Maternal DHA Supplementation and Childhood Blood Pressure

BY

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Abstract

Background: Blood pressure (BP) in childhood is positively associated with BP in adulthood; it is important to understand influential factors of childhood BP. Child race, body mass index (BMI), and diet are well studied, but intrauterine exposures that may influence BP long term are not well explored.

Objective: To determine if DHA supplementation during pregnancy leads to lower blood pressure in offspring at 4-5.5 years of age.

Methods: We measured BP longitudinally at 6-mo intervals from 4 to 5.5 years of age in a cohort of 179 children whose mothers were randomized to either supplementation with the omega-3 fatty acid DHA (600 mg/d) or placebo during pregnancy.

Results: Black race, child BMI and child salt intake were positively associated with systolic and diastolic BP. Maternal DHA status at delivery was associated with significantly lower BP at 4 and 5 years of age. Black children whose mothers were assigned to placebo had higher BP across all ages compared to white children and black children whose mothers were supplemented with DHA. Similarly, maternal DHA supplementation protected against higher BP observed in children who were overweight or obese compared to healthy weight children at 4 and 5 years of age; and protected BP of those whose average sodium intake across all ages exceeded 1.9 g/day.

Conclusion: Improving fetal DHA status through maternal DHA supplementation during pregnancy appears to protect against several risk factors for higher BP in childhood.

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Jamie Hilton

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Chapter 1: Introduction

Fish consumption and DHA intake is much lower in the US compared to European and Asian countries (1),(2). Consequently, pregnant and lactating women in the US have significantly lower DHA levels in their blood and breastmilk (3). This is significant because DHA has been shown to lower blood pressure levels in both hypertensive and normotensive individuals. And some research has shown that breastfeeding leads to lower blood pressure in childhood (4). Because human milk contains DHA, it has been suggested that the effect may be due to the DHA content of milk (5). One study looked at the effect of consuming a formula with DHA and arachidonic acid during infancy on childhood blood pressure and found that the addition of these fatty acids to formula resulted in lower blood pressure in childhood with an effect size similar to breastfeeding in infancy (5). Therefore, long chain polyunsaturated fatty acids (LCPUFA) exposure early in development may program a lower blood pressure in childhood. Assuming early LCPUFA exposure that includes DHA, does have a programming effect, one could suggest a similar effect of exposing the fetus to DHA during pregnancy. However, no research has been done to examine the impact of maternal DHA supplementation during pregnancy on blood pressure during childhood.

Justification. Because on average pregnant women in the US consume less DHA than European and Asian women, US women might benefit from DHA supplementation during pregnancy. This benefit may extend to the health of the offspring even into childhood. If this were the case, improving maternal DHA intake could be a way to prevent chronic disease and improve the health of the growing population.

Overall, the purpose of my study was to investigate the impact of maternal DHA supplementation during pregnancy on childhood blood pressure between the ages of 4 and 5.5 years compared to a vegetable oil placebo.

Research question: Does 600 mg of DHA supplementation during pregnancy lead to lower blood pressure in children between 4 to 5.5 years of age?

Chapter 2: Literature Review

Diet and Blood Pressure

Blood pressure is the force applied by the blood onto the arteries as blood is pumped by the heart (6). As blood pressure rises the heart must work harder to pump blood throughout the body (6). This increased workload can lead to damage of the heart and outcomes such as myocardial infarction (MI), stroke, heart failure, kidney failure, and aneurisms (6). High blood pressure or hypertension is defined as having a systolic pressure $\geq$ 140 mm Hg or a diastolic pressure $\geq$90 mm Hg (6). Primary hypertension is the most common form of high blood pressure, and multiple factors contribute to the development of primary hypertension including diet, lifestyle factors, age, gender, and race or ethnicity (7).

Diet is a major contributor to high blood pressure. There are multiple components that contribute to high blood pressure including excessive sodium intake, low potassium intake, and increased alcohol consumption (8). The Dietary Approaches to Stop Hypertension (DASH) study developed a diet to target these areas of nutrition that contribute to hypertension (9). The DASH dietary pattern includes 8-10 servings of fruits and vegetables per day, 2-3 servings of low fat dairy, and less than 2.3 grams of sodium per day (9). The DASH diet was found to reduce systolic and diastolic blood pressure by 11.4 mm Hg and 5.5 mg Hg in those with hypertension and by 3.5 mm Hg and 2.1 mm Hg in those without hypertension (10). The American Heart Association recommends limiting sodium consumption to less than 2400 mg per day, limiting alcohol consumption to 1 drink for women and 2 drinks for men per day, and eating fish at least twice per week to improve heart health and help lower blood pressure (11). Making dietary changes such as appropriate intake of key nutrients related to blood pressure regulation can lead

to reductions in blood pressure. Specifically, increased intake of omega 3 fatty acids like docosahexaenoic acid (DHA: 22:6n-3) and eicosapentaenoic acid (EPA: 20:5n-3) has been shown to lead to a slight improvement in blood pressure in both normotensive and hypertensive individuals with the most benefit in individuals with hypertension (12).

EPA and DHA are omega 3 fatty acids that have important roles in fetal development and have protective roles in cardiovascular health (13). Fish is a common source of these essential fatty acids, but the body is capable of synthesizing EPA and DHA from a precursor known as alpha-linolenic acid (ALA) (14). ALA is found in flaxseeds, walnuts, canola and some other vegetable oils, as well as other fortified foods and plants (14). However, less than 0.2% of ALA consumed is converted to EPA and less than 0.05% is converted to DHA, so good dietary sources such as fish and fish oil supplements may be necessary, particularly during development (15). The amount of DHA and EPA varies by type of fish, with fattier fish having more omega 3 fatty acids than leaner fish (16). Omega 3 fatty acid content can also vary based on whether the fish was farm raised or wild, and based on growing conditions like water temperature and season (16). In a 3 ounce serving of salmon, there is 0.71 g EPA and 0.51 g of DHA (17). Cod liver oil provides the greatest amount of EPA and DHA, containing 6.2 g and 9.9 g per 3 ounce serving, respectively [10]. Because DHA and EPA levels vary by fish species, there is a large variability in DHA consumption per serving as a result.

Within the western diet, fish is less commonly consumed. According to the 2009 census conducted by the US Census Bureau, the average person consumes 15.8 pounds of fish per year (1). The average yearly consumption amounts to roughly 4.8 ounces of fish per week. Consuming 4.8 ounces of salmon in a week, provides approximately 1.08 g EPA and 0.78 g of DHA or 2 grams of omega 3 fatty acids, however, most fish consumed in the US is in the form of

low DHA and EPA sources such as fish sandwiches, shrimp, etc. (2). The Food and Nutrition Board of the Institute of Medicine set an adequate intake level of 1.1 g/d for females and 1.6 g/d for males of ALA (18) but as noted above, the body is inefficient at converting ALA into DHA and EPA (15). From fish consumption alone, Americans are not meeting their needs for omega-3 fatty acids, and they are not meeting the recommendation to consume 8-12 ounces of fish per week. Increasing DHA and EPA intake could improve blood pressure levels in adults, and consequently improve disease outcomes.

Extensive research has been done to assess the impact of DHA and EPA supplementation in normotensive and hypertensive adults. In 2014, Miller et al. (19) conducted a meta-analysis of over 70 studies that evaluated both fish oil supplementation as well as diet interventions. Miller et al. (19) found that fish oil supplements were more effective in lowering blood pressure than high fish intake diets. Specifically, supplementation of less than 2 g of EPA and DHA per day was effective in lowering systolic blood pressure, but not diastolic blood pressure; and supplementation of greater than 2 g per day led to a decrease in diastolic blood pressure (19). Furthermore, the impact of EPA and DHA supplementation was greater in hypertensive individuals compared to normotensive individuals (19). The results of this most recent meta-analysis are comparable to the meta-analysis conducted by Campbell et al. in 2013 (20). In this analysis, Campbell et al. (20) did not include studies that looked at diet and only included 17 studies in the analysis. Campbell et al. (20) also found that fish oil supplementation lowered systolic blood pressure, but could not recommend fish oil supplementation as a sole treatment for hypertension. These findings are important because individuals with high blood pressure even when treated are at greater risk of mortality and morbidity than individuals with normal blood

pressure levels (21). Thus it is important to target preventive measures and focus on preventing hypertension starting at a young age (21).

The prevalence of high blood pressure during childhood is on the rise partly due to the increasing rates of obesity during childhood. Weight as well as other factors like race, gender, sex, and disease, primarily heart and kidney disease, impact blood pressure levels during childhood (22). A study published in 2015, measured the prevalence of elevated blood pressure in children ages 2-17 using medical records of over 20,000 children (22). The study methods defined high blood pressure as > 90^{th} percentile for weight, age and height or >120/80 mm Hg (22). The study found that 36% of subjects had blood pressure above the 90^{th} percentile at ≥ 1 visits during that year, and 3% of subjects had blood pressure above the 90^{th} percentile at ≥ 3 visits during a single year (22). Within the population who had ≥ 3 high blood pressure measurements, 62% of those subjects had a BMI > 85^{th} percentile (22). As in adults, the risk for hypertension in childhood increases with body weight. Importantly, a meta-analysis by Chen et al. (23) found that BP tracks from childhood to adulthood, confirming that having higher blood pressure in childhood increases one's risk for hypertension (24) and atherosclerosis (25) in adulthood. These findings highlight the need for preventive measures even in childhood. With growing obesity rates, hypertension in childhood is on the rise, and alternative treatment methods like diet and lifestyle modifications are needed to address this growing problem.

As with adults, high sodium intake is also a contributor to hypertension in childhood. Correia-Costa et al. (26) studied the relationship between sodium intake, gender and obesity and its impact on blood pressure in a cross sectional cohort study of 298 Portuguese children between 8-9 years of age (26). The study found that males consumed significantly more sodium than females (6.8 ± 2.4 g/d vs 6.1 ± 1.9 g/d, p-value 0.018), and overweight/obese children consumed

significantly more sodium than normal weight children (6.8 ± 2.4 g/d vs 6.1 ± 2.0 g/d, p=0.006). In addition, the study found that systolic blood pressure was positively associated with both sodium intake and BMI, and for every 1 gram increase in sodium intake they saw a 1.00 mm Hg increase in SBP in overweight/obese males (26). This effect was not seen in normal weight boys or in girls of any BMI category. Yang et al. (27) analyzed the data from the 2003-2008 NHANES survey consisting of 6235 subjects, and also found an association between sodium intake, BMI and BP in children and adolescents 8-18 years of age (27). Specifically, Yang et al. (27) found for every 1 gram increase in sodium intake there was a 1.0 mm Hg increase in SBP among all subjects and a 1.5 mm Hg increase in SBP among all overweight/obese participants. In this population, the average sodium intake was 3387 mg/d, with males consuming more sodium than females, normal weight subjects consuming more than overweight/obese subjects, and non-Hispanic whites consuming more than non-Hispanic black subjects (27). These findings suggest that both BMI and sodium intake are positively associated with SBP, and children with elevated BMI are more susceptible to hypertension when consuming a high sodium diet.

Studies linking DHA supplementation to lower blood pressure in adulthood highlight the need to investigate the impact of DHA supplementation on hypertension in childhood especially for children exposed to high risk factors for hypertension like having a BMI >85th percentile and consuming high amounts of sodium. Moreover, unlike in adults who have lower BP while taking omega 3 fatty acids, several studies in childhood suggest early exposures may lead to lower BP long after supplement action ceases (28), (29). Some studies have found a connection between breastfeeding and lower blood pressure in childhood. Specifically, past research has identified a positive impact of breastfeeding on blood pressure that was not seen in formula fed infants (4), (29), (30), (31). A physiological difference found in childhood linked to early feeding implies

early developmental programming related to some difference in feeding. One factor that distinguishes human milk from old formulations of term and preterm formula is DHA content suggesting that improving early DHA status could have long term benefits in reducing blood pressure (5, 32). The purpose of this review is to investigate the effects of DHA supplementation during pregnancy on blood pressure in childhood.

Breastfeeding and Childhood Blood Pressure

Some research has found that children who were breastfed as infants have lower blood pressure than partially breastfed or exclusively formula fed infants. The following studies demonstrate the benefits of breastfeeding. Wilson et al. (29) found that children who were exclusively breastfed for > 15weeks had significantly lower blood pressure at 7.2 years of age (measured at 6.9-10 years) than children who were breastfed < 15 weeks or who were formula fed. Singhal et al. (30), compared donor breastmilk to preterm formula and standard formula and measured blood pressure at ages 7-8 and at ages 13-16. No difference in blood pressure was found at 7-8 years but blood pressure was significantly lower in both systolic and diastolic blood pressure at 13-16 years of age in the donor breastmilk group (30). In addition, Singhal et al. (30) found that the amount of breastmilk the infant received had a significant impact on blood pressure. The difference in findings between the two ages could be due to response bias, as 81 percent of subjects participated in the 7-8 year follow up but only 26 percent participated in the 13-16 year follow up (30). Another study by Lawlor et al. (31) found that at 5 years of age, children who were breastfed for more than 6 months had significantly lower blood pressure (-1.19 mm Hg) than children who were breastfed less than 6 months or who were formula fed. Finally, Martin et al. (4) conducted a meta-analysis of 15 studies that looked at the impact of breastfeeding for more than 2 months on blood pressure levels in childhood and found an overall

1.4 mm Hg decrease in systolic blood pressure and 0.5 mm Hg decrease in diastolic blood pressure. These studies suggest that both the length of breastfeeding and the exclusivity of breastfeeding have an impact on blood pressure in childhood.

One commonality among the above-mentioned studies is that none of the control formula groups were supplemented with DHA or arachidonic acid (ARA). Human milk is known to contain DHA and ARA, and the absence of DHA and ARA in the control formula could explain the differences in blood pressure between the two groups. The earliest age in which a difference in blood pressure can be seen cannot be determined from current research. No major differences were apparent in the methods used for measuring blood pressure, but not all studies found differences in blood pressure at the same ages. These discrepancies suggest that more research needs to be done to pinpoint the age at which blood pressure differences could be found.

Although, some research suggests there is an association between breastfeeding and blood pressure, there have been several studies published in recent years that have found no benefit of breastfeeding on blood pressure. For example, de Jong et al. (33) found no effect of breastfeeding on blood pressure at 9 years of age. The study included 341 subjects randomized to a control formula group (no DHA or AA), a LCPUFA formula group (0.45% AA and 0.30% DHA), and a breastfed group (33). The study only required participants to breastfeed for 2 months, a much shorter period than what is currently recommended. The result is comparable to children that were breastfed for a short period of time (< 6 months) in the studies conducted by Lawlor et al. (31) and Wilson et al. (29). Similarly, Martin et al. (34) and Kramer et al. (35) found no differences in blood pressure at 6.5 (n= 13,889) and 11.5 (n=13,616) years of age in the Promotion of Breastfeeding Intervention Trial (PROBIT). The purpose of the PROBIT study was to increase length and exclusivity of breastfeeding, and although the study resulted in great

increases in breastfeeding length and exclusivity at 3 months of age, they found no differences in blood pressure between the intervention and control groups. But as mentioned before, length of breastfeeding seems to be inversely associated with BP, and 3 months of breastfeeding is a shorter period of time than in the studies that found a benefit of breastfeeding. In addition, the PROBIT trial was only designed to educate and encourage breastfeeding in the intervention group, and did not require exclusive breastfeeding for any period of time to participate in the study. Horta et al. (36) conducted a systematic review on the association between breastfeeding and blood pressure and found systolic blood pressure was lower in breastfed groups but this association was not present in larger studies, and there was no association between breastfeeding and diastolic blood pressure.

Overall, the available research is not in agreement. Some randomized control trials have found a benefit of breastfeeding on blood pressure, but large observational studies have not shown an association when socioeconomic status is controlled for. More recent studies suggest differences in findings could be due to publication bias, small sample size, and confounding factors such as socioeconomic status that are not properly controlled for in observational studies. Although, there are many studies published on this topic, they vary by study type, sample size, age of first blood pressure measurement, and income status of the populations. With such variability it is difficult to come to a conclusion on the benefits of breastfeeding on blood pressure in childhood.

Impact of DHA supplementation on blood pressure in children

Supplementation during Infancy: Human Milk. Although studies have shown that DHA supplementation lowers blood pressure in normotensive and hypertensive individuals, Larnkjear et al. (37) and Asserhoj et al. (38) found no benefit to maternal supplementation of DHA during breastfeeding on blood pressure at 2 to 7 years of life. Larnkjear et al. (37) recruited 122 Danish mothers who consumed < 0.4 g/d of omega 3 long chain polyunsaturated fatty acids ((n-3) LCPUFA) (37). This level is below the Danish national median for (n-3) LCPUFA intake, with the highest intake averaging around 0.82 g/d (37). The intervention group received 0.6 g EPA and 0.8 g DHA per day and the other group received olive oil capsules. Supplementation occurred during the first 4 months of life and the mothers were asked to exclusively breastfeed during that time. Because blood pressure data of children who were not exclusively breastfed for the entire 4 month period were included in the analysis, degree of breastfeeding was considered a covariate. Blood pressure was measured at 2.5 years of life (37) and at 7 years of life (38), and at neither time point did blood pressure differ between the two groups. Maternal DHA supplementation during lactation did not lead to a greater reduction in blood pressure at 2.5 or 7 years of age, however, all human milk provides DHA and the studies were done in countries where adults consume more DHA than in the US. It is possible that milk of women not supplemented provided adequate DHA without supplementation. However, no studies have looked at the impact of maternal DHA supplementation during lactation in the US population. A benefit may exist in populations that do not consume enough DHA from diet alone.

Although no benefit for child blood pressure was seen in this population, children of US woman may still benefit from supplementation as US fish intake is much lower than Danish fish

intake. More research needs to be done to determine if US populations would benefit from this intervention.

DHA in Formula and Childhood Blood Pressure. It has been hypothesized that the presence of DHA in breastmilk has contributed to the lower blood pressure levels in children who were exclusively breastfed. Prior to 2002, formula was not supplemented with DHA (39). The addition of DHA to formula after 2002 may have helped to improve blood pressure levels in exclusively formula-fed children. To test this hypothesis, Forsyth et al. (5) conducted a study to compare term formula to term formula supplemented with DHA (0.15%-0.25% DHA). Forsyth et al. (5) found diastolic blood pressure and mean blood pressure at 5.8 years of age was lower in the LCPUFA formula group compared to the non-supplemented formula group. In addition, the diastolic blood pressure of the LCPUFA formula group was comparable to the breastfed infant control group.

Prenatal DHA Supplementation and Childhood Blood Pressure. The impact of maternal DHA status during pregnancy on early fetal programming is not well studied. Vidakovic et al. (40), conducted a prospective cohort study assessing maternal second-trimester n-6: n-3 PUFA ratios using plasma glycerolphospholipid concentrations. Childhood blood pressure was then measured at 6 years of age. The study found that children of mother's with high n-3 PUFA (wt%) during the second trimester, specifically related to high DHA (wt%) had lower systolic blood pressure at 6 years of age (40). No association was found with diastolic blood pressure. Since there has only been one study conducted in this area, there is a need for more research on this topic specifically for randomized control trials. Research in this area is necessary to better understand the benefits of DHA supplementation and its role on childhood blood pressure, as well as to determine mechanisms of DHA action. Current research shows that

DHA is required for fetal development and is preferentially provided to the infant during pregnancy. DHA first starts to accumulate in the fetus at 24 weeks gestation, but the amount of DHA that is transferred varies greatly from mother to mother depending on the mother's own DHA intake (3). The variability in DHA transfer makes it difficult to design a strong randomized controlled trial and could impact the findings of the study (3).

Summary

My review of the literature suggests that DHA intake in infancy does have some impact on blood pressure in childhood. The research on the impact of breastfeeding on blood pressure is extensive, but inconclusive. Some studies suggest that children that were breastfed have lower blood pressure during childhood compared to children who were partially breastfed or exclusively formula fed. However, recent studies have not found an association between breastfeeding and blood pressure and argue that previous study findings were due to small sample size and confounding factors such as socioeconomic status. The studies in this review looked at a wide range of ages ranging from 2 to 17 years of age, and lower blood pressure was evident at these ages in some studies but not in all. Only one study compared infant formula supplemented with DHA, formula without DHA, and breastfeeding, and infants receiving DHA formula had lower blood pressure compared to infants not receiving DHA formula. However, more studies need to be done to confirm this finding.

Maternal DHA supplementation during pregnancy could also increase DHA supply at a critical early period of development; however, the impact of increasing fetal supply on childhood blood pressure is less widely studied. A prospective cohort study did associate higher DHA plasma levels during pregnancy with lower systolic blood pressure at 6 years of age (40), but no

research has looked at the impact of maternal DHA supplementation during pregnancy and blood pressure in childhood.

The literature suggests that DHA has an important role in lowering blood pressure, and that excessive sodium intake and elevated BMI both play a role in raising blood pressure. What we do not know is whether supplementing DHA during pregnancy will lead to lower blood pressure in childhood and if DHA supplementation might lower the offspring's risk of high blood pressure when exposed to risk factors such as excessive sodium intake or elevated BMI. The results of this randomized controlled trial can start to answer both of these questions.

Chapter 3: Methods

Subjects

The purpose of this study is to determine whether DHA supplementation during pregnancy leads to lower blood pressure in children between 4 and 5.5 years of age. The KU DHA Outcomes Study (KUDOS) was a phase III placebo controlled randomized clinical trial that looked at the effects of DHA supplementation during pregnancy on gestational duration, birth size, length, and maternal and newborn DHA status. Mothers were recruited from the Kansas City Metropolitan Area between January 2006 and November 2009. To participate in the study, mothers had to speak English, be between 8 and 20 weeks gestation, 16 - 35.99 years of age, and planning to deliver in a hospital in the Kansas City area. Exclusion criteria were as follows: pre-existing DM, hypertension, preexisting health condition such as HIV/AIDs, hepatitis, lupus, cancer, and alcohol or drug dependency that could impact growth and development, and morbid obesity (BMI>40). The study followed an intent to treat protocol and included 3 females with a BMI >40.

The NIH also funded developmental follow up of the children to age 6. A total of 179 children (of 350 mothers enrolled) continued to participate in follow up visits after age 4. Blood pressure measurements were taken every 6 months starting at 4 years of age and continued through 6 years of age.

Setting

Study subjects were recruited from the Kansas City metropolitan area between January 2006 and November 2009. Data continues to be collected as the remaining participants reach 6 years of age.

Ethics

The KUDOS study was approved by the University of Kansas Medical Center Human Subjects Committee # 10186 and 11406. Both the research protocol and informed consent process were in compliance with the Declaration of Helsinki, and were approved by the Institutional Review Boards/Human Subjects Committee at the University of Kansas Medical Center, the University of Missouri-Kansas City; and St Luke's Hospital. The proposed project is covered by the existing protocol.

DHA Supplementation and Blood DHA Analysis

Pregnant participants were randomized into one of two groups. To account for potential bias of age at enrollment, randomization was stratified by age (16-25.99 or 26-35.99). The experimental group received 600 mg/d DHA in the form of 3 capsules each containing 200mg of DHA. The placebo group also received 3 capsules per day containing equal parts soybean oil and corn oil. Orange flavoring was used to conceal the identity of the placebo and DHA capsules. The participants were instructed to take 3 capsules per day until delivery. Each month the subjects were mailed a month's worth of capsules and were supplied a self-addressed stamped envelope to return the previous bottle. Upon receiving the sealed envelope the pharmacy staff counted the remaining capsules, and this count was used to assess total DHA intake and compliance at the end of the study.

Prior to randomization, a blood sample was drawn from the mother. Fatty acids were analyzed as described previously (41). Briefly, red blood cell (RBC) lipids were extracted by a modified procedure of Folch et al. (42) and RBC phospholipids were separated using thin-layer chromatography (43). RBC phospholipids were then trans-methylated using boron trifluoride-methanol resulting in fatty acid methyl esters (FAMES) (44). Next the FAMES were separated using a Varian 3900 gas chromatograph with a 100 meter capillary column (SP-2560; Sigma Aldrich). Peak integration and analysis was performed using a Star 6.41 Chromatography Workstation (41). Qualitative standards (PUFA 1 and PUFA 2; Sigma Aldrich) and a weighed standard mixture (Supelco 37 Component FAME mix) was used to identify individual peaks and to calculate a final percentage weight of total fatty acids (41). Individual RBC-phospholipid fatty acid levels were reported as a percentage of total fatty acid by weight (wt %). Following delivery, a second blood sample was drawn and analyzed for fatty acids. Change in DHA wt% was used examine the effect of the intervention (placebo vs DHA).

Blood Pressure Assessment and Reporting

Beginning at 4 years of life, blood pressure measurements were taken every 6 months in the offspring. Blood pressure was measured using a GE Carescape V100. At the end of a 60 minute cognitive assessment, the child was wheeled to the room where blood pressure was measured. The child remained seated while their blood pressure was measured in triplicate. Child and toddler size cuffs were used to conduct blood pressure measurements unless a larger size was necessary. The cuff was applied by wrapping it around the upper arm, aligning the arrow marked artery with the patient's brachial artery (45). Appropriate cuff size was chosen by making sure the Index line on the cuff fell between the range marks on the cuff as the cuff was wrapped around the patient's arm (45). If the index line did not fall between the range marks a

larger or smaller cuff was chosen (45). Measurements were taken 3 times by research staff trained in this procedure. Average systolic and diastolic measurements were calculated using the last two measures, coefficient of variance (CV) was then calculated for each average. If the CV was >0.095, the two measures closest in number were selected to calculate the average, rather than the last two. The method was applied to 9.2% of SBP measurements and 19.4% of DBP measurements. This method was applied across all age groups. Reasons for missing BP data include irritated or restless children, or mechanical failure. The measurements with only one data point were excluded from analysis (n= 23).

Blood pressure and BMI percentiles were calculated using the EZ BMI/BP calculator software version 2013. The software uses the child's age, gender, and height to determine the BP percentile, and age and gender are used for BMI percentile calculations.

Dietary Intake

Food, formula, and human milk intake were obtained by a registered dietitian using a standardized multipass-24-hour recall method at each visit throughout the study (46). In infancy, recalls were obtained at 6 weeks, 4, 6, 9 and 12 months. From age 1 to 5.5 years, recalls were obtained at 6 month intervals. A dietary recall kit that included bean bags, bowls, cups, measuring cups and spoons, as well as food pictures was used to assist the parents in completing the dietary recall. Recalls were entered into Nutrition Data System for Research (NDSR) (versions 2008-2014, University of Minnesota), and assessed for accuracy by a second registered dietitian. A dietary recall was deemed unreliable when the parent or child was unable to recall multiple meals in the 24 hour period. In addition, dietary recalls were assessed for quality assurance when the calorie intake was above 2100 calories or below 912 calories. If it was found

that the calorie intake was inaccurate due to the parent or child being unable to recall one or more meals the recall was considered unreliable. These recalls were excluded from analysis. Recalls were included in analysis when a child consumed less than usual due to illness.

Dietary sodium intake from 12 months to 5 years of age was averaged across ages. For analysis, participants were grouped by sodium intake above and below 1.9g/d based on US Dietary Reference Intakes (DRI) for children 4-8 years of age (DRI for children 4 - 8 years of age is < 1.9g Na/d) (47).

Database

The database includes average blood pressure at each age, maternal characteristics including age, BMI, DHA supplement intake, DHA status at enrollment and delivery, smoking status, race and weight gain in pregnancy; and data on the child including birthweight, diet from birth to 5.5 years of age, BMI percentile at 2-5 years of age, body composition at age 5, and sex. Treatment group was added by one of the study primary investigators in possession of the randomization data as the study is still masked to staff.

Statistical Analysis

First we looked at the effect of supplementation on blood pressure using an intent to treat analysis and a one tailed t-test. Then we examined a number of potential covariates that had been associated with blood pressure including maternal characteristics [race, BMI, gestational weight gain, DHA status (RBC phospholipid DHA wt %), smoking status] and child characteristics (BMI percentile, percent body fat at 5 years, sex, average sodium intake from 12 months-5.5 years, and length of breastfeeding) by calculating the mean and standard error of the mean for each variable. In addition, we performed one tail t-tests on several variables including race,

sodium intake, maternal smoking status, breastfeeding days, and child BMI percentile at 4 and 5 years. We also looked at body fat percentage at 5 years. From this we identified three variables that seemed to have the greatest impact on blood pressure: maternal race, child BMI percentile, and child sodium intake. Next we ran a correlation matrix and found relationships among those three variables. The statistical software SAS version 9.4 was used to run a PROC MIXED repeated measures ANOVA. PROC MIXED repeated measures were performed to allow for missing data since all participants were included that participated in blood pressure measurement at a minimum of one age time point. Several models were created: 1) model BP=maternal race, treatment and treatment by year; 2) model BP= Na intake >1.9g/d, treatment, and treatment by year 3) model BP = 5 year BMI percentile, treatment, and treatment by year 4) model BP= treatment, maternal race, gestation smoke, and treatment by year. Each model was run for SBP, DBP as well as SBP percentiles and DBP percentiles. Finally PROC frequency measures were performed to determine if there were any differences in the frequency of high sodium intake by race within treatment groups.

Chapter 4: Results

Our hypothesis was that DHA supplementation would lead to lower BP in childhood. Participant characteristics were similar between the placebo and DHA group. Maternal and child characteristics are given in Table 1. The total number of BP measurements at each age group is depicted in Figure 1.

Maternal LCPUFA status at enrollment and postpartum

Maternal LCPUFA (wt %) can be found in Table 2. DHA supplementation resulted in an increase in total n-3 LCPUFA (wt %) (p-value < 0.0001), specifically an increase in DHA (wt %) (p-value < 0.0001), as well as a decrease in both total n-6 LCPUFA (wt %) (p- value <0.0001) and total ratio of n-6: n-3 LCPUFA (p-value < 0.0001).

DHA Supplementation and Child Blood Pressure

The DHA group had lower average SBP at 4, 4.5 and 5 years, and also had lower average DBP at all ages (Table 3). The intervention lead to an average drop in SBP of 1.383 mm Hg (P-value = 0.031) and an average drop in DBP of 1.72 mm Hg (P-value = 0.0075) before controlling for other variables. Six percent of children in the placebo group and 3 % of children in the DHA group had pre-hypertensive BP (>90th percentile) (Table 4). In addition, 5 % of children in the placebo group and 2 % of children in the DHA group had hypertensive BP (>95th percentile) (Table 4).

BMI percentile at 5 years and Blood Pressure

We saw a significant effect of 5 year BMI percentile on DBP percent and DBP (P-values = 0.0379 and 0.0022 respectively). The same effect was observed for SBP percent and SBP as

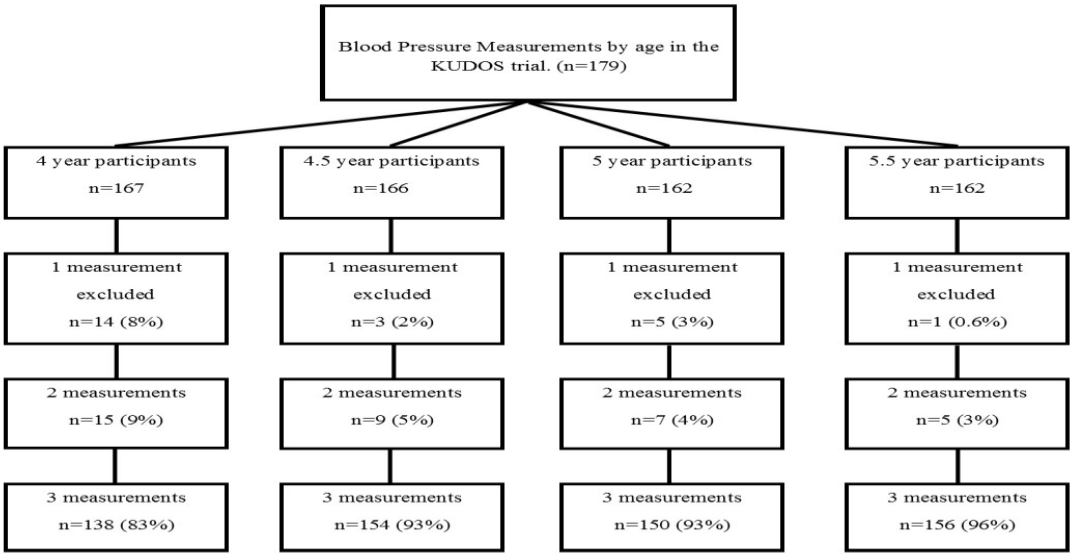
Table 1 Characteristics of mothers and their children participating in the KUDOS study (n=179)

	Value	
Maternal Characteristics	**Placebo group**	**DHA group**
Age at enrollment (y)	26 ± 4.8	26.4 ± 4.8
Gestational age at enrollment (wk)	15.0 ± 3.5	14.4 ± 3.5
Maternal BMI	25.3 ± 5.1	25.9 ± 5.0
Pregnancy weight gain (lbs)	35 ± 15.5	39 ± 15.1
Black, (n)	28	25
Not-black, (n)	58	68
Mother Hispanic, (n)	6	4
Ever smoked (%)	44	49
Preeclampsia, (n)	2	3
Total capsules taken (#)	427.5 ± 122.1	426.7 ± 112.0
Capsules (#/wk)	17.1 ± 4.0	17.5 ± 3.9
Breastfeeding (d)	190 ± 221.3	205.9 ± 245.5
Formula (d)	254.7 ± 161.9	279.7 ± 138.5
Additional DHA intake (n)	17	13
Additional DHA (mg)	221.7 ± 91.2	227.7 ± 80.6
Childhood Characteristics		
Males/Females (n)	48/38	41/52
2yr BMI percentile	61.6 ± 29.8	65.6 ± 24.0
3yr BMI percentile	60.9 ± 26.2	66.4 ± 26.6
4yr BMI percentile	62 ± 27.2	70.1 ±26.1
5yr BMI percentile	64.2 ± 26.2	69.6 ±25.0
5yr Fat Mass (%)	25.0 ± 6.5	24.8 ± 5.2
Sodium intake (mg/d)*	1977 ± 514.3	1901 ± 544.6
Birth weight (g)	3266 ± 522.3	3441 ± 485.1

* Sodium intake was calculated as the average across all ages studied (12mo -5years).

* Values represent mean ± SD

Figure 1: Blood Pressure Measurement Flow Chart



(%) = percentage of subjects that participated in any BP measurements at that age .

Table 2 Maternal RBC LCPUFA* concentrations at enrollment and postpartum in the Placebo and DHA groups of the KUDOS study (n=179)

RBC LCPUFA concentrations	**Placebo group**		**DHA group**	
	Enroll	**PP**	**Enroll**	**PP**
	FAs, wt %		FAs, wt %	
Total n-3 LCPUFAs	**6.4 ± 1.4**	**6.3 ± 1.3**	**6.3 ± 1.3**	**9.0 ± 2.1**
20:3n-3 Eicosatrienoic acid	0.1 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
20:5n-3Eicosapentaenoic acid	0.3 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
22:5n-3 Docasapentaenoic acid	1.6 ± 0.3	1.4 ± 0.3	1.5 ± 0.3	1.0 ± 0.3
22:6n-3 Docosahexaenoic acid	4.3 ± 1.2	4.6 ± 1.0	4.4 ± 1.1	7.6 ± 2.0
Total n-6 LCPUFAs	**20.9 ± 2.7**	**19.0 ± 2.5**	**20.9 ± 2.5**	**17.3 ± 2.8**
20:3n-6 DGLA	1.8 ± 0.5	1.8 ± 0.5	1.7 ± 0.6	1.7 ± 0.5
20:4n-6 Arachidonic acid	14.9 ± 2.3	13.2 ± 1.8	14.9 ± 1.9	12.4 ± 2.0
22:2n-6 Docosadienoic acid	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
22:4n-6 Docosatetraenoic acid	3.4 ± 0.7	3.1 ± 0.8	3.5 ± 0.8	2.5 ± 0.7
22:5n-6 Docosapentaenoate	0.8 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	0.6 ± 0.3
Total n-6:n-3 LCPUFA ratio	**3.3 ± 0.8**	**3.1 ± 0.6**	**3.4 ± 0.7**	**2.1 ± 0.7**

*LCPUFA: Long chain polyunsaturated fatty acids

**Values represent mean ± SD

Table 3 Average childhood blood pressure in control and DHA groups between ages 4 and 5.5y in the KUDOS study (n=179)

Blood Pressure	Age	Placebo (mm Hg)	DHA (mm Hg)	P-value
SBP	4y	99.8 ± 11.6	97.2 ±7.7	0.058
	4.5y	100.7 ± 9.7	99.1 ± 7.8	0.136
	5y	99.2 ± 7.5	97.0 ± 6.4	0.026
	5.5y	102.0 ± 10.9	102.7 ± 10	0.328
DBP	4y	61.2 ± 10.9	57.9 ± 6.6	0.016
	4.5y	62.6 ± 10.4	60.4 ± 5.5	0.053
	5y	58.4 ± 6.3	57.6 ± 5.2	0.196
	5.5y	62.5 ± 11.1	61.8 ± 10.5	0.339

* Values represent mean ± SD
SBP: Systolic Blood Pressure
DBP: Diastolic Blood Pressure

Table 4 Pre-hypertensive and Hypertensive blood pressure percentiles.

	Placebo		DHA	
Blood Pressure Percentile	>90%	>95%	>90%	>95%
4y SBP n (%)	5(7)	4(6)	4(5)	2(2)
4.5y SBP n (%)	5(6)	8(10)	4(5)	2(2)
5y SBP n (%)	3(4)	5(7)	2(2)	0(0)
5.5y SBP n (%)	5(7)	7(9)	6(7)	7(8)
4y DBP n (%)	7(10)	6(9)	2(2)	2(2)
4.5y DBP n (%)	7(9)	6(8)	3(4)	2(2)
5y DBP n (%)	4(5)	2(3)	1(1)	0(0)
5.5y DBP n (%)	7(9)	4(5)	3(4)	4(5)

*BP above the 90th percentile is considered pre-hypertension, and above the 95th percentile is considered hypertension in childhood.)

well (p-values = 0.0042 and 0.0003). Average BMI percentile for black and not-black subjects is reported in Table 5. Children in the placebo group with a BMI percentile > 85th percentile at 5 years had a significantly greater SBP at 4, 5 and 5.5 years and a significantly greater DBP at all ages when analyzed with a 1-tailed t-test. This relationship is depicted in Figure 2. We also looked at 4 year BMI percentile but a significant difference in blood pressure was not observed.

Maternal Race and Blood Pressure

We found a significant effect of race on DBP percentile (p-value = 0.0318) and a trend on DBP (p-value = 0.1108) after running a PROC Mixed repeated measures ANOVA. There was a similar trend with Race and SBP percentiles (p-value = 0.1573) and SBP (p-value = 0.0543). Figure 3 shows the relationships between race and blood pressure. In general, black children in the placebo group had significantly higher DBP than placebo white, DHA white, and DHA black children at ages 4y and 4.5y. SBP was higher in black children in the placebo group as well but this was not significant.

Average Sodium intake and Blood Pressure

We found no significant effect of average sodium intake on blood pressure. Consuming an average sodium intake above 1.9 g/day had no effect on DBP or DBP percentile (p-value = 0.3275 and 0.3356 respectively) or on SBP or SBP percentile (p-value =0.1140 and 0.4098 respectively). More black children in the placebo group consumed >1.9 g Na/day (64.3%) than black children in the DHA group (48%), however, the difference was not significant.

Table 5: Child Characteristics by Race from KUDOS study (n=179)

	Placebo		DHA	
Child Characteristics	**Black**	**Not-black**	**Black**	**Not-black**
Sample size, (n)	28	58	25	68
Sodium intake (mg/d)	2128 ± 481.7	1904.5 ± 517.7	2008.8 ± 862	1861.5 ± 367.7
Sodium >1.9g/d (%)	64.3	44.8	48	45.6
2yr BMI percentile	48.4 ± 23.4	68.4 ± 30.9	60.2 ± 24.5	67.1 ± 24
3yr BMI percentile	61.3 ± 22.5	60.6 ± 28.1	65.8 ± 29.6	66.6 ± 25.7
4yr BMI percentile	66.8 ± 24.6	59.7 ± 28.3	66.2 ± 28.8	71.6 ± 25.1
5yr BMI percentile	73.6 ± 21	59.5 ± 27.4	70.7 ± 26.3	69.1 ± 24.8
5yr Body Fat (%)	23.9 ± 7.2	25.5 ± 6.2	23.7 ± 6.9	25.1 ± 4.6
Breastfeeding (d)	100 ± 197.4	235 ± 220.4	81.1 ± 160.3	251.8 ± 256.2
Males/Females (n)	16/12	32/26	14/11	27/41
Smoking during pregnancy, (%)	46	31	36	26
Smoking during pregnancy, (d)	77.8 ± 118.3	35.6 ± 83.4	80.4 ± 122.9	35.1 ± 83.8
Birth weight (g)	3253.7 ± 426.8	3272.4 ± 566	3182.6 ± 533.3	3535.7 ± 432.6

Values represent means ± SD

*: p-value ≤ 0.05
†: p-value ≤ 0.1

Figure 2 Relationship between 5 year BMI percentile above the 85th percentile and blood pressure

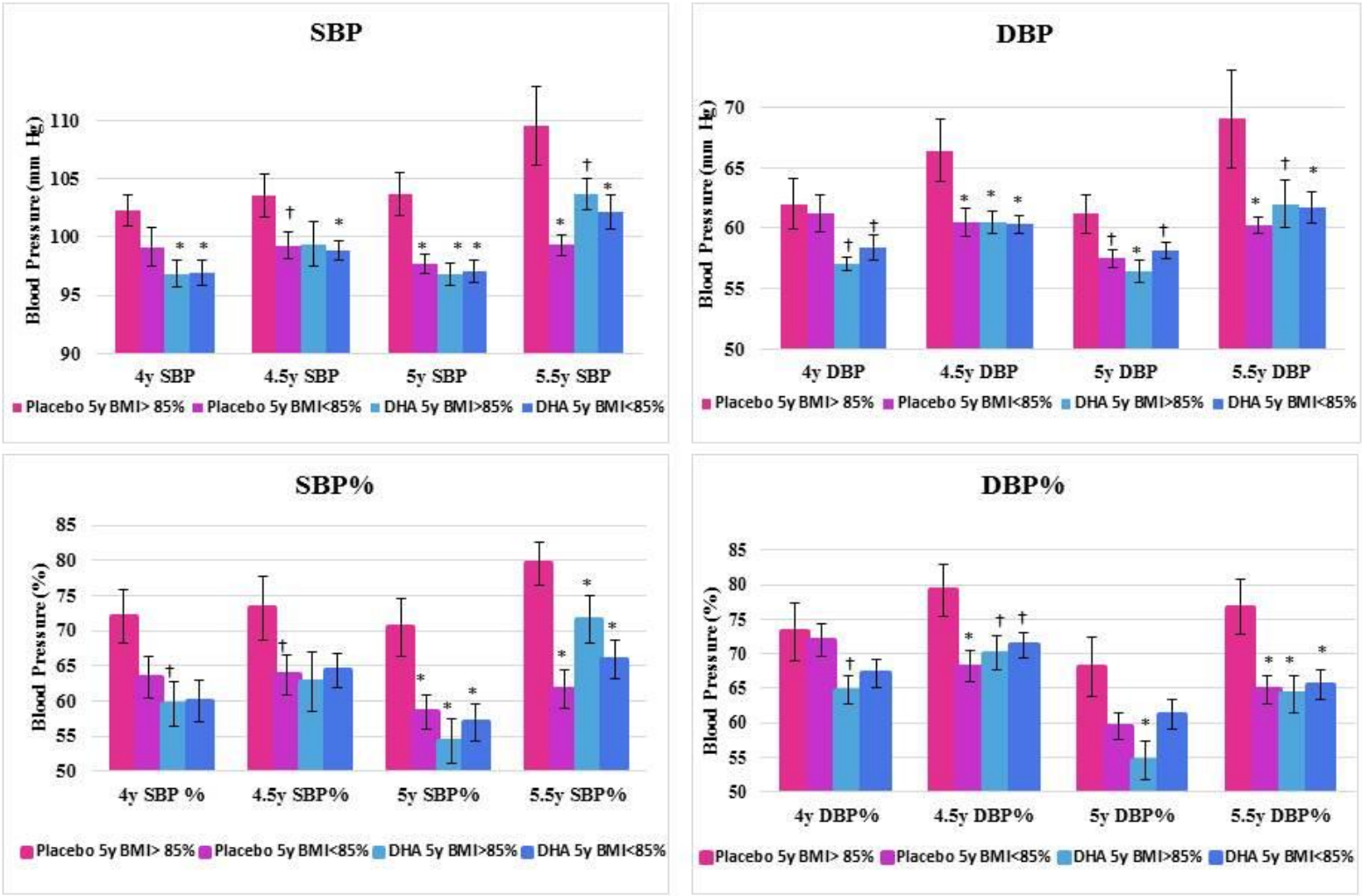
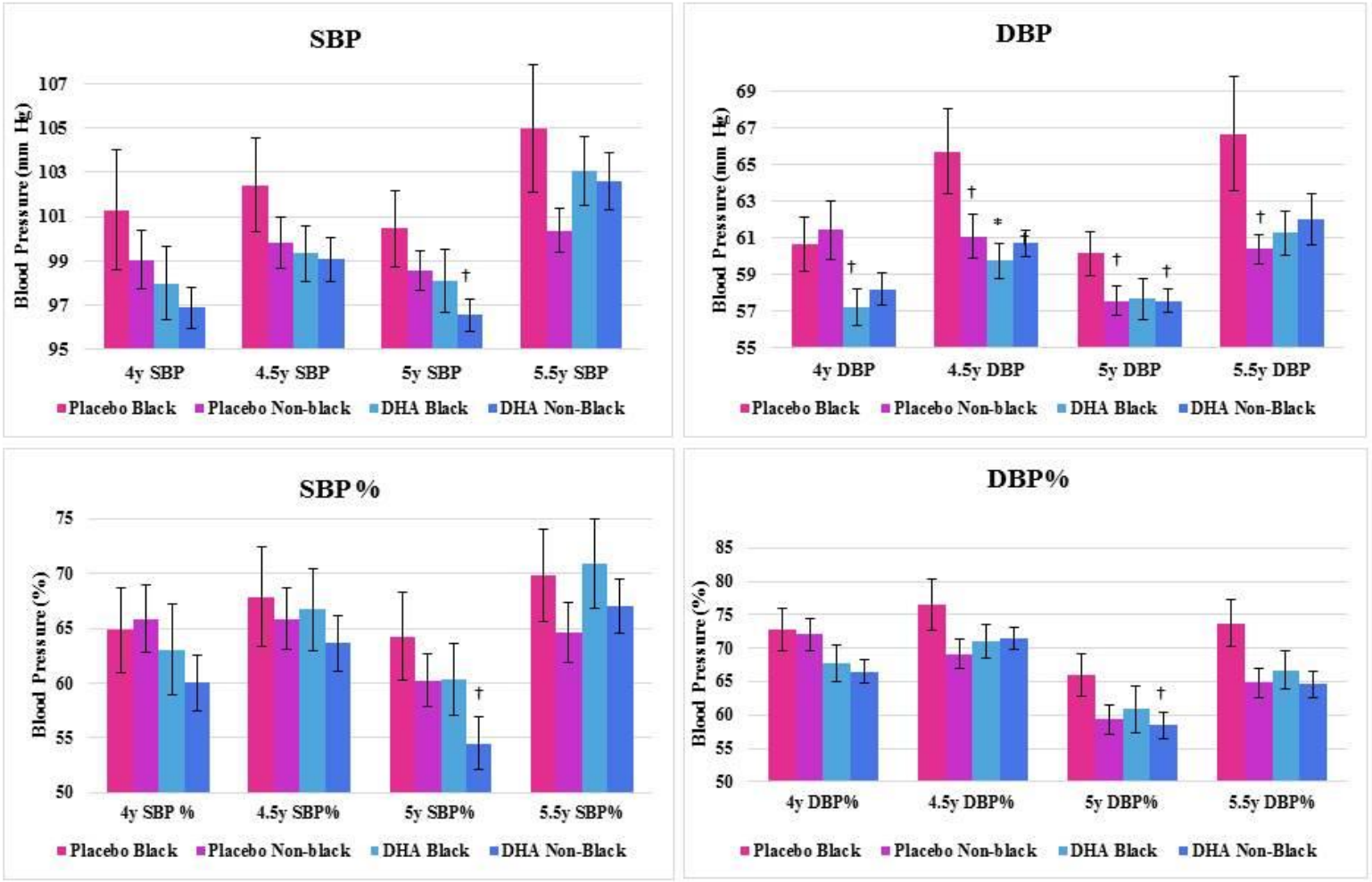


Figure 3 Relationships between maternal race and blood pressure.

*: p-value ≤ 0.05
†: p-value ≤ 0.1



Black children consumed >1.9 g Na/d than not-black children in the placebo group (64.3% vs 44.83%, p-value = 0.09087), but there was no difference in the proportion of children whose sodium intake was above 1.9 g/d between black and not-black children in the DHA group (45.59% >1.9 g Na/d not-black vs 48% >1.9 g Na/d black, p-value 0.8362).

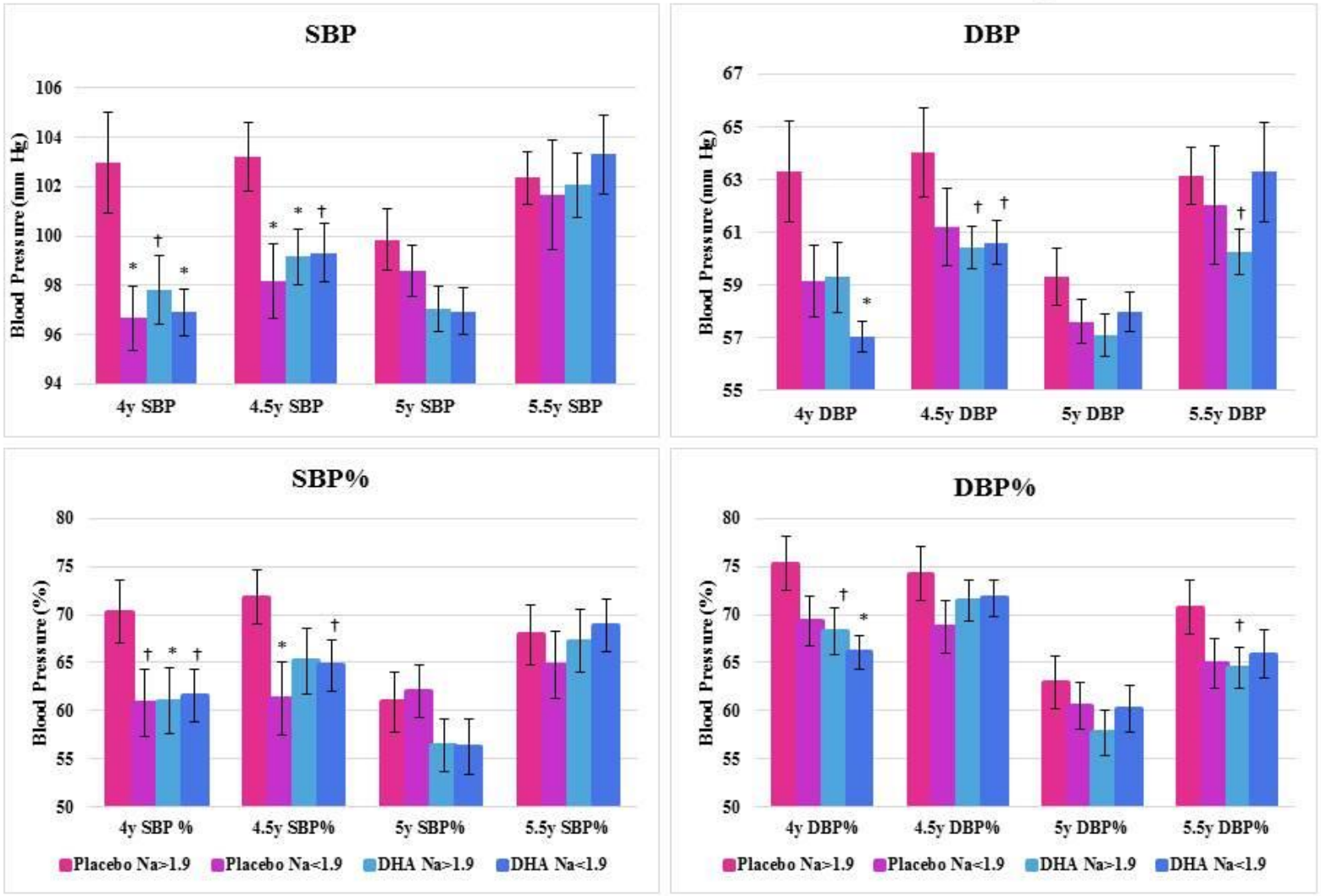
Figure 4 depicts the differences in average blood pressure between the two treatment groups based on average sodium intake above 1.9 g/d. Blood pressure of children who consumed on average more than 1.9 g/d sodium had significantly higher blood pressure than the placebo group that did not consume greater than 1.9 g sodium/ day and both DHA groups, whether they consume >1.9 g/d or not for SBP at ages 4, 4.5 and 5 years, and for DBP at 4.5 and 5.5y.

Differences in Child Characteristics by Race

We noted some differences in characteristics between treatment groups of the black children. First, 64% of black children in the placebo group consumed >1.9 g Na/day (mean = 2128 g/d) whereas in the DHA group, only 48% of black children consumed >1.9 g/d (mean = 2008.8 g/d). No difference in average sodium intake was seen in not-black children between the DHA and Placebo groups. In black children, BMI percentiles were similar in both placebo and DHA groups at 4 and 5 years, but in white children BMI percentiles were much higher in the DHA group at 4 and 5 years. In addition, not-black mothers breastfed twice as long as black mothers in both the DHA and placebo groups.

Figure 4 Relationship between sodium intake above 1.9g/d and blood pressure

*: p-value ≤ 0.05
†: p-value ≤ 0.1



Maternal Smoking and Blood Pressure

Smoking during pregnancy did not have significant impact on blood pressure in either group. Relationships between maternal smoking status during pregnancy and blood pressure can be seen in Figure 5.

Breastfeeding and Blood Pressure

We looked at the effect of breastfeeding days on blood pressure, and assessed differences in blood pressure based on breastfeeding for > 60 days and for >6 months. We found a negative correlation between length of breastfeeding and blood pressure at several time points, but this was not significant.

Body fat % at 5 years and Blood Pressure

The mean body fat percentage at 5 years of age was 24.8 ± 5.2 for the DHA group and 25.0 ± 6.5 for the placebo group. Using this information we looked at blood pressure of children with greater than and less than 25 percent body fat in each treatment group. At 5 years of age the children with body fat >25% in the placebo group had slightly higher blood pressure than the other 3 groups but this was not significant, and this was not seen at 5.5 years.

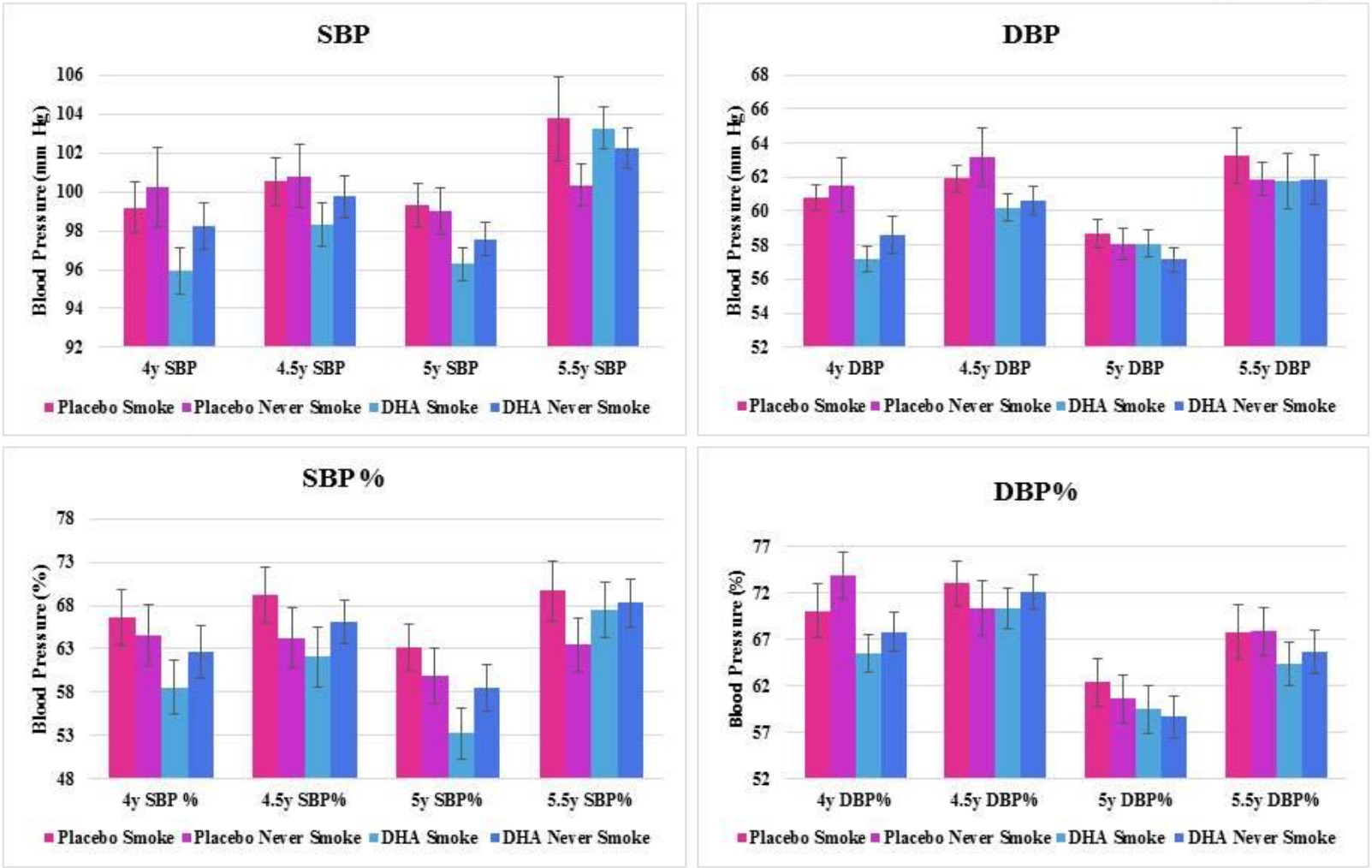
Table 6: Correlation Matrix

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*
1. 4yrSBP%	1.00																					
2. 4.5yrSBP%	**0.34**																					
3. 5yrSBP%	**0.43**	**0.34**																				
4. 5.5yrSBP%	**0.27**	**0.35**	**0.31**																			
5. 4yrDBP%	**0.46**	**0.22**	**0.21**	**0.14**																		
6. 4.5yrDBP%	**0.21**	**0.46**	**0.33**	**0.34**	**0.31**																	
7. 5yrDBP%	**0.16**	**0.20**	**0.40**	**0.17**	**0.17**	**0.27**																
8. 5.5yrDBP%	-0.03	**0.26**	0.13	**0.42**	0.08	**0.36**	**0.26**															
9. 4yrSBP	**0.84**	**0.23**	**0.29**	**0.21**	**0.47**	**0.23**	0.04	-0.04														
10. 4.5yrSBP	**0.25**	**0.87**	**0.29**	**0.30**	**0.24**	**0.47**	**0.15**	**0.22**	**0.26**													
11. 5yrSBP	**0.37**	**0.27**	**0.90**	**0.27**	**0.20**	**0.36**	**0.33**	0.10	**0.34**	**0.35**												
12. 5.5yrSBP	0.06	**0.23**	0.10	**0.78**	0.04	**0.29**	-0.02	**0.41**	0.11	**0.27**	**0.17**											
13. 4yrDBP	**0.45**	**0.16**	0.09	0.08	**0.82**	**0.18**	0.04	-0.02	**0.63**	**0.19**	0.12	0.04										
14. 4.5yrDBP	**0.17**	**0.42**	**0.29**	**0.27**	**0.28**	**0.80**	**0.22**	**0.28**	**0.31**	**0.55**	**0.38**	**0.24**	**0.31**									
15. 5yrDBP	**0.15**	**0.19**	**0.38**	0.14	0.12	**0.29**	**0.96**	**0.20**	0.07	**0.18**	**0.36**	0.01	0.05	**0.28**								
16. 5.5yrDBP	-0.14	**0.17**	-0.05	**0.37**	-0.11	**0.26**	0.02	**0.74**	-0.09	**0.17**	-0.01	**0.69**	-0.09	**0.22**	0.03							
17. PregDaysSmo	0.00	0.05	-0.02	**0.26**	-0.05	0.05	-0.01	0.06	-0.06	-0.03	-0.05	**0.24**	-0.05	-0.02	-0.04	0.10						
18. BFDays	-0.08	0.14	-0.08	-0.05	**-0.15**	0.02	0.00	-0.09	-0.03	0.12	-0.08	-0.08	-0.13	0.04	0.02	-0.07	**-0.31**					
19. 4yr_BMIageP	**0.17**	0.10	0.13	**0.16**	0.08	**0.19**	0.07	0.05	0.14	**0.16**	**0.16**	0.11	0.02	**0.23**	0.11	0.06	-0.01	0.02				
20. 5yr_BMIageP	0.13	0.11	0.12	**0.22**	0.11	**0.19**	0.04	0.06	0.14	**0.17**	**0.18**	**0.21**	0.07	**0.24**	0.09	0.12	-0.01	0.02	**0.88**			
21. Gender	0.01	-0.03	-0.02	-0.09	**-0.28**	-0.11	-0.03	-0.14	-0.09	**-0.18**	**-0.16**	**-0.16**	-0.06	-0.06	0.05	-0.03	-0.05	0.06	0.01	-0.03		
22. MotherRace	0.03	0.06	0.12	0.09	0.04	0.11	0.13	**0.16**	0.09	0.08	0.12	0.11	-0.03	0.12	0.11	0.13	**0.21**	**-0.30**	0.01	0.13	-0.09	
23. Avg Na intake	**0.19**	**0.15**	0.02	0.09	0.14	0.11	0.06	0.08	**0.22**	0.13	0.06	0.05	**0.17**	0.13	0.06	-0.02	**0.22**	**-0.23**	0.13	0.10	-0.13	**0.16**

R=0.147 is significant at p value 0.05; Gender: 0= Male, 1 = female; Maternal race: 0 = not black, 1 = black

Figure 5 Relationship between maternal smoking status during pregnancy and childhood blood pressure.

*: p-value ≤ 0.05
†: p-value ≤ 0.1



Correlations

Several other relationships between smoking during pregnancy, breastfeeding, and Na intake and BMI were found. There was a negative correlation between total breastfeeding days and smoking during pregnancy ($r=-0.311$, $p <0.00001$). We also found a positive correlation between days smoked during pregnancy and average sodium intake ($r = 0.224$, p-value = 0.001). In addition, we found a negative correlation between total breastfeeding days and average sodium intake ($r = -.232$, p-value = 0.0009). A positive though not significant correlation was also found between BMI and sodium intake at 4 ($r = 0.13$, p-value = 0.083) and 5 years of age ($r = 0.10$, p-value = 0.183). The results from the correlation matrix can be found in Table 6.

Chapter 5: Discussion

DHA Supplementation and Blood Pressure

In this study, we found that DHA supplementation during pregnancy led to lower SBP at 4, 4.5 and 5 years of age and lower DBP at all ages. This confirms our hypothesis that DHA supplementation would lead to lower blood pressure in childhood. Despite this lowering effect, we did not see an effect on SBP at 5.5 years of age. Overall, there was a lot of variability in the blood pressure measurements, and our analysis methods helped to reduce this variability, but not completely.

In our analysis we also looked at a number of factors that are known to be associated with BP including BMI, Na intake, race, breastfeeding, and maternal smoking status. What we found was a protective effect of DHA against higher SBP and DBP in children carrying these risk factors specifically BMI >85th percentile, black race and Na intake >1.9g/d (Figures 2, 3 and 4).

Maternal PUFA status and Blood Pressure

DHA supplementation resulted in a significant increase in maternal DHA postpartum, as well as a decrease in total PUFA n-6: n-3 ratio. Our findings that DHA supplementation leads to improved LCPUFA profiles in the mother, and lower SBP in the child confirms the observational study findings of Vidakovic et al. (40). This is only the second study to look at the impact of maternal LCPUFA status during pregnancy and its impact on blood pressure in childhood, and it is the first randomized control trial to assess the impact of DHA supplementation during pregnancy on childhood blood pressure. Our findings suggest that supplementing with DHA during pregnancy may cause an intrauterine programming effect that leads to lower BP in

childhood. This finding is important as hypertension and obesity in childhood is increasingly more common, and certain populations are at greater risk for these health issues than others.

5 year BMI percentile and Blood Pressure

Research has shown that being overweight or obese increases one's risk for hypertension. In our study we found that the BP of children in the placebo group whose BMI was above the 85th percentile (the marker for overweight and obesity in childhood) was significantly greater than children with BMI less than the 85^{th} percentile in both groups and the children in the DHA group with a BMI percentile greater than the 85^{th}. This finding was significant at 4.5, 5, and 5.5 years in both SBP and DBP. Overall, BMI percentile at 5 years was the most powerful variable that significantly predicted both SBP and DBP.

Race and Blood Pressure

Individuals of African descent are known to have a greater risk for hypertension in life. In our study we found that black children in the placebo group had the highest DBP among all the groups at all ages, and the highest SBP at 4.5, 5 and 5.5 years of age. However, we found that black children whose mothers received DHA had blood pressure that more closely resembled that of not-black children from the placebo group and from the DHA group. This difference with supplementation is suggestive of a possible protective effect of DHA on blood pressure, and it highlights a method of prevention that clinicians could use to lower the risk of hypertension in this race.

Sodium intake and Blood pressure

Sodium intake was higher among black children than among not-black children (mean: 2.1g/d vs 1.9g/d respectively). Like with race, the placebo group that consumed >1.9g Na/d had higher DBP and SBP at 4, 4.5, and 5 years of age and the difference was significant at 4, 4.5, and 5 years for SBP and it was significant at 4.5 and 5.5 years for DBP compared to the BP of children in the DHA group who consumed > 1.9 g/d. The blood pressure of children in the DHA group consuming more than 1.9 g Na/day more closely matched the blood pressure of children consuming less than 1.9g/d on average.

Maternal Smoking and Blood Pressure

Several studies have found a positive association between maternal smoking during pregnancy and childhood blood pressure (48), (31), (49), (50). However, Bergel et al. (51) found no association and our results are in agreement with Bergel et al. More research is needed in this area, and although we did not find an association between DHA supplementation and smoking this remains an interesting area of study.

Breastfeeding and Blood pressure

We found a negative but not significant correlation between breastfeeding days and BP. Our findings confirm the most recent studies as we did not find any significant differences in blood pressure between children that were breastfed greater than 6 months and children breastfed less than 6 months. One reason for this could be due to socioeconomic differences as well as differences in length of breastfeeding between black and not-black participants (Mean: 91 days vs 243 days respectively) since some research has shown that length of breastfeeding plays a role in blood pressure outcomes.

Implications of Findings

Our findings that maternal DHA supplementation led to lower blood pressure in childhood emphasizes the importance of DHA supplementation as a preventive measure for future disease. Our results suggest that n-3 LCPUFA supplementation in US pregnant women has a greater impact on childhood health than previously known. With the rise in childhood obesity rates, childhood blood pressure is also on the rise. Because DHA supplementation during pregnancy led to lower blood pressure in childhood especially in children exposed to risk factors for hypertension, this highlights the need for greater intake of DHA during pregnancy to protect against the risk for hypertension in childhood. Achieving optimal health and nutrition status during pregnancy is one approach to lowering risk for future disease.

Future Studies

More randomized control trials in this area are necessary to confirm our findings. Furthermore, studies are needed to determine what minimum dose of DHA is necessary to achieve this effect on BP in childhood. The dose used in this study (600 mg/d) is much higher than current doses found in prenatal vitamins (250 mg DHA is most common in prenatal supplements). The results of the study suggest that DHA may protect against risk factors for high blood pressure including high sodium intake, race, and obesity.

Limitations

One major limitation of the study is the method used to measure blood pressure. Because blood pressure was not a primary outcome, the method used was not as precise as methods used in previously conducted studies. Children were not asked to remain still for any period of time although they had been sitting for another test prior to blood pressure measurement and were wheeled to the assessment room. Furthermore, the measurements were taken successively with no rest time in between. Because blood pressure was not a primary outcome, the measurement method likely contributed to the variability in measurement data.

Another limitation of the study was that women who were already taking <300mg/d of DHA prior to the KUDOS study were not excluded from the study. In fact 17% of women in the placebo group and 10% of the women in the experimental group were taking DHA that was not given as part of the study. This may explain why we found higher correlations between some variables and the difference in DHA status between enrollment and delivery of the child when using an intent-to-treat analysis.

Conclusions

Overall, we found that BMI > 85th percentile, Na intake >1.9g/d and black race were significant risk factors for high BP, but from these relationships we cannot say what is the cause of higher blood pressure in the high risk groups. However, these relationships suggest that DHA protects against higher BP in the high risk groups. In conclusion, DHA supplementation during pregnancy leads to lower blood pressure in the offspring, and protects against well known risk factors for hypertension including race, high sodium intake, and BMI.

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