P-value calculated using Z-test for two independent population proportions.
*Missing maternal allergy data for 13 participants (not included in this analysis).

Chapter 5

DISCUSSION

The results of this study show that prenatal supplementation with 600 mg DHA during the second and third trimester does not provide protection against allergic illness in the first six years of life. The lack of effect was consistent across all periods examined: the first year of life, the second through sixth years of life, and from birth to six years of age. Previous studies have shown conflicting evidence of protection against both allergies and asthma.

Exploratory data analysis showed several interesting findings, which may be areas for further research. Offspring of women who did not report having an allergic illness were less likely to have an allergic illness during the first six years of life, and the difference almost reached a significant level (RR: 0.78, CI: 0.61-1.00). Males were significantly more likely to have had a wheeze/asthma diagnosis in the first six years of life than females (RR: 1.69, CI: 1.01-2.85). Children who were not black had a significantly lower risk of both wheeze/asthma and skin allergy in the first six years (wheeze asthma RR: 0.60, CI: 0.37-0.98; skin allergy RR: 0.57, CI: 0.41-0.80). DHA supplementation did not seem to provide additional protection against allergies or asthma in children who were black, males, or offspring of women with reported allergies.

A strength of the study was its double-blind, placebo-controlled design. Many of the subjects were still coming to the study center for progress visits at the time of this project, so there was continued contact with these participants. Another strength was that only medically documented diagnoses of allergies and asthma were included, so parent reports were excluded. This increased the likelihood that only true allergic illnesses were examined in the study.

A limitation of the study was incomplete data for certain variables that were being examined, including paternal allergy data. Another limitation was that visual and neural development were the primary outcomes being studied in the KUDOS cohort, not incidence of asthma and allergies. Since only medically documented incidence of allergies and asthma were included in the analysis, the research heavily relied on complete and thorough medical examination and documentation from a wide variety of doctors. It was difficult to obtain medical records for certain subjects, and others dropped out of the study before six years of age. Sometimes parents took their child to multiple clinics or hospitals, so records may not have been received from all locations of care. Thus, while every effort was made to ensure complete medical records data collection and coding, some allergic events may have been missed.

The lack of a protective effect could be due to the timing of supplementation. A previous study at the University of Kansas Medical Center showed protective effects of DHA when supplemented in formula during infancy (35). Since that trial, most formula companies have added DHA to their products. The subjects in the current trial who were breast-fed for less than 100 days (n= 114 (49.6%)) were presumably formula-fed during part of their infancy, thus receiving supplemental DHA regardless of treatment group. When human milk fed, all infants also received DHA. This exposure to DHA may have obscured the protective effects of supplemental DHA which had been shown in previous studies.

Alternatively, it is also possible that the protective effect of DHA against asthma and allergies is a more immediate effect, compared to the developmental programming effect that was hypothesized in this study. Future studies could examine the effect of DHA intake throughout childhood on the incidence of allergies and asthma.

In conclusion, the results of this study do not support the hypothesis that prenatal supplementation with 600 mg of DHA during the second and third trimesters of pregnancy protects against allergies and asthma up to six years of age. This result was consistent for incidence of skin allergies, wheeze/asthma, other allergies, or any allergic diagnosis. The effects did not differ during the first year of life, the second through sixth years of life, or from birth to age six.

Chapter 6

SUMMARY

There is some evidence to support the theory that fetal development may be a critical for the development of allergies and asthma later in life. DHA may play a key role in the development of the immune system. This study aimed to investigate the effects of DHA supplementation during this time period on the incidence of allergies and asthma up to six years of age.

Women in the KUDOS cohort were given 600 mg of DHA or a placebo during their second and third trimesters of pregnancy, and their offspring were followed up to six years of age. Two-hundred thirty infants were included for analysis. Their medical records were collected until six years of age or until their records could no longer be acquired if the infant dropped out before six years of age. All records were coded and allergic diagnoses were examined for this study. These diagnoses were grouped into three categories, skin allergies, wheeze/asthma, and other allergies.

Associations and relative risks were calculated for each type of allergy diagnosis and no significant associations were found. DHA supplementation during the prenatal period did not show a protective effect against asthma or allergies during the first year of life, the second through sixth years of life, or from birth to six years of age for any allergy, skin allergies, other allergies, or wheeze/asthma.

Limitations of the study included a different primary outcome for the KUDOS cohort and issues inherent in using medical records. Strengths included the randomized, double-blind, placebo-controlled design and medically documented-allergic illnesses. Further research could investigate continued DHA intake during childhood on the incidence of allergies and asthma.

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