Spring 4-2011

PRESCHOOL CHILDREN'S WILLINGNESS TO TRY AND PREFERENCE FOR DOCOSAHEXAENOIC ACID OMEGA-3 FOODS

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by

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A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Interdepartmental Area of Nutrition

Under the Supervision of Professor Nancy M. Lewis

Lincoln, Nebraska

May, 2011

PRESCHOOL CHILDREN'S WILLINGNESS TO TRY AND PREFERENCE FOR DOCOSAHEXAENOIC ACID OMEGA-3 FOODS

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University of Nebraska, 2011

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During the preschool years, obesigenic eating habits are formed which are strongly associated with risk of developing chronic diseases later in life particularly cardiovascular disease. Docosahexaenoic acid (DHA) omega-3 fatty acid is a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development. Current intakes of DHA are below recommended levels. United States (US) Midwestern children may be particularly at risk for suboptimal intakes of DHA. However, foods fortified with life'sDHA^{TM}, a product of Martek Biosciences and omega-3 eggs may provide culturally acceptable sources of DHA to Midwestern children.

Very little is known about the consumption and awareness of DHA omega-3 of US Midwestern low socio-economic preschool children and their parents or preschoolers' willingness to try and prefer foods rich in DHA. Therefore, methodology was developed including the Eating Habits of Preschool Children questionnaire and a taste test experiment to fill this knowledge gap.

Children 3-5 years enrolled in a Midwestern Head Start/Early Childhood Toddler Educare (ExCITE) program were recruited for this study. Parents were asked to complete two questionnaires, one on their children's and their familiarity and awareness of

omega-3 foods and one on familial and child neophobia. The children participated in a taste test experiment in which their willingness to try 14 foods rich in DHA as well as their preference for these foods were recorded. T-tests and McNemar nonparametric tests as well as a correlation were conducted.

Forty-seven children and their parents participated. Of the fish listed, only tuna was classified as a typical food consumed. Awareness and perceived knowledge of omega-3 in general was more frequent than that of DHA. Children were willing to try most foods but least likely to try mackerel and sardines ($P < .05$). Children preferred fortified foods over fish and typical foods over novel foods ($P < .05$). Novel fish was the least palatable to the children. Children with reported greater food neophobia were less likely to try new foods offered ($P < .05$). DHA fortified foods typical of the Midwestern diet may be the most likely way to get US Midwestern children to consume adequate amounts of DHA for chronic disease prevention.

ACKNOWLEDGEMENTS

I sincerely wish to thank my advisor Dr. Nancy Lewis for seeing me through my doctorate. I appreciate your advice, support and wealth of experience. Thank you to my Doctoral Supervisory Committee: Dr. Kaye Stanek-Krogstrand for your nutrition and writing expertise, Dr. Kent Eskridge for your statistical expertise, and Dr. Julia Torquati for your early childhood expertise.

My heartfelt appreciation goes to Lincoln Public Schools: Pat Schmidt, the Head Start/ExCITE teachers, and the preschool students and their parents who worked with me at Arnold, Saratoga and West Lincoln elementary schools.

I wish to thank the Nutrition & Health Sciences Dept. Staff, the best staff on campus: Connie Wieser, Donna Hahn, Jeannie Pittam, and Lori Rausch for all their assistance and kindness. Thank you to my friends and laboratory partners especially the encouragement and camaraderie of Mili, Paula, Martha, Jennifer, Narissa, Melissa, and Kris.

Thank you to all my friends and family (old and new) who cheered me on as I moved, got married and finished a PhD. I couldn't have done it without you! Aunt Glory, Jess, Trista and Krissy thanks for encouraging me to go on and get my PhD. Thank you to the Murrays – Mom and Dad, Andrew, Laura & Felicity for your endless love, listening ears and prayers, as well as the prayers and support of my new family the Bettenhausens and Hedstroms. Thank you from the bottom of my heart to my best friend and husband Matt who spent many late nights in the den or on campus during our first months of marriage unconditionally loving and supporting me in every way – I love you Sweetheart. Finally, to my Lord and Savior Jesus for making this effort worthwhile; He's the reason I live.

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INTRODUCTION

Within the past 30 years, childhood obesity prevalence has doubled for preschool children aged 2-5 years (1). Childhood obesity is defined by the Centers for Disease Control and Prevention (CDC) as a Body Mass Index (BMI) at the 95th percentile or above as plotted on the CDC BMI-for-age growth charts (2). According to the National Health and Nutrition Examination Survey (NHANES), 12.4% of preschool children can be classified as obese (3). A disproportionate number of those with low socio-economic status (SES) are classified as overweight or obese (2, 4-5).

One of the biggest contributors to childhood obesity is diet (6). The establishment of eating patterns begins during the preschool years, aged 3-5 years (7-9). It is during these years that food behaviors such as food neophobia and food pickiness need to be overcome since both behaviors are strong predictors of future healthful eating habits (10). Food neophobia has been defined by Pliner and Hobden (1992) as "the reluctance to eat and/or avoidance of novel foods" (11). Children with food neophobia are unwilling to try new foods. Food pickiness, a similar yet distinct behavior, has been defined by Galloway, Lee, and Birch (2003) as the "unwillingness to eat many familiar foods" (9). Children with food pickiness have limited variety in their diets due to their strong preferences for only a limited number of foods.

During these key years, some children begin to gain excess weight which often predicts, by age five, their future weight patterns and thus obesity-associated risk (12). In addition, during these 3-5 age years, obesigenic eating habits are formed which are strongly associated with the risk of developing chronic diseases later in life (13). One

consequence of childhood obesity is that it places children at an increased risk of early adult death from cardiovascular disease, the leading cause of death in the United States (US) (14).

Docosahexaenoic acid (DHA) omega-3 fatty acid is a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development; it is a key nutrient in the preschool years (15). The consumption of DHA and DHA-rich omega-3 fish has a well-established preventative effect against the development of cardiovascular disease in adults (16). DHA may also have beneficial effects on depression, cognition, immune function, Attention Deficit Disorder, metabolic disorders and reproductive health, but studies are not conclusive (17-18). This fatty acid is considered to be conditionally essential because of the very low conversion rates in the human body from the long-chain alpha linolenic acid (ALA) form to the long chain DHA form (17).

Currently, there is not an age-specific amount of DHA recommended for preschool children; more research is needed (18). However, it is recommended that children two years and older consume 1-2% of their energy as omega-3 fatty acids, and 500-1000 mg of DHA and eicosapentanoic acid (EPA) per week in order to help protect them against future cardiovascular disease (19). Many researchers believe that dietary recommendations for children aged 2-12 years should parallel those of adult populations by the inclusion of 1-2 fatty fish meals every week or foods containing the combined DHA and EPA equivalent (18).

National consumption of DHA omega-3 is below recommended levels; the NHANES, 1999-2000 reported a mean intake of only 100 mg/day combined of DHA and EPA (20). Likewise, adequate consumption of DHA and EPA is of concern worldwide (21-22).

US Midwestern children do not typically consume foods rich in DHA omega-3 fatty acids. These fatty acids are found naturally primarily in fish, but they are also found in seafood such as shrimp, typically not foods commonly consumed in the Midwest (23-24). Although US Midwestern preschool children's intake of DHA has not been separately quantified, a study conducted with central inland Canadian children aged 4-7 years reported that 74% of the children had DHA intakes less than 30 mg/day (25). Concerns about methyl mercury contamination of fish and marine products leading to a decrease in the IQ of young children and other negative cognitive effects may further deter the US Midwestern population from eating DHA rich fish and feeding it to their young children (26).

Recently, some foods deemed more acceptable to Midwesterners have been fortified with DHA. Foods fortified with life'sDHA^{TM}, a product of Martek Biosciences Corporation, include a wide variety of foods ranging from tortillas to stick butter (27). These products are fortified with DHA produced from an algae source. Therefore, they do not have the "fishy" taste or smell unlike products fortified with DHA extracted from fish-based sources. Because they are completely plant-based, these products are also excellent alternative sources of DHA for all vegetarians including vegans. Furthermore, there is no risk of mercury contamination in contrast to fish and marine-derived sources of DHA with these products. DHA and EPA can safely be consumed up to 3 g/day as established by the US Food and Drug Administration, and consuming DHA from

fortified foods and beverages rather than supplements decreases the risk of unsafe levels of consumption (22). A list of the foods and beverages fortified with life'sDHA™ that can be found in US Midwestern city grocery stores is presented in Appendix A: Table 1: life'sDHA™ Omega-3 Fortified Foods. These fortified foods and beverages may provide an acceptable solution for low consumption of DHA in the Midwestern preschool population leading to the consumption of heart-healthy fats and decreasing the risk for obesity-related chronic disease.

Very little research has been conducted specifically on preschoolers' willingness to try and preference for foods rich in the DHA form of omega-3 fatty acids. Willingness to try foods rich in DHA (overcoming food neophobia) and the development of preferences for foods rich in DHA (overcoming food pickiness) are both important in the incorporation of these beneficial foods as a part of a culturally accepted diet. Accepted foods are foods which are considered to be "normal" and palatable to individuals. These are strongly tied to culture as well as individual food preferences based on taste, texture and appearance (28).

An experimental study was conducted with a low SES preschool population of US Midwestern Head Start/Early Childhood Toddler Educare (ExCITE) children for the primary purpose of exploring parental and child DHA and omega-3 exposure, reported child food neophobia, and child willingness to try and preference for specific foods rich in DHA omega-3 fatty acids.

This study had three primary objectives and two secondary objectives.

The ***primary objectives*** of this study were the following:

1. **To determine the willingness of Head Start/ExCITE preschoolers to try typical and novel foods rich in DHA omega-3 fatty acids.**
2. **To determine the preference of Head Start/ExCITE preschoolers for typical and novel foods rich in DHA.**
3. **To explore the relationship between willingness to try novel foods rich in DHA omega-3 fatty acids and reported child food neophobia.**

The ***secondary objectives*** of the study were the following:

1. **To describe the exposure of Head Start/ExCITE preschoolers to omega-3 foods, and to describe the exposure and awareness of the caregivers of Head Start/ExCITE preschoolers to omega-3 foods and information.**
2. **To describe potential willingness of US Midwestern families to accept DHA rich foods into their diets.**

The ***long-term goal*** of this study was to change the dietary eating habits of US Midwestern low SES children during the key preschool years to accept foods and beverages rich in DHA omega-3 fatty acids in order to reduce their risks of developing obesity-related cardiovascular disease as adults.

REVIEW OF LITERATURE

Food Preference Development

Many factors influence preschoolers' willingness to try and their preference for new foods. Leading researchers in the area of child development and food acceptance behaviors believe the development of food preferences and eating patterns results from a combination of genetic predispositions shaped by a multitude of environmental factors. Food neophobia tends to be related to child and maternal temperament (general neophobia) in contrast to food pickiness which is more related to environmental factors such as food exposure and variety (9).

Genetic factors

Research on the biological mechanisms which determine taste and lead to food preferences is still very limited compared to research on the environmental factors which influence children's food preferences. However, genetic research has determined the role of innate taste preferences, preference inheritability and the contributions of personality and age.

Innate taste preferences

Studies have shown that infants are genetically wired to prefer sweet tastes even without prior exposure (29). Contrarily, infants have predispositions to resist consuming foods which taste bitter or sour. Infants also seem to show a preference for salt, but this preference does not typically emerge until the age of four months (29).

Genetically-determined sensitivity to the bitter compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) classifies individuals into categories of supertasters, tasters and nontasters (30). Nontasters cannot taste these bitter compounds; they comprise about 30% of individuals (31), These individuals may be less particular about foods than their supertaster counterparts who are more sensitive to bitter and sour foods and may also be more sensitive to sweet tastes, spicy flavors, alcohol and fat textures (31). These genetically-determined sensitivities may lead to supertasters' avoidance of certain food items such as certain bitter-tasting fruits and vegetables (31-34). Many of the avoided items are rich in bitter tasting chronic-disease preventative phytochemical compounds including polyphenols, isoflavones, flavonoids and glucosinolates (30).

Mennella et al. (2005) examined the genetic and cultural connections to child and adult preferences for sweet and bitter tasting foods (35). Cheek cell genomic DNA was extracted from mothers and their children aged 5-10 years (n = 143) to group participants by alleles of TAS2R38, a taste gene which determines sensitivity to bitter tastes from the compound PROP and sweet tastes from compounds such as sucrose. Sensitivity to PROP was determined by having the participants classify samples with various concentrations of PROP as "yucky" or as tasting like water. The participants' facial expressions were also recorded. Participants were then asked to pick between samples containing various amounts of sucrose to determine their sweet sensitivity. Food preferences were obtained by asking the participants to name their favorite cereals and beverages. In addition, the mothers completed a child temperament questionnaire.

Results indicated that alleles of TAS2R38 did influence bitter taste perception in children and adults and preferences for sweet foods and beverages in children. Participating children with bitter-sensitizing alleles of TAS2R38 had stronger preferences for higher concentrations of sucrose, reported liking cereals with higher concentrations of sugar, and were less likely to list milk or water as favored beverages than children with bitter-insensitive alleles. Adult preference for sweet foods seemed to have a stronger cultural relationship particularly race and ethnicity than a genetic association (35). This finding is supports other studies that report that ethnicity, age and sex are all linked to taste perception (30).

Leann Birch, a pioneer in child feeding development research provided a comprehensive refutation of the "wisdom of the body" theory which has been widely circulated, but has not been supported by research. The premise of the "wisdom of the body" theory is that the body will innately crave foods rich in the nutrients for which it is lacking. Therefore, by encouraging children to eat what they crave, parents will be providing them with a body-regulated, appropriate diet. Birch maintained that not only is this theory unfounded, scientific studies have shown that left to their own preferences, children select obesigenic foods, foods high in total calories, fat, sugar and salt when healthful and non-healthful choices are provided (28).

Heritability

Food preference inclinations are partially inherited. Breen et al. (2006) used a twin approach to determine the heritability of food preferences. The researchers recruited families with same-sex preschool twins ages 4-5 years old (n = 214) (36). Approximately half of the twins were monozygotic (MZ, 103) and half were dizygotic (DZ, 111). The

mothers of the children were given food frequency questionnaires (FFQ)s and asked to rank their children's likes and dislikes. The food frequency questionnaires included 95 foods which could be grouped into four categories: vegetables, fruits, desserts, meat and fish. The study excluded 18 foods because they were familiar to fewer than 25% of the children (36).

The mean liking scores for each of the four food groups were compiled for the MZ twins as well as for the DZ twins. Then genetic model fitting techniques were used to generate correlations coefficients and confidence intervals for each of the four food groups. Shared environments versus non-shared environments were included in the analysis. The researchers reported stronger correlations for MZ twins than DZ twins for nearly all foods (72 out of 77). Heritability was more detectable with food groupings. The study found that heritability was highest for the meat and fish grouping and modest for the other three food groups. Shared environments most influenced the desserts, fruits and vegetable groups. As would be expected, non-shared environments had little effect on food preferences (36).

Personality

Certain personality-related factors influence children's willingness to try and preference for new foods. In fact, questions related to children's reactions to new foods have been included in instruments developed to assess children's temperaments for many years. The results of a study conducted by Rowe and Plomin (1977) with twin children aged five months to nine years (n = 182) indicated that reactions to food are significantly associated with a child's emotionality (37). This finding was further confirmed by a study conducted by Pliner and Loewen (1997) which indicated that there is a positive

correlation between food neophobia and the temperament components of emotionality, shyness, and negative reactions to food in children aged 5-11 years (n = 81 sibling pairs) (38).

Food neophobia has been positively associated with children who are more anxious or neurotic (9). In addition, food neophobia occurs less often in children who tend to be more adventurous in other areas of life. Children (n = 72) aged 8-11 years who expected to dislike novel foods were less likely to favorably rate these foods when they had tried them in contrast to children who expected to like the novel foods (39). This study indicated that the reluctance to try new foods based on a negative perception of the foods may itself become a barrier to children's liking the foods even if they are favorably rated by other children (39). Studies on the influence of gender on children's willingness to try and accept new foods remain inconclusive (40).

Age

Food variety, defined as the number of different kinds of foods consumed, has been shown to correlate with child age. Variety is limited during the first 4-6 months of life with exclusive breast milk or infant formula feeding. Variety then increases up to the age of three years, when studies show that children tend to have a slight decrease in their variety. At the age of three, preschool children are most likely to be neophobic toward foods (8).

Two articles published in 2005 examined the role of age in food selection from the ages of 2-3 years (41-42). Both studies used data from a pool of 456 children who were attending a nursery for hospital employees in Dijon, France. The children's food selections between a variety of eight dishes and their consumption intakes were recorded

by trained research assistants. The estimated food variety and energy intake was then calculated for each child. In addition, data were collected on the children's duration of breastfeeding when they were infants, and the children's present BMIs were calculated. A follow-up was conducted with the some of the participants (n = 339) 1-19 years after the initial study to measure variety seeking and food neophobia using a food neophobia questionnaire (scores of 10-70) adapted from the Food Neophobia Scale (11), the Food Attitude Survey (43) and a prior adaptation of the Food Neophobia Scale (44). The conclusion from the follow-up was that food variety prior to age four influences food variety later in life and is tied to food neophobia (41-42).

Although the children varied widely in their individual food choices, the researchers reported that overall, energy intake increased from age two to age three; however, food variety decreased. This finding is consistent with other studies that have shown there is an increase in pickiness and neophobia from age two to age three. The studies also indicated that during this three year old age, preschoolers tend to favor particular food groups and preparations. The authors' found that the data used for these two studies pointed to the children's overall preference for animal products (sausages, dry sausages, muscle meat, warm fish, offal and eggs), starchy foods (hot starchy dishes, bread and dry beans), and combination dishes (usually composed of animal products and starchy foods). Cream cheese and yogurts were selected more often than spreadable or mature cheeses. In addition, during this age, the vegetable group was the least popular and most avoided (41-42).

The classification of children as "picky eaters" begins early in the child/parent relationship. The results of the Feeding Infants and Toddlers Study (FIT), a large cross-

sectional study involving 3,022 infants and toddlers, indicated that caregivers begin to identify some of their children as “picky eaters” as young as four months old (45). By 24 months of age, half of the children were identified as “picky eaters” by their caregivers. Pickiness was positively associated with older children (age two) and lower weight for height percentiles. The food groups most avoided were the vegetable and meat groups. The study found that regardless of pickiness, all children within the study met their basic recommended nutritional needs (45).

The study also reported that the majority of caregivers offered their children new foods 3-5 times before giving up on their infant or child eating that food. A quarter of the caregivers reported only offering new foods 1-2 times. These findings show the need for further food introduction education because studies have reported that it may take 8-15 exposures for a child to accept a new food (45).

Early childhood eating patterns influence older children’s reported dietary variety (46). An eight year longitudinal study examined infant and toddler eating patterns to determine if these patterns were predictive of grade school dietary variety. The study followed a sample of children (n = 70) from birth to age eight. Food records collected by a trained interviewer were obtained frequently during the first three years of life and then biannually up to age eight (46). The study concluded that early food experiences from birth to age two have an effect on later school-age dietary variety, although variables may differ for each food group. The results indicated the only significant influence on school-age vegetable variety was maternal vegetable preferences. The authors noted that this finding supports existing studies on maternal preference as an influencer for foods

including milk, types of fat and dietary restrictions. Fruit variety and exposure during infancy significantly predicted school-age dietary fruit variety (46).

Environmental factors

There have been quite a few studies conducted to examine the influence of environmental factors on the development of food preferences in preschool children. Contributing environmental factors include the following: prenatal diet, breastfeeding duration, parenting style, preschool and daycare programs, feeding strategies, adult modeling and advertising.

Prenatal diet

There is evidence that the development of food preferences and therefore, the initial formation of eating patterns begin in the womb prior to birth (47). It has been established that fetuses develop their sense of taste and smell prior to delivery. Several studies have reported that certain spices and flavors such as cumin, curry, and garlic found in the maternal diet are detectable in the maternal amniotic fluid (48-49). This fluid is swallowed regularly by the fetus throughout the pregnancy. Therefore, it is hypothesized that babies begin to form preferences based on environmental exposure to certain flavors prior to birth. Research on this prenatal taste development is still limited; however, a study conducted in 2001 reported that infants whose mothers drank large amounts of carrot juice during their last trimester responded more favorably to carrot flavored cereal postnatally than the control group (50).

Breastfeeding duration

The American Academy of Pediatrics recommends exclusively breastfeeding babies throughout the first six months of infancy (51). During this time period, breastfed infants are exposed to a range of flavors determined by the maternal diet. In her review of parental influence on eating patterns during infancy, Savage notes that some specific flavors evident in breast milk include vanilla, alcohol, carrot juice and garlic (47). This flavor exposure may further contribute to food preference development and the later acceptance of dietary variety. Prolonged breastfeeding (the recommended six months) has been shown to have a significant positive relationship with school-aged increased dietary variety and a significant negative relationship with child pickiness and food neophobia (9, 46).

In addition, studies suggest that breastfed infants may be at a lower risk for developing obesity later in life. One study involving well-educated, middle-class mothers (n = 24) and healthy, non-Hispanic 12-13 month old infants (n = 31) examined the relationships between breastfeeding, maternal control and toddler energy balance (52). The study's findings supported the hypothesis that mothers who breastfeed are less controlling of their infants' later feeding practices from 12-18 months. This decreased maternal control was positively associated with appropriate energy intakes in their children leading to appropriate child body mass. The authors hypothesized that breastfeeding tends to encourage appropriate toddler energy intakes by increasing shared feeding cues and regulation between infants and mothers. Furthermore, they hypothesized that toddlers who are breastfed may be more sensitive to their internal hunger cues since their intakes

were less regulated earlier in infancy. The study reported that although breastfed toddlers tended to have higher energy intakes, they tended to be the tallest and leanest (52).

Parenting style

Parenting style classification has been shown to predict preschoolers' food preferences. For example, a study conducted with African American and Hispanic caregivers (n = 231) who had at least one 3-5 year old child enrolled in Head Start examined the connection between preschool food consumption and parenting style (53). The caregivers completed a questionnaire to determine their primary parenting style: authoritative versus authoritarian. Parents who provided a variety of healthful foods to their children and then allowed the children to make choices from these foods were classified as authoritative. Parents who strongly controlled eating through the restriction of some foods and the force-feeding of others were classified as authoritarian. Additionally, the caregivers answered questions which addressed how often the children were served dairy, fruits, and vegetables; how often these foods were encouraged or pushed; how often the children consumed these foods; and the caregivers' and children's BMIs. Results indicated that authoritative parenting was positively associated with healthful eating behaviors including access to fruits and vegetables; parental encouragement to eat dairy, fruits and vegetables; and the preschoolers' consumption of these food groups. In contrast, preschoolers with authoritarian parents tended to eat fewer vegetables than their counterparts (53).

Preschool and daycare programs

Although parental influence seems to be most strongly correlated with food preference development and eating habit maintenance, the contribution of preschool and daycare programs such as the Head Start Program on food preference development should not be overlooked. The enrollment of preschoolers in these programs is increasing nationally. A review conducted by Savage, Fisher, and Birch (2007) noted that the current generation of preschool children is living in a culture where both parents are increasingly working outside the home (47). Just under half of these children will be placed in daycare, and many of these children will be served restaurant or fast foods in much greater amounts than previous generations (47).

The US Census Bureau estimates that one third of preschool children in the US receive at least one meal provided from a non-parental caregiver, such as a daycare, each day (54). In 2002, the US Census Bureau found that the average US preschool child spends an average of 32 hours each week in child care (55). In addition, it is calculated that only approximately half of parents eat breakfast with their preschool child (56). Therefore, it is important for both parents and childcare workers to carefully monitor the foods they are providing the preschool children under their care. There is a growing need for childcare workers to be trained alongside parents to ensure their knowledge and practice of feeding behaviors and appropriate mealtime modeling encourages preschool children's healthful food preference development.

Feeding strategies

Studies have shown that specific feeding strategies used by parents and childcare providers also influence the food preferences of preschool children. Specific parent feeding strategies can be counter-productive to encouraging the adoption of healthful eating patterns. For example, a study conducted by Fisher and Birch (1999) compared preschoolers' preference for and selection of snack foods under two conditions: food restriction or food access (57). The preschool children were given a control food ad libitum during snack time, while a restricted food (neither preferable nor non-preferable) was sitting in the center of their table in a sealed, transparent container. The children were given access to the restricted food for a short period of time. The children showed a preference for the restricted food and consumed more of that food than they had prior to the experiment and more than the ad libitum food provided during the experiment. This study cautions parents and caregivers against emphasizing food restriction because this well-intentioned feeding practice may increase the food's desirability and thus consumption (57).

Two similar studies also confirm the benefits of providing exposure and access to healthful foods to children rather than using a pressure or reward feeding strategy. A study conducted with five year old girls (n = 191) reported that the consumption of fruits and vegetables was highest in girls whose parents modeled the behavior and lowest in girls whose parents tried to pressure them to eat more of these food groups (58). Likewise, a study conducted with 5-7 year old children in the United Kingdom reported that children (n = 49) repeatedly exposed to an unfamiliar vegetable (sweet red pepper)

were more prone to like and consume the vegetable over children who were rewarded if they would try the vegetable (59).

Childcare feeding strategies which employ an intervention approach have been shown to be effective in changing preschoolers' eating patterns. For example, a pilot study conducted with Head Start preschool children (n = 46) assessed the effectiveness of a food neophobia intervention (60). There were three main parts to the 12-week intervention: child participation in a social marketing nutrition education program entitled The Food Friends: Making New Foods Fun for Kids, increased exposure to a variety of novel foods, and repeated exposure to specific indicator foods. All children were given the opportunity to try novel foods three times per week throughout the program in order to increase their exposure to a variety of new foods. In addition, these children were given the same two novel indicator foods (Gouda cheese and Daikon radish) weekly throughout the 12 weeks of the intervention in order to repeatedly expose them to specific new foods. Foods were introduced to the children during the lunch meal or snack times.

Children in the experimental group participated in the Food Friends program, which included weekly teacher-led activities, story-books, a puzzle, a theme song, and a memory game all encouraging the children to try new foods. Seven food characters were developed to increase the children's enthusiasm for the program. Parents of children in the experimental group were sent newsletters which reinforced the program's message (60).

The children's reduction of food neophobia was measured by comparing the change in the children's willingness to try and consume novel foods offered as well as the children's rankings of the novel and familiar foods from pre-assessment to post-

assessment and again at a follow-up assessment completed 10 days post-intervention. Children were asked to rank nine foods (five familiar and four novel foods) using three cartoon faces: smiling, neutral and frowning. Any eating behaviors exhibited during the taste test were recorded (60).

Results indicated that the children in the experimental group had an increased preference for the Daikon radish indicator food signifying that the social marketing nutrition education strategy enhanced the acceptability of this food. Both groups of children showed an increased preference for the Gouda cheese indicator food with repeated exposure. Likewise, refusals to try new foods decreased significantly in both groups with time, and the children tended to rate unfamiliar foods more favorably with increased variety exposure. These findings indicate that the social marketing strategy paired with the food interventions was the most effective for increasing acceptance of novel foods. However, the food intervention strategies (increasing variety and repeated exposure) being used were also significantly effective in reducing food neophobia by themselves (60).

Adult modeling

Multiple studies have indicated that adult modeling is a strong influencer on preschoolers' food preference development. For example, in a United Kingdom fruit and vegetable consumption study, the strongest predictor for increasing fruit and vegetable consumption among children aged 2-6 years was parental fruit and vegetable consumption (61). The children's level of food neophobia was a secondary prediction. A study which recruited the primary caregivers of children aged 2-5 years (n = 434) reported that both the children's consumption of and preference for vegetables was

predicted by parental consumption of the same foods and eating homemade foods (62). From these and other studies, it can be inferred that appropriate parental modeling and control are essential in both helping children overcome their fear of new foods and increasing their consumption of fruits and vegetables.

Adessi et al. (2005) examined the influence of adult role-modeling on two to five year old children's willingness to try and accept new foods (63). Preschool children (n = 27) were divided into three experimental groups. Foods were offered prior to lunch as a snack to the children separate from their classmates and given the new foods to try with a familiar adult model. The foods the children were served was semolina with cumin and yellow dye, semolina with caper paste and green dye, and semolina with anchovy paste and red dye. The children were served 100 g of one of these three new foods in a clear cup (63).

At each of the food trials, what the adult models ate or did not eat was an experimental variable. On one of the trials, the adult models ate semolina dyed with the same color as that given to the child; on another trial, the adult models ate semolina dyed with a different color from that given to the child, and on third trial, the adult models did not eat anything with the children. The three experimental groups differed in the order of which colors and flavors were served with which adult model behavior. The children's behaviors were videotaped and their leftover food was weighed in grams (63).

Statistical analyses were conducted to compare the three experimental groups, the children's behavior during the three separate trials and the three different colored foods. Also, analysis was completed to determine whether factors such as age or classroom affected the children's willingness to try or consume the new foods. The researchers

reported that the children were most willing to try new foods when the adult models ate the same colored food. There was no significant difference between the children's willingness to try and consume the new foods when the adult models ate a different colored food or did not eat any food at all. Children were more likely to eat more of the new food in the second and third trials regardless of experimental condition. The authors' attributed this increased consumption as a response to the repeated exposure of the food; by the third trial, the food was more familiar to the children. No statistical significance was found for the children's preference for any one particular color of food, age, or classroom (63).

Galloway, Lee and Birch (2003) examined the effects of food pickiness and food neophobia on vegetable consumption in seven year old girls (n = 192) (9). The girls' food neophobia was measured using a combination of neophobia questionnaires. The girls' food pickiness was assessed through a questionnaire given to the girls' parents that assessed parental attitudes and perceptions about the girls' willingness to eat various familiar foods during mealtimes. The girls' vegetable intake was estimated based on three 24-hour food recalls (one weekend and two weekdays picked at random). The study measured some typical predictors of food neophobia and pickiness including child anxiety scores, parental food neophobia, maternal vegetable intake, and maternal food attitudes about having enough time to eat healthfully.

Results indicated the girls' and mothers' vegetable consumptions were well below the recommended amounts. However, girls who had food neophobia, food pickiness or both behaviors consumed significantly fewer servings of vegetables than the other girls who did not have these food behaviors. Maternal (but not paternal) neophobia was associated

with child neophobia. Likewise, maternal vegetable pickiness and perception that healthful foods take too long to prepare were predictors of child pickiness. Girls who were more anxious tended to have more food neophobia (9).

The positive correlation between maternal and sibling food neophobia with children's food neophobia is inconclusive in contrast to food pickiness since food neophobia has a stronger link to personality. Pliner and Loewen (1997) conducted a food neophobia study of 81 sibling pairs aged 5-11 years and their mothers (38). Siblings were individually asked to select foods they would like to try out of a list of 20 novel and familiar foods and five additional foods reported to be well-liked by most children. The children's behavioral neophobia was quantified by calculating a ratio based on the number of novel foods each child selected divided by the number of familiar foods selected. The children were then asked to try and rate the foods using a five face hedonistic scale. After the initial behavioral neophobia task, the children were asked to taste and rate six foods that were not a part of the initial taste test. These foods were divided into good tasting familiar foods, good tasting novel foods and bad-tasting foods (38).

The mothers of the children completed five separate questionnaires. First they completed the Parent's Willingness to Taste questionnaire to assess their food neophobia of the same foods their children were being offered. They then completed questionnaires designed to assess their temperament as well as the temperaments of each of their children. They also filled out a Food Neophobia Scale for themselves and their children (38).

Results indicated that food neophobia was strongly correlated with temperament. While maternal and child food neophobia were correlated when assessed using the data

from the Food Neophobia Scale questionnaires, familial food neophobia was not indicated with the behavioral data. Therefore, maternal reporting of child food neophobia may not be wholly accurate, and behavioral taste testing may still be warranted (38). Parents may also be less likely to predict the willingness of older elementary school children aged 7-12 years than using self-report child neophobia instruments such as the Food Situation Questionnaire with the children themselves (7).

Results of an early study conducted by Pliner (1994) indicated that parents tended to be particularly neophobic of foods of animal origin compared to their children who did not have this food categorical neophobic behavior (64). This finding may further indicate the role of cultural norms and exposure influencing willingness to try specific foods deemed culturally less desirable.

Advertising

There has been much debate over the past 30 years on the contribution of televised food advertisements (ads) to child eating patterns and the current childhood obesity epidemic (65). The Federal Trade Commission (FTC) estimates that in 2004 children aged 2-11 years viewed 75% of all food ads on television, viewing 38.5 hours containing 5,538 food ads (66). Several comprehensive content analyses have been conducted on television ads commonly viewed by 2-11 year old children. These analyses have indicated that most food ads directed to children are high in fat, sugar, sodium and low in nutrients (66-68).

Children begin to recognize and prefer advertised brands as young as 2-3 years old particularly for fast food chains and Coca Cola (69). A study conducted with preschool

children aged 2-6 years (n = 46) from a Head Start program examined the effect of television food commercials on children's food preferences (70). The children were shown a 30 minute video of age-appropriate television programming either with or without interspersed 30 second food commercials ranging from brand-name juice to fast-food chicken. The study found that the preschool children who watched the programming with the food commercials were more likely to show a preference for the advertised products than the control group (70).

Likewise, a study conducted with Head Start children (n = 40) aged 4-6 years reported that the children were more likely to rate the taste of identical food items (carrots, graham crackers and gummy snacks) more favorably when a licensed popular cartoon character was on the package (71). This indicates the influence of food packaging on young children's taste perception and thus food preference and selection.

These preferences may be further strengthened when combined with the influence of peers (72). Four to six year old overweight children seem to be particularly influenced by food branding over their healthful weight counterparts (73). There is evidence that televised food ads increase children's requests for the advertised products which may lead to increased purchase and consumption of these unhealthful foods (74-76). Furthermore, research has reported that children will eat more of the advertised foods when these foods are available (77-79). There is preliminary evidence that there may be a direct correlation between children viewing unhealthful food ads and their body weight (80). Although the research cited supports the television food ad-childhood obesity link, it should be noted that the FTC reported that televised food ads in 2004 contained the

same nutrient-poor products and messages as 1977 ads which were televised prior to the obesity epidemic, and food ads have decreased 9% during that time period (66).

Health Implications of Food Preference Development

The health implications of early food preference development are both immediate and long-term. Food preferences and eating patterns fluctuate throughout life, and healthful eating patterns can be learned later in life. However, establishing healthful eating patterns starting from birth not only immediately gives the infant and young child a nutrient-rich diet, but they also create fewer unhealthful habits which may be difficult to unlearn later in life. The early development of healthful eating patterns has implications for food group and nutrient balance and for the prevention of chronic diseases such as obesity and cardiovascular disease.

Food group and nutrient balance

Preschool children identified as "picky eaters" have diets that favor a limited number of food groups, which may lead to nutrient imbalances. A large study conducted in Quebec, Canada examined the change in eating behaviors of preschool children (n = 2,103) at age 2.5, 3.5, and 4.5 years old (81). The researchers analyzed data collected from 24 hour food recall interviews from the Longitudinal Study of Child Development. They reported that overall, preschool children who were identified as "picky eaters" consumed less fat, energy and protein than their less particular counterparts. In addition, these children had sub-optimal intakes of fruits, vegetables, meat and other meat alternative foods (80).

Obesity

According to the American Dietetic Association's Committee on the Prevention of Obesity in Children and Youth's Executive Summary, within the past 30 years childhood obesity prevalence has doubled for preschool children, aged 2-5 years (1). A disproportionate number of those overweight and obese children come from low SES families (5).

One of the biggest contributors to childhood obesity is diet (6). Inverse relationships have been found between the early consumption of specific healthful foods including fruits, vegetables and milk and childhood obesity (46, 61). Skinner et al. reported that children tend to maintain the same calcium intake over time stressing the importance of beginning healthful eating patterns as early as possible (46).

The 2002 Feeding Infants and Toddlers Study reported that as the toddlers' transitioned to table foods at 19 to 24 months of age, a quarter of the children were consuming high-calorie, high-fat, salty foods such as potato chips on a daily basis (82). Polyunsaturated fat intake has been negatively associated with obesity, and total and saturated fat have been positively associated with obesity (46). These findings stress the importance of preschool children consuming foods with the right kinds of fat.

While energy dense foods may factor into a preschoolers diet when added occasionally, if these foods continue to be offered at every meal, the preschooler will end up with an increased energy intake. Over weeks, months, and years, this increased energy intake, without additional energy expenditure, could lead to significant inappropriate weight gains.

Cardiovascular disease

A pediatric branch of the Bogalusa Heart Study was conducted with infants and preschool children birth to four years of age (83). The aim of the study was to identify the development of early eating patterns that might place children at risk for later cardiovascular disease. Numerous dietary recall records were collected on the children throughout their first four years of life. The results indicated that by ages 2-4 years old, the children had begun to consume excess intakes of energy, total fat, saturated fat and cholesterol – all dietary risk factors for cardiovascular disease. The early establishment of these unhealthful eating patterns placed the children at risk for the development of chronic diseases (83).

Children aged 4-6 years in a Head Start population (n = 358) were reported to have dietary intakes of total fat, saturated fat and cholesterol exceeding the recommendations of the National Cholesterol Education Program (84). A study conducted using USDA data reported that US Midwestern children (n = 161) aged 2-4 years consumed the majority of their energy from foods high in saturated fat and sugar and consumed less than 2% of their fat intake from fish sources (85). Likewise, children (n = 329) in rural Oklahoma aged 1-6 years consumed the majority of their energy, protein and fat from foods that are high in saturated fats, and fish was not a major contributor (less than 2%) to dietary protein and fat intake (86).

Omega-3 Intake & Food Preference Development

Most of the food preference development research has focused primarily on the consumption of dairy products, fruits and vegetables. Very little research has been conducted specifically on preschoolers' willingness to try and preference for foods rich in

the DHA and EPA forms of omega-3 fatty acids which are the most beneficial forms during the preschool years for heart health, brain cell structure, and retinal development (15).

McManus et al. interviewed Australian mothers of preschool children (n = 38) on their children's acceptance and consumption of fish and seafood (87). Middle class mothers were recruited into one of seven focus groups in which they answered questions about familial intake and attitudes toward fish and seafood. Focus group interviews were transcribed and then analyzed in order to find themes across the groups.

Results indicated that the majority of the children had tried a variety of fish and seafood products including tuna, prawns, fish and chips (French fries), fish fillets and white fish flesh. Crustaceans were the least popular fish or seafood. The children in the study preferred fish and seafood that were served plain or with chips rather than foods cooked with sauces or mornays. Intake was also lower in larger families and families containing teenagers (87).

The mothers reported that they usually like to purchase their fish and seafood from the same markets rather than trying new places. Likewise, many of the mothers would cook the same foods, rather than trying new recipes. They wanted to prepare one meal for their families instead of preparing separate foods for the children or modifying fish recipes to suit their children's needs; for example, cooking a less spicy rendition of a dish for their children (87).

The study participants listed barriers to their fish and seafood consumption. The most commonly reported barriers included price, availability, familial food preferences, lack of

preparation/cooking skills and the smell of the fish. The majority of the mothers reported that they lacked confidence in preparing fish for their families. Many perceived preparation and cooking to be barriers to preparing fish, particularly fish still containing the bones. The familial attitudes toward fish and seafood greatly influenced the mothers' choices to include these foods regularly. The study found that the mothers' husbands/male partners greatly influenced the types and frequency of fish and seafood included in the families' diets. Likewise, if the mother did not like fish or seafood herself, she was unlikely to include it as part of the family's menu (87).

The mothers mentioned several strategies for getting their children to try and consume fish and/or seafood. Many of the mothers encouraged their children to try and eat the fish or seafood by likening it to chicken, a familiar food. Some of the mothers mentioned that they would entice the children to eat fish by associating it with particularly desirable foods such as chips (87).

Limited research has been conducted on preference of adult consumers for foods and beverages fortified with omega-3. These products began with omega-3 fortified eggs and now include meats, poultry, milk, margarine, dip, muffins, pancakes, oat cereal, salad dressing, chocolate, muesli and soup to name a few (88). Many of these products have been either fortified with the ALA short-chain form of omega-3 or DHA and EPA obtained from fish or other marine sources. The ALA form of omega-3 does not show as many preventative properties as the longer chain DHA and EPA forms of omega-3 (89). DHA and EPA foods fortified with fish or marines-based sources share similar smells and tastes with their source foods that may not be palatable to consumers who originally avoided these undesirable organoleptic root dietary sources (88). One solution is

microencapsulation technology which has improved preference ratings for all forms of omega-3 (88). In addition, omega-3 fortified products may have short shelf-lives which require the addition of antioxidants (88). The bioavailability of algal-oil compared to cooked salmon has been reported to be comparable (90). Therefore, products fortified with algal forms of DHA may provide a viable solution to these problems.

A study using 4-12 year old children (n = 31) reported increased levels of plasma phospholipid DHA after supplementation with algal-derived microencapsulated DHA fortified orange juice (91). The children were given either 50 mg/day or 100 mg/day of DHA over six weeks. At baseline, a FFQ was used to determine consumption of DHA and EPA. The results of this FFQ indicated that the children were only consuming about 18% of the US Dietary Guidelines Advisory Committee's recommendations for DHA and EPA. The juice was well accepted leading to a 96% participant adherence rate and rated favorably as "good" or "very good" by 93% of the participating children (91). This study lends credence to the use of algal-derived DHA fortified foods as a palatable way to increase DHA consumption and plasma phospholipid content.

Main Research Questions & Alternative Hypotheses

1. **Will US Midwestern Head Start/ExCITE Program preschoolers be more willing to try typical or novel foods rich in DHA omega-3 fatty acids?**

   Based on previous food preference studies (9, 11, 38), it was hypothesized that the sample of US Midwestern Head Start/ExCITE preschool children would be more willing to try typical foods rich in DHA omega-3 fatty acids than novel foods rich in DHA omega-3 fatty acids.

2. **How will US Midwestern Head Start/ExCITE Program preschoolers rate various foods rich in DHA omega-3 fatty acids on a food preference scale?**

   Based on documented barriers to getting preschool-age children to eat fish and seafood even in coastal areas (87), it was hypothesized that the US Midwestern Head Start/ExCITE preschool children would more favorably rate typical foods and beverages fortified with DHA omega-3 fatty acids than novel foods rich in DHA omega-3 fatty acids.

3. **What will the relationship be between US Midwestern Head Start/ExCITE preschoolers' willingness to try novel foods rich in DHA omega-3 fatty acids and their reported food neophobia?**

   Based on previous food preference studies (9, 11, 38), it was hypothesized that there would be a negative relationship between US Midwestern Head Start/ExCITE preschool children's willingness to try novel foods rich in DHA omega-3 fatty acids and reported food neophobia.

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Manuscript I:

Development of a Methodology to Measure US Midwestern Preschool Children's Willingness to Try and Preference for Foods Rich in Docosahexaenoic Acid Omega-3

ABSTRACT

This article describes the development of methodology to measure US Midwestern preschool children's willingness to try and preference for foods rich in docosahexaenoic acid (DHA) omega-3. A review of existing methodology that measures children's willingness to try and preference for novel and typical foods is provided. The rationale behind developing this specific methodology, the development of the survey questionnaire and taste testing experiment, the data collection procedures, and data analysis are described. The described methods build on existing measures of child preference development particularly food neophobia for low socio-economic Midwestern preschool children. These methods can be used to help fill a critical gap in understanding how to best get Midwestern children to consume foods rich in DHA in order to meet their optimal nutritional needs and establish chronic disease preventative eating habits.

The establishment of healthful eating patterns from birth gives the infant and young child a nutrient-rich diet and creates fewer unhealthful habits which may be difficult to change later in life (1). The early development of healthful eating patterns has implications for food group and nutrient balance and for the prevention of chronic diseases such as obesity and cardiovascular disease (2-3). These eating patterns are established in children as young as age 3-5 years (4-6).

It is during these years that food behaviors such as food neophobia and food pickiness need to be overcome because the absence of both behaviors are strong predictors of future healthful eating habits (7). Food neophobia has been defined by Pliner and Hobden (1992) as "the reluctance to eat and/or avoidance of novel foods" (8). Children with food neophobia are unwilling to try new foods. Food pickiness is a similar yet distinct behavior that has been defined by Galloway, Lee, and Birch (2003) as the "unwillingness to eat many familiar foods" (6). Children with food pickiness have limited variety in their diets due to their strong preference for only a limited number of foods.

Midwestern children do not typically consume adequate levels of Docosahexaenoic acid (DHA) omega-3, a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development (9). These fatty acids are found naturally primarily in fish but are also found in seafood such as shrimp, typically not foods commonly consumed in the Midwest (10-11). Due to their novelty, these foods may not be as likely to be tried or preferred by Midwestern children. Recently, foods and beverages, some typical and some atypical of the Midwestern diet, such as margarine spread and soymilk have been fortified with algal-derived DHA (12). A measure was needed to determine Midwestern preschool children's willingness to try and preference for these algal DHA

fortified foods and beverages compared to their willingness to try and preference for DHA omega-3 fortified eggs and naturally rich sources of DHA available in the Midwest.

The purpose of this article is to provide a review of existing methodology that measures children's willingness to try and preference for novel and typical foods and then to provide an in-depth description of the development of methodology to specifically measure Midwestern preschool children's willingness to try and preference for foods rich in DHA omega-3. For an overview of reviewed and developed methods see Figure 1.

REVIEW OF METHODS

To develop appropriate methodology for measuring Midwestern preschool children's willingness to try and preference for foods rich in DHA omega-3, existing methodology that measures children's willingness to try and preference for novel and typical foods was reviewed.

Adult & Older Child Measures of Food Neophobia

Pliner and Loewen have been leaders in research on adult and child food neophobia. They have conducted numerous studies to develop and validate food neophobia questionnaires against observed laboratory behavior in taste tests. These questionnaires include the Food Neophobia Scale which has been validated for adults (8) and later for parents filling out this questionnaire for their children, aged 5-11 years (13); and the Food Situations Questionnaire which was a self-report measure validated for use with 7-12 year old children (4).

A good example of these taste test experiments used to validate questionnaire measures of food neophobia is a temperament and food neophobia study conducted with

81 sibling pairs, aged 5-11 years, and their mothers (14). Siblings were individually asked to select foods they would like to try out of a list of 20 total novel and familiar foods and five additional foods reported to be well-liked by most children. The children's behavioral neophobia was quantified by calculating a ratio based on the number of novel foods each child selected divided by the number of familiar foods selected. The children were then asked to try and rate the foods using a five face hedonistic scale. After the initial behavioral neophobia task, the children were asked to taste and rate six foods that were not a part of the initial taste test. These foods were divided into good tasting familiar foods, good tasting novel foods and bad-tasting foods (14).

The mothers of the children completed five separate questionnaires: the Parent's Willingness to Taste questionnaire to assess their food neophobia of the same foods their children were being offered, two questionnaires designed to assess their temperament as well as the temperaments of each of their children and two Food Neophobia Scale questionnaires for themselves and their children. Results indicated that food neophobia was strongly correlated with temperament (14). Although valid and reliable, the food neophobia instruments developed from studies such as this one have been limited to adults and children above the age of five years.

Review of Existing Early Childhood Food Neophobia Measures

Food Records & Variety Food records have been used in longitudinal studies to report the influence of early childhood eating patterns on these children's later reported dietary variety. For example, an eight year study examined infant and toddler (n = 465) eating patterns to determine if these patterns were predictive of grade school dietary variety (2). The study concluded that early food experiences from birth to age two have

an effect on later school-age dietary variety, although variables may differ for each food group (2).

Food Variety, Food Pickiness & Food Neophobia Measures A study conducted in Dijon, France (n = 465) estimated food variety in 2-3 year old children (7, 15). Children at a nursery were given choices for each food group from eight dishes and variety seeking scores were calculated. A follow-up study was conducted with some of the participants (n = 339) 1-19 years after the initial study to measure variety seeking and food neophobia using two questionnaires. The food neophobia questionnaire was scored on a scale of 10-70. This questionnaire was adapted from the Food Neophobia Scale (8), the Food Attitude Survey (16) and a prior adaptation of the Food Neophobia Scale (17). The behavioral food variety scores correlated to later food variety scores and food neophobia scores (7, 15).

Food pickiness and food neophobia were measured to compare parental influence on vegetable consumption in seven year old girls (n = 192) (6). The girls' food neophobia was measured using a combination of 16 questions from two neophobia questionnaires: the Food Neophobia Scale for Children adapted from the Food Neophobia Scale used for adults (8) and the Food Situations Questionnaire (4) completed by their parents. The girls' food pickiness was assessed through a questionnaire given to the girls' parents that assessed parental attitudes and perceptions about the girls' willingness to eat various familiar foods during mealtimes. The girls' vegetable intake was estimated based on three 24-hour food recalls. The study measured some typical predictors of food neophobia and pickiness including child anxiety scores, parental food neophobia, maternal vegetable intake and maternal food attitudes about having enough time to eat healthfully. Results

indicated girls who had food neophobia, food pickiness or both behaviors consumed significantly fewer servings of vegetables than the other girls who did not have these food behaviors. Maternal (but not paternal) neophobia and pickiness were associated with child neophobia. Girls who were more anxious tended to have more food neophobia indicating a stronger tie to personality than food pickiness (6).

Taste Test Food Neophobia Measures Food neophobia reduction was measured in a study conducted with preschool children (n = 46) enrolled in a Head Start Program (18). Children assigned to the experimental group participated in a 12-week intervention program entitled The Food Friends: Making New Foods Fun for Kids, which used social marketing techniques along with repeated exposure and increased variety to specific indicator foods to encourage the children to try new foods. The children's reduction of food neophobia was measured by comparing the change in the children's willingness to try and consume novel foods offered as well as the children's rankings of the novel and familiar foods from pre-assessment to post-assessment and again at a follow-up assessment completed 10 days post-intervention. Children were asked to rank nine foods (five familiar and four novel foods) using three cartoon faces: smiling, neutral or frowning. Any eating behaviors exhibited during the taste test were recorded. The significant change over the 12 weeks indicated that the social marketing strategy paired with the food interventions was effective in increasing acceptance of novel foods. However, the food intervention strategies (increasing variety and repeated exposure) used were also significantly effective in reducing food neophobia by themselves (18).

In another study, the influence of adult role-modeling on 2-5 year old children's willingness to try and accept new foods was measured (19). Preschool children (n = 27)

were given three new foods to try prior to their lunch or snack times – one per week: semolina with cumin dyed yellow, semolina with caper paste dyed green, and semolina with anchovy paste dyed red. Each food trial was with a different social influence: an adult model who ate the same color-dyed semolina as the child, an adult model who ate different color-dyed semolina from the child, and an adult model who did not eat anything with the child. The children's behaviors were videotaped, and their leftover food was weighed in grams. The researchers reported the children's willingness to try and consume new foods with each condition as well as with subsequent trials (19).

Children were most likely to try and consume the food when the adult model was eating the same color (19). In addition, children were more likely to eat more of the new food in the second and third trials regardless of experimental condition. The authors' attributed this increased consumption as a response to the repeated exposure of the food; by the third trial the food was more familiar to the children. No statistical significance was found for the children's preference for any one particular color of food, or statistical significance for age or classroom (19).

Results of an early study conducted by Pliner (1994) indicated that parents tended to be particularly neophobic of foods of animal origin compared to their children who did not have this food categorical neophobic behavior (13). This finding may further indicate the role of cultural norms and exposure influencing willingness to try specific foods deemed culturally less desirable.

DEVELOPED METHODS

An in-depth description of the methodology developed for two instruments the Eating Habits of Preschool Children questionnaire and a taste test experiment designed

specifically for measuring low socioeconomic status (SES) US Midwestern preschool children's willingness to try and preference for DHA is provided.

Rationale

Most of the food neophobia and preference research specific to the preschool age population has focused primarily on the consumption of dairy products, fruits and vegetables. Very little research has been conducted specifically on preschoolers' willingness to try and preference for foods rich in DHA omega-3, a key nutrient during the preschool years (9). A measure was needed to assess willingness to try and preference for DHA omega-3 foods specific to the low SES US Midwestern population.

Sampling Methodology

The UNL Institutional Review Board and Lincoln Public Schools (LPS) Research Review Committee approved the study protocol, all participating parents provided written informed consent and children provided verbal assent. Please see Appendix B for the LPS permission request letter and Appendix C for the LPS approval letters.

The convenience sample of parents and preschool children aged 3-5 years old was recruited from the fall 2010 semester enrollment in one of eight LPS Head Start/Early Childhood Toddler Educare (ExCITE) Program classrooms located at three schools. This sample was selected because of the sample's low SES which placed the children at increased risk for obesity and thus obesity-related complications including the risk for cardiovascular disease later in life (20-22). In addition, the sample was selected because part of the mission of Head Start includes a health and nutrition component so this study would serve the needs of the target population (23).

Study participants included boys and girls of various racial and ethnic backgrounds. Classrooms were selected at schools that had demographic populations paralleling the US Midwestern population. Children with any reported food allergies were excluded from the study for safety reasons. Recruitment took place at parent-teacher conferences. Each eligible parent received a folder containing a recruitment letter, informed consent form, and two questionnaires. Please see Appendix D for the parental recruitment letter and Appendix E for the UNL Institutional Review Board Informed Consent Form. Consenting parents completed the informed consent form and two questionnaires under the supervision of their children's classroom teachers. These forms were obtained prior to the taste test experiment. Each child was given a code which was placed on the informed consent form, both questionnaires and experimental affective rating sheet to assure the confidentiality of individuals' results.

Sample Size Justification Calculations for sample size were made based on tables from Cochran & Cox, 1957 (24). For $\alpha = .05$, sample size was determined to have at least a .8 chance (power) of detecting a difference between two foods regarding the probabilities of the children's willingness to try the foods. A sample of > 45 children was determined to be ample to detect percentage when calculating for the worst case scenario of a 50/50 proportion for one of the two foods. The children's food preference rating was on a three-point Likert scale ("yummy"-1, "just okay"-2, and "yucky" -3). The mean scores of this quantitative variable were used to determine food preference. A sample of 49 children with an estimated standard deviation of 0.15 had an 80% chance of detecting a 12% difference of food preferences between two foods. Finally it was determined that a sample size of > 45 children $\alpha = .05$ will have a better than 50% chance to detect an

expected correlation of 0.3 between the children's reported food neophobia as reported by the children's parents using the food neophobia questionnaire and the children's willingness to try novel foods rich in DHA determined by the taste test experiment.

Questionnaires

A parent or primary caregiver of the participating preschool children completed two questionnaires upon their enrollment in the research study. Please see Appendix F for the Eating Habits of Preschool Children Questionnaire and Appendix G for the Food Neophobia Questionnaire.

Eating Habits of Preschool Children Questionnaire The first questionnaire, Eating Habits of Preschool Children contained 12 questions. The questionnaire was designed to determine the child and parental consumption of and familiarity with foods naturally rich in DHA (the four types of canned convenience fish rich in DHA most available in the Midwest) or foods with DHA-fortified counterparts.

Food Neophobia Scale Questionnaire The second questionnaire, the Food Neophobia Scale Questionnaire, contained 25 questions about the families' and children's eating behaviors. The purpose of this questionnaire was to determine the families' and children's food neophobia. Ten of the questions were directly from and 10 were modified from the Child Food Neophobia Scale, an instrument developed by Pliner & Hobden (1992) with satisfactory test-retest reliability and internal consistency $r(38) = 0.91$ and $r(31) = 0.87$, ps <0.01 (8). The Child Neophobia Scale completed by parents has been significantly associated with behavioral trait neophobia observed in taste test studies as well as parental predictions of willingness to eat foods (13). Five of the questions were

directly from the "Reaction to Food" section of the "Colorado Child Temperament Inventory," a parental rating instrument designed to assess temperament in children ages 1-6 years old developed by Rowe & Plomin (1977) (25). These five instrument items correlate with sociability and emotionality dimensions of temperament and have reliability ratings of 0.77 for alpha and 0.74 for retest (25). All instruments were used with permission from the instruments' developers.

Questionnaire Pilot Testing The Eating Habits of Preschool Children questionnaire was developed and modified using feedback from a child development specialist and two mothers of preschool age children. The Eating Habits of Preschool Children and Food Neophobia questionnaires and experimental taste test procedures were pilot tested using a small sample (n = 7 pairs) of parents and 3-5 year old preschool children enrolled at the UNL Ruth Staples Child Development Laboratory. Participants were recruited for the pilot study using a recruitment letter and informed consent form sent home in the children's take home folders. Consenting parents completed the two questionnaires and were contacted via telephone to discuss the clarity, accuracy and ease of use of the questionnaires. Parents responded favorably to the questionnaires; therefore, only minor changes were made to improve question clarity.

Experimental Taste Test

Taste Test Pilot Testing The experimental taste testing procedures were pilot tested with the children recruited from the UNL Ruth Staples Child Development Laboratory. From this pilot test, modifications were made. Taste testing procedures were changed to increase validity and developmental appropriateness. For example, it was planned that the

children would affectively rate each food item and then rank order them at the end of the taste test; however, this was too difficult of a task for the children as evidenced by the children's inability to complete the task. Furthermore, the ranked results completed were unreliable as evidenced by children routinely selecting the last food tried or pointing to a leftover portion cup, but not being able to verbally tell which samples were their favorite. Sample preparation and storage procedures were modified to increase the timeliness and efficiency of the experiment, the standardization of the samples, as well as to address food safety concerns. One child refused to participate in the taste test experiment; therefore, a more clearly defined procedure was established for inviting the children to participate and responding to children's refusals although during the actual study no children refused to participate. Also, the pilot test confirmed the necessity to test both 2% and whole DHA-fortified milk due to a difference in food preference ratings by some children.

Sample Preparation The participating Head Start/ExCITE children completed a food preference taste testing experiment in order to document their willingness to try and preference for 14 foods rich in DHA: white bread with margarine spread, white bread with olive oil, 2% milk, whole milk, chocolate milk, plain soymilk, strawberry and carrot yogurt, banana and sweet potato yogurt, blueberry pomegranate juice, hard boiled eggs, mackerel, salmon, sardines and tuna. DHA fortified foods and beverages chosen were any items which could be readily purchased off the shelf in a non-specialty Midwestern city grocery store, and the amount of DHA could be obtained from the label or manufacturer. Fish was selected based on the four most readily available convenience canned fish in Midwestern city grocery stores. It was assumed from previous experience

that most low SES Midwesterners would be the most likely to purchase and prepare DHA rich fish from a can rather than fresh or frozen products.

The same products including brands were purchased for each test to decrease sample variation. Samples were prepared ahead of the taste test by placing a small amount of each food into two ounce clear plastic condiment containers and sealing each container with a plastic lid. Foods were prepared and stored in a refrigerator as close to the taste test as feasible to assure freshness. The utmost care was taken to make sure the foods were prepared in a food safe environment and kept at appropriate temperatures from preparation to the testing.

The DHA fortified white bread slices were cut into sixths so that each piece had a crust and non-crust side to it and either placed in a condiment cup for dipping in the olive oil (packaged separately) or buttered with the DHA fortified margarine spread. The DHA omega-3 eggs were boiled for 20 minutes until they were hard, peeled and sliced into eighths so that each egg sample contained both white and yolk. The cow's milk, soymilk, juice, and olive oil samples were poured into the condiment containers. The yogurt was spooned into the condiment containers. The canned fish were drained, and chunks were broken up before spooning into the containers. Separate utensils were used for each food item to make sure that there was not cross-contamination of flavors. Strong smelling items such as the mackerel were stored separately from the other food items until just before the taste test. All food items were transported to the schools in a 50 quart rolling cooler that could keep ice stored up to five days even at external temperatures of 90ºF. The investigator sanitized the taste testing area before the experiment began.

Children were tested between meals and snacks to avoid satiety. They were invited to participate one at a time by their teachers who asked them if they would like to be a helper, helping by trying some foods. After the children's verbal assent was obtained, the children were taken one at a time to a quiet room away from the other children, teachers and staff.

In order to record the children's food preferences, a three cartoon face hedonistic scale was used: a frowning face for foods considered "yucky," a neutral face for foods considered "just okay" and a smiling face for foods considered "yummy." Please see Appendix H for the Three Face Hedonistic Scale. Hedonistic face scales are a well accepted means of assessing food preference in young children (14). Three face hedonistic scales have been used as a developmentally appropriate tool for taste test experiments with preschool age children (18).

The validity of the cartoon face hedonistic scale was tested with each child prior to starting the official taste test experiment. Before they viewed the food samples, the children were asked to name their favorite food, a food they thought was "yummy." They were asked to name a food that they did not like at all, a food they thought was "yucky." The cartoon face hedonistic scale was explained to the children – the happy face was for foods the children liked or "yummy" foods, the frowning face was for foods the children did not like at all or "yucky" foods, and the middle (neutral) face was for foods that the children did not really like or dislike, or foods that were "just okay." The children were then asked to point to the face that matched their favorite food and then the face that matched the food they did not like at all which they had stated earlier. This procedure

was used to determine that children understood the response format before the taste test was conducted.

During the taste test, the children were asked to try 14 DHA-rich food and beverage samples, 10 DHA fortified and four fish samples. The children were offered the foods one at a time starting with the juice which is a commonly accepted food by this age group. This allowed the children to have a positive first experience, making them more likely to participate in the rest of the taste test. Each additional food item was then offered to the children in a varied order. Children were allowed to drink the fluids directly from the condiment cups and eat the hardboiled egg, bread dipped (right before offered) in olive oil and bread with margarine spread with their fingers. They were given a small plastic taste testing spoon (a new spoon per food item) for each of the other foods. If the children tried the food item, they were immediately asked to point to the face which matched what they thought of it. The validity of their assessment was verbally checked by stating out loud their choice and checking for their confirmation. For example, if a child tried the canned mackerel and pointed to the frowning face, the researcher would say, "You pointed to the frowning face. Is this a food you don't like at all? Do you think it's a "yucky" food?" The children's willingness to try each food along with their affective rating of it were recorded. A summary of the taste test experimental procedure is provided in Figure 2. Water was provided ad libitum during the taste test. If the children tried two foods in a row they rated "yucky," they were encouraged to take a drink of water in order to get the bad taste out of their mouths. Paper towels were kept on hand along with sanitizing wipes to clean up any messes made during the taste test.

In addition, the children's food-related behaviors observed during the food preference taste test experiment were recorded including any touching, smelling, playing with and/or tasting the food without swallowing. The taste test took about 10-15 minutes per child to complete.

Data Processing and Analysis

Pilot data were analyzed using SPSS (version 19.0, 2010, IBM Corporation, Somers, NY).

DISCUSSION AND CONCLUSIONS

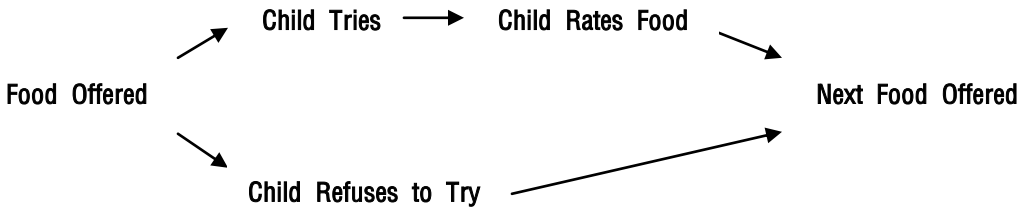
Measuring food preference in preschool age children can be difficult due to these children's limited developmental abilities to explain their preferences and distinguish between many degrees on a scale. Therefore, food preference measurement was limited to a three point scale. Foods and beverages fortified with algal-derived DHA are new to the market; therefore, there were also a limited number of foods and beverages from which to choose. The taste test methods particularly the foods and beverages selected were designed to measure willingness to try and preference for DHA omega-3 foods and beverages specifically for the US Midwestern low SES population. These procedures and foods and beverages may need to be modified for other populations. The developed methods build on existing measures of child preference development establishing food neophobia measures for the US Midwestern low SES population. These methods can be used to help fill a critical gap in understanding how to best get Midwestern children to consume foods rich in DHA in order to meet their optimal nutritional needs and establish chronic disease preventative eating habits.

FIGURES

Figure 1: Food Neophobia Instruments and Methods

Instrument/Method	Measure	Reference
Food Neophobia Scale	Food neophobia: self-report adults, parental-report children aged 5-11 years	(8, 13)
Food Situations Questionnaire	Situational food neophobia: self-report children aged 7-12 years	(4)
Parents Willingness to Taste	Willingness to try specific novel and familiar foods: self-report parents of children aged 5-11 years	(14)
Food Attitude Survey	Willingness to try and preference for specific novel, familiar and fictitious foods: self-report adults	(16)
Food Neophobia Scale for Children	Adapted from Food Neophobia Scale, parental-report children aged 7 years	(6)
Food Friends Taste Test Assessment	Change in willingness to try novel and familiar indicator foods: Head Start children aged 3-5 years	(18)
Eating Habits of Preschool Children Questionnaire	Consumption of and familiarity with DHA omega-3 foods: self-report and parental-report parents of children aged 3-5 years	Developed by Bettenhausen, 2010
DHA Taste test experiment	Willingness to try and preference for foods rich in DHA omega-3: US Midwestern, low SES children 3-5 years	Developed by Bettenhausen, 2010

Figure 2: Taste Test Experimental Procedures



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Manuscript II:

Exposure of US Midwestern Preschool Children and Their Parents to Foods Naturally Rich in Docosahexaenoic Acid (DHA) Omega-3 and Foods with DHA Omega-3 Fortified Counterparts and Parental Awareness of Omega-3

ABSTRACT

Although research indicates that US children are consuming too much of the wrong kinds of fats and too little of the fats known for their chronic disease preventative properties such as docosahexanoic acid omega-3 (DHA), little research has been conducted to classify familiarity with foods naturally rich in and fortified with DHA in the US Midwestern population. The Eating Habits of Preschool Children questionnaire was developed and administered to determine parental and children's familiarity and consumption of four foods naturally rich in DHA and 10 foods which have DHA fortified counterparts. The questionnaire also asked questions concerning the awareness and perceived knowledge of omega-3 and DHA fatty acids and fortified products. A convenience sample of parents (n = 47) of 3-5 year old children enrolled in a Lincoln Public Schools Head Start/Early Childhood Toddler Educare (ExCITE) Program classroom completed the 12-item questionnaire. The data were analyzed to find descriptive frequencies, means and standard deviations. Parental consumption of foods was similar to their children's consumption. Results indicated that of the DHA rich foods listed in the questionnaire, only tuna was a typical food consumed in the Midwest. Awareness and perceived knowledge of omega-3 in general was more frequent than that of DHA. Very few participants could list valid foods fortified with omega-3 or DHA. Results from this questionnaire provided descriptive data which contribute to the understanding of Midwestern Head Start/ExCITE child and parental exposure to and awareness of omega-3 foods. Results were also used to categorize a series of foods as typical or novel to the Midwestern population in order to use these foods for a follow-up taste test experiment.

Docosahexanoic acid (DHA) omega-3 fatty acid is a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development, and is a key nutrient in the preschool years (1). Currently, there is not an age-specific amount of DHA recommended for preschool aged children. However, it is recommended that children two years and older consume 1-2% of their energy as omega-3 fatty acids and 500-1000 mg of DHA and eicosapentanoic acid per week in order to protect them against future cardiovascular disease (2). The consumption of DHA and DHA-rich omega-3 fish has a well-established preventative effect against the development of cardiovascular disease in adults (3). Polyunsaturated fat intake has been negatively associated with obesity, and total and saturated fat have been positively associated with obesity (4). These findings stress the importance of preschool aged children's consumption of foods with the right kinds of fat.

However, the 2002 Feeding Infants and Toddlers Study reported that as the toddlers' transitioned to table foods at 19 to 24 months of age, a quarter of the children were consuming high-calorie, high-fat, salty foods such as potato chips on a daily basis: consuming too much of the wrong types of fat (5). Likewise, a pediatric branch of the Bogalusa Heart Study conducted with infants and preschool children identified early eating patterns that placed children at risk for later cardiovascular disease (6). The results indicated that by 2-4 years of age, the children had begun to consume excess intakes of energy, total fat, saturated fat and cholesterol – all dietary risk factors for cardiovascular disease.

National consumption of chronic disease preventative DHA omega-3 is below recommended levels (7). Furthermore, US Midwestern children may be at an even greater

risk of low consumption of DHA because these children do not typically consume foods rich in DHA omega-3 fatty acids. These fatty acids are found naturally primarily in fish and in seafood such as shrimp, which are not foods typically consumed in the Midwest (8-9). Even in coastal cultures in which fish is more available, it can be challenging to get preschool age children to consume foods naturally rich in DHA (10).

Recently, some foods deemed more acceptable to Midwesterners have been fortified with DHA. Foods fortified with life'sDHA^{TM}, a product of Martek Biosciences Corporation, include a wide variety of foods ranging from juice to tub margarine (11). These products are fortified with DHA produced from an algae source. Therefore, these products do not have the strong "fishy" taste or smell of some products fortified with DHA extracted from fish-based sources. These fortified foods and beverages may provide an acceptable solution for increasing consumption of DHA in the US Midwestern preschool population leading to the consumption of heart-healthy fats and decreasing the risk for obesity-related chronic diseases.

During the preschool years, ages 3-5, eating habits are formed which can either establish healthful preventative patterns or establish patterns strongly associated with the risk of developing chronic diseases later in life (12-15). These patterns can help protect or increase children's risk of an early adult death from cardiovascular disease, the leading cause of death in the US (16).

Very little research has been conducted on US Midwestern preschool children's and their parents' consumption of DHA omega-3 foods or the awareness and exposure of these children and their parents to these foods and DHA omega-3 information. The

purpose of this study was to describe the exposure of Midwestern Head Start/Early Childhood Toddler Educare (ExCITE) preschool children to foods naturally rich in DHA omega-3 and foods and beverages containing DHA fortified counterparts, and to describe the exposure and awareness of the caregivers of these preschooler children to omega-3 foods and information.

METHODS

Rationale

A 12-item questionnaire, The Eating Habits of Preschool Children, was used to determine the parental and child familiarity and frequency of consumption of four foods naturally rich in DHA and 10 foods and beverages which have DHA-fortified counterparts. This questionnaire provided descriptive data which contributes to the understanding of US Midwestern Head Start/ExCITE enrolled child and parental exposure to and awareness of omega-3 foods.

Sampling Methodology

A convenience sample of parents of 3-5 year old children enrolled for the fall 2010 semester in one of eight Lincoln Public Schools (LPS) Head Start/ExCITE Program classrooms was used for this study. The Head Start/ExCITE population was targeted due to low-socioeconomic status which increases this population's risk of chronic disease (17). Although participants recruited included both genders and various racial and ethnic backgrounds, classrooms were selected for demographic populations paralleling the US Midwestern population. Recruitment and parental completion of the informed consent took place at school during the fall parent-teacher conferences. Parents had the option to

include their relationship to the child, their phone number and the best time to reach them on the informed consent form.

Development of Survey Questionnaire

The Eating Habits of Preschool Children questionnaire was developed using the expertise of a child development specialist and the experience of two mothers of preschool age children. The questionnaire was pilot tested with parents of preschool children enrolled at the UNL Ruth Staples Child Development Laboratory. Pilot test feedback was used to modify the questionnaire to improve the clarity of the questions.

Questionnaire Description

The questionnaire had six pages containing 12 questions. Questions one and two were in tabular form with 18 identical foods on the vertical axis and consumption frequencies on the horizontal axis. Included foods were those which have a DHA-fortified version on the market or were a type of fish rich in DHA. These foods are listed in Table 1. All listed foods and beverages were readily available in Midwestern city grocery stores. Parents completing the questionnaire were instructed to check how often their preschool children (question one) or they (question two) ate or drank the foods and beverages. The frequency choices included nine categories: never tried, less than once per month, once per month, 2-3 times per month, 1-2 times per week, 3-4 times per week, 5-6 times per week, once per day, and more than once per day.

Questions three and four “Has your child ever had any fish?” and “Does your child have any known food allergies or intolerances?” were used as an additional safety measure to omit children who might have any risk of an allergic reaction from a follow-

up taste testing experiment. Questions five and six contained two parts each to gather information concerning omega-3 and DHA omega-3 awareness and perceived knowledge. Question five asked, “Have you heard of omega-3 fatty acids?” Question six asked, “Have you heard of docosahexaenoic acid (DHA)?” After each question, a follow-up question asked, “If yes, how knowledgeable would you say you are about omega-3 fatty acids?” (or DHA for question six). Four choices ranged from “very knowledgeable” to “not at all knowledgeable.” Question seven asked generally about omega-3 fortified products, “Are you aware of any food or beverage products fortified with omega-3?” Question eight asked specifically about life’s DHA™ products, “Have you seen the life’s DHA™ logo on any foods or beverages which you have purchased?” An example of the logo appeared to the right of this question. Questions seven and eight had a qualitative follow-up, “If yes, please list.” Questions nine through twelve gathered demographic information including number of children at home, ages of children in the household, race/ethnicity, and highest level of education. Participants were allowed to check all racial/ethnic categories they felt applied to them, and the question also contained an “other” category in which participants could write their answers.

Questionnaire Administration

Parents completed the questionnaire under the supervision of their children’s classroom teachers at parent teacher meetings. Teachers had been instructed prior to the conferences on how to administer the questionnaire. Any missing or unclear data were obtained via phone by the researcher.

Data Processing and Analysis

IBM SPSS (version 19.0, 2010, IBM Corporation, Somers, NY) was used to obtain means, standard deviations and frequencies for the questionnaire. There were no missing data. Qualitative answers to questions seven, eight and the "other" category of the race/ethnicity question were compiled and quantified.

The UNL Institutional Review Board approved the study protocol, the questionnaire, and all participating parents provided written informed consent.

RESULTS AND DISCUSSION

Forty-seven preschool children and their parents participated in the study. The findings from questions one and two are presented in Table 1 and Appendix I. Foods and beverages which were consumed less than one time per month or which the participant or their child had never tried were classified as "novel" foods for this US Midwestern sample. Eleven foods and beverages were classified as "typical" foods and seven foods and beverages were classified as "novel" foods. This classification of foods as "typical" or "novel" was needed for the behavioral taste testing portion of this study. These findings matched study predictions which were in alignment with experimental food classification used in a series of Canadian child food neophobia studies that used tuna fish, cow's milk and cheddar cheese as familiar foods (18-20). Parental and child consumption patterns were similar for the foods listed. This finding supports existing studies that reported that child eating patterns tended to mirror those of their parents (15, 21-23). In addition, this finding is supported by other omega-3 related studies which reported that familial attitudes toward fish and seafood greatly influenced the mothers'

choices to include these foods regularly in the household's menus as well as the maternal and paternal preferences for fish and seafood regulating household consumption patterns (10). These food consumption results further supported other studies that reported foods naturally rich in DHA omega-3 are lacking in the Midwestern diet (8-9).

Questions three and four provided an additional safety net to make sure that children at risk for potential allergic reactions to foods that were going to be offered as part of the taste test were omitted from the study. No children at risk were found.

The findings from questions five and six are summarized in Figure 1. As expected, more parents reported awareness and perceived knowledge of omega-3 in general than DHA. Over half of the parents had never heard of DHA compared to a quarter who had never heard of omega-3. Only one parent felt they were very knowledgeable about omega-3 and DHA. Three fourths of the parents reported that they were not aware of any products fortified (n = 35) with DHA nor had they seen the life'sDHA™ symbol on any products (n = 37). Twelve parents reported an awareness of omega-3 fortification, 11 listed products: six listed fish, two listed flax seeds, one listed walnuts, and fortified products listed included yogurt, toddler Gerber® products, butter spread, eggs and cereals. Of the 10 parents who reported that they had seen the life'sDHA™ logo on foods, five listed products: three listed infant formula and two listed juice. Very few parents could list specific products fortified with omega-3 and even fewer could list products with the algal form of DHA.

The majority of parents (n = 32, 68%) reported having two or three children at home. The range for children at home was 1-6 children. The ages of these children within the

household ranged from four months to 17 years. Additional parental and child demographics are reported in Table 2 and were obtained from questions 11 and 12 and the informed consent form.

The data collected accomplished its purpose of gathering descriptive information to inform a subsequent taste testing experiment. However, the findings are limited due to the small, convenience sample. In addition, self-reported consumption is not always reliable.

CONCLUSIONS

This study questionnaire assessed US Midwestern Head Start/ExCITE children and their parents' familiarity and frequency of consumption of four foods naturally rich in DHA and 10 foods and beverages which have DHA-fortified counterparts. This questionnaire provided descriptive data which contribute to the understanding of Midwestern Head Start/ExCITE child and parental exposure to and awareness of omega-3 foods. This questionnaire provided vital information to categorize the foods into "typical" and "novel" Midwestern foods. This and other descriptive information was needed for a follow-up taste test experiment with the same preschool children.

TABLES

Table 1: Mean Frequency of Midwestern Child and Parental Food Consumption per Month

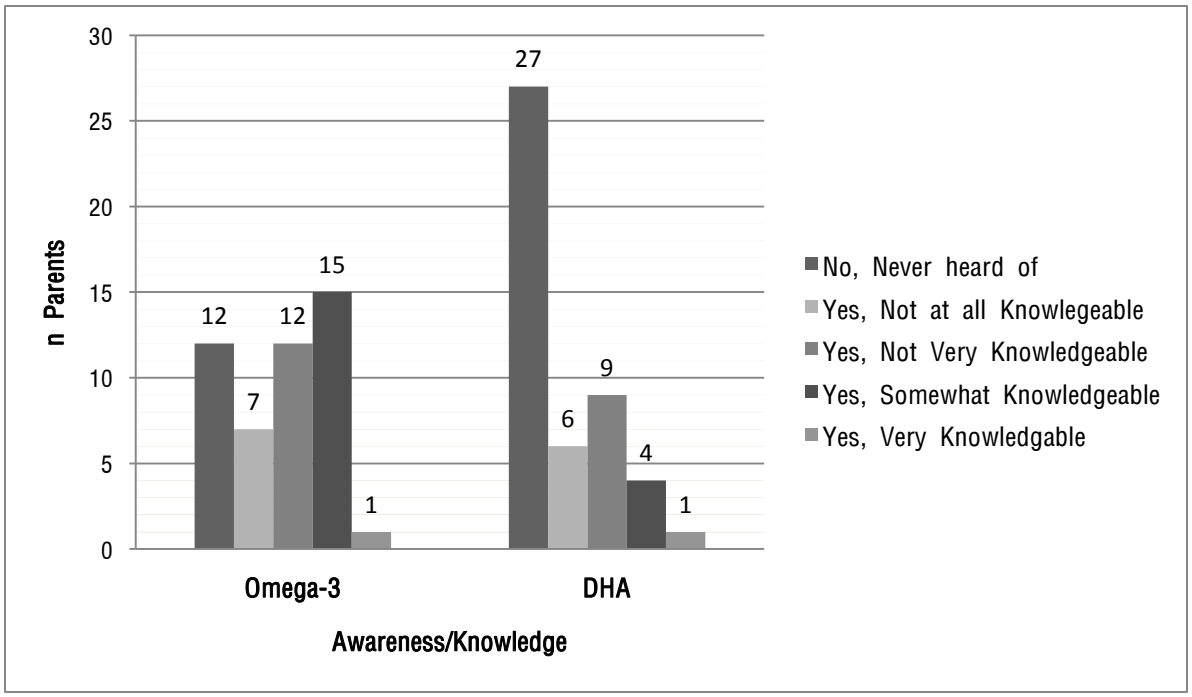
	Mean Frequency Food Consumption per Month	
Typical Foods	Children	Parents
Margarine Spread	6	6
Olive Oil	1	1
2% Milk	22	6
Whole Milk	2.5	1
Chocolate Milk	6	1
Hardboiled Egg	1	1
Albacore Tuna	1	2.5
Bread	6	6
Cheese	6	6
Non-Citrus Juice (ex. grape, apple, cranberry)	14	6
Yogurt	6	2.5
Novel Foods		
Plain Soymilk	<1	<1
Strawberry Carrot Yogurt	<1	<1
Banana Sweet Potato Yogurt	<1	<1
Blueberry Pomegranate Juice	<1	<1
Mackerel	<1	<1
Salmon	<1	<1
Sardines	<1	<1

Table 2: Parent and Child Demographics

	n	%
Parental Relationship to Child		
Mother	42	89.4
Father	4	8.5
Grandmother	1	2.1
Child Gender		
Male	21	44.7
Female	26	55.3
Child Age		
3 Years	9	19.1
4 Years	37	78.7
5 Years	1	2.1
Ethnicity/Race		
African American/Black	2	4.3
American Indian	0	0
Asian or Pacific Islander	2	4.3
Caucasian/White	28	59.6
Hispanic/Latino	4	8.5
More than One	10	21.2
Missing Data	1	2.1
Parental Education		
< High School	6	12.8
High School/GED	13	27.7
Some College	13	27.7
Associates Degree	9	19.1
Bachelor's Degree	4	8.5
Graduate Degree	2	4.3

FIGURES

Figure 1: Awareness and Perceived Knowledge of Omega-3 and DHA



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Manuscript III:

US Midwestern Preschool Children's General Willingness to Try and Favorable Preference for Foods Rich in Docosahexaenoic Acid Omega-3

ABSTRACT

Within the past 30 years, childhood obesity prevalence has doubled for preschool children aged 2-5 years. A disproportionate number of those with low socio-economic status are classified as overweight or obese. One of the biggest contributors to childhood obesity is diet, and the establishment of eating patterns begins during the preschool years. During these years, obesigenic eating habits are formed which are strongly associated with risk of developing chronic diseases later in life particularly cardiovascular disease. Docosahexaenoic acid (DHA) omega-3 fatty acid is a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development, a key nutrient in the preschool years. Current intakes of DHA are below recommended levels. Very little research has been conducted specifically on preschoolers' willingness to try and accept foods rich in the DHA form of omega-3 fatty acids.

Preschool children enrolled in an US Midwestern Head Start/Early Childhood Toddler Educare (ExCITE) program were recruited for this study. Parents were asked to complete two questionnaires, one on both their child's and their own familiarity and awareness of omega-3 foods and one on familial and child neophobia. The children participated in a taste test experiment in which their willingness to try 14 foods and beverages rich in DHA as well as their preference for these foods was recorded. T-tests and McNemar nonparametric tests were conducted to detect statistical differences between willingness to try pairs of foods; t-tests were used to detect differences in preferences between groupings of foods. A correlation was calculated between willingness to try new foods and reported food neophobia.

Data were collected in fall 2010 with 47 children and their parents. The children were willing to try most foods but were least likely to try mackerel and sardines compared to other foods offered. Food preference findings indicated that the children preferred fortified foods over fish and typical foods over novel foods. Novel fish was the least palatable to the children. Children who were reported to have greater food neophobia were significantly less likely to try new foods in the taste test experiment. DHA fortified foods typical of the Midwestern diet may be the most likely way to increase DHA consumption in US Midwestern children.

Within the past 30 years, childhood obesity prevalence has doubled for preschool children aged 2-5 years (1). According to the National Health and Nutrition Examination Survey (NHANES), 12.4% of preschool children can be classified as obese (2). A disproportionate number of those with low socio-economic status (SES) are classified as overweight or obese (3-5).

One of the biggest contributors to childhood obesity is diet (6). The establishment of eating patterns begins during the preschool years, children aged 3-5 years (7-9). It is during these years that food behaviors such as food neophobia and food pickiness need to be overcome since both behaviors are strong predictors of future healthful eating habits (10). Food neophobia is the fear of trying new foods while food pickiness is the reluctance to eat a variety of familiar foods (7, 11).

During these key years, some children begin to gain excess weight which has been reported to predict, by age five, future weight patterns and thus obesity-associated risk (12). In addition, during these 3-5 age years, obesigenic eating habits are formed which are strongly associated with the risk of developing chronic diseases later in life since early childhood eating patterns tend to be predictive of adult eating patterns (13-14). One consequence of childhood obesity is that it places children at an increased risk of early adult death from cardiovascular disease, the leading cause of death in the US (15-16).

Docosahexaenoic acid (DHA) omega-3 fatty acid, a polyunsaturated fat with known benefits for heart health, brain cell structure, and retinal development, is a key nutrient in the preschool years (17). The consumption of DHA and DHA-rich omega-3 fish has a

well-established preventative effect against the development of cardiovascular disease in adults, yet child and adult consumption is below recommended levels (18-19).

US Midwestern children do not typically consume foods rich in DHA omega-3 fatty acids. These fatty acids are found naturally primarily in fish but are also found in seafood such as shrimp, not typically foods consumed in the Midwest (20-21).

Some DHA fortified foods and beverages recently introduced in US Midwestern city grocery stores use an algal form of DHA, life'sDHA^{TM}, a product of Martek Biosciences Corporation (22). These fortified foods and beverages may provide an acceptable solution for low consumption of DHA in the Midwestern preschool population leading to the consumption of heart-healthy fats and decreasing the risk for obesity-related chronic disease.

Very little research has been conducted specifically on preschoolers' willingness to try and preference for foods rich in the DHA form of omega-3 fatty acids. Willingness to try foods rich in DHA (overcoming food neophobia) and the development of a preference and willingness to consume foods rich in DHA (overcoming food pickiness) are both important in the incorporation of these beneficial foods as a part of a culturally acceptable diet. Accepted foods are foods which are considered to be "typical" or "normal" and palatable to individuals. These foods are strongly tied to culture as well as individual food preferences based on taste, texture and appearance (23).

Food preference development is influenced by both genetic and environmental factors. Food neophobia tends to be related to child and maternal temperament (general

neophobia) in contrast to food pickiness which is more related to environmental factors such as food exposure and variety (7).

The purpose of this article is to describe the willingness of Head Start/Early Childhood Toddler Educare (ExCITE) Program preschoolers' willingness to try and preference for "novel" and "typical" Midwestern foods and beverages rich in DHA omega-3 fatty acids. A third purpose of the article is to explore the relationship between willingness to try "novel" and "typical" Midwestern foods and beverages rich in DHA omega-3 fatty acids and reported child food neophobia. The findings may be used to help describe potential willingness of US Midwestern families to accept DHA rich foods into their diets.

METHODS

Study Design and Participants

A convenience sample of 3-5 year old children enrolled for the fall 2010 semester in one of eight Lincoln Public Schools Head Start/ExCITE Program classrooms and their parents was used for this study. Participation eligibility was not based on racial or ethnic background; however, classrooms were selected for demographic populations paralleling the US Midwestern population. Both genders of children and parents were equally recruited. Classroom fall parent-teacher conferences were where recruitment and parental completion of the informed consent took place.

Measures

The study design included the parents' completion of two questionnaires. The first questionnaire, Eating Habits of Preschool Children, was used to determine child and parental consumption of and familiarity with a list of foods naturally rich in DHA (four types of canned convenience fish most available in the Midwest) and 10 foods and beverages with DHA fortified counterparts. This information was used to categorize foods as "novel" or "typical" foods. Questionnaire development and findings are related in detail in Manuscript II: Exposure of US Midwestern Preschool Children and Their Parents to Foods Naturally Rich in Docosahexaenoic Acid (DHA) Omega-3 and Foods with DHA Omega-3 Fortified Counterparts and Parental Awareness of Omega-3. The second questionnaire, the Food Neophobia Scale Questionnaire was used to determine the families' and children's food neophobia.

After the parental questionnaires were completed, the children participated in a taste test experiment in which they were asked to try 14 foods and beverages naturally rich or fortified with DHA. The children used a three point cartoon face hedonistic scale to affectively rate the foods as "yummy," "just okay" or "yucky." The children's willingness to try each food as well as their preference for each food were recorded. The food preferences were coded three for "yummy," two for "just okay" and one for "yucky."

Statistical Analysis

Data were analyzed using IBM SPSS (version 19.0, 2010, IBM Corporation, Somers, NY). Foods were categorized into "typical" or "novel" foods based on parent-reported

data obtained from the Eating Habits of Preschool Children questionnaire. “Typical” foods were defined as those which 70% or more of the parents reported their children had tried at home; “novel” foods were defined as those reported to have not been tried by 70% or more of the children at home. The children’s willingness to try typical or novel foods was calculated as the number of “yes” or “no” responses out of the total seven foods offered in each category. The mean of the children’s willingness to try the typical foods was tested against the mean of the children’s willingness to try novel foods using a paired samples t-test. To determine the willingness to try (yes or no) novel foods compared to typical foods, McNemar nonparametric tests were conducted for all (49 tests) novel food and typical food pairs. McNemar nonparametric tests were then conducted for all (40 tests) fish versus fortified food pairs to see if tuna, a typical fish, pairs were significant. McNemar tests were then conducted for all (25 tests) mackerel and sardine pairs.

The mean of the children’s preference for the typical foods was tested against the mean of the children’s preference for the novel foods using a paired samples t-test. Comparisons of the means of typical versus novel foods were also conducted after removing mackerel and sardines from the novel foods. Paired t-tests were also used to compare the preference means of fortified DHA foods with those of DHA rich fish.

A total food neophobia score was calculated for each child by summing the scores of two previously validated children’s food neophobia measuring questionnaires completed by the children’s parents. Scores could range from 15-95 – the higher the score, the greater the child’s food neophobia. A nonparametric Spearman’s rho correlation was

conducted between the mean of the children's willingness to try the typical foods and the mean preschoolers' food neophobia score.

The UNL Institutional Review Board approved the study protocol, all participating parents provided written informed consent and children provided verbal assent. A detailed description of the development and protocol of the methods are reported in Manuscript I: Development of a Methodology to Measure US Midwestern Preschool Children's Willingness to Try and Preference for Foods Rich in Docosahexaenoic Acid Omega-3.

RESULTS AND DISCUSSION

Participant Characteristics

Study participants included 47 preschool children (M = 21, F = 26) and their parents (M = 4, F = 43). Children ranged from 3-5 years of age with the majority (n = 37, 79%) being four years old. The majority of participants considered themselves to be solely Caucasian/White (n =28, 60%) with the next largest group checking more than one racial/ethnic category (n = 10, 21%). The majority of the parents reported that they had either completed high school/GED (n = 13, 28%), some college (n = 13, 28%). Nine had completed an associate's degree (19%).

Willingness to Try Foods

The children were significantly more ($t = 2.381$, $df = 46$, Sig. 1-tailed .0105, $P<0.05$) likely to try typical foods (6.55, SD ± 1.27) than novel foods (6.26, SD ± 1.67). Of the 49 novel food-typical food pairs, only those which contained mackerel or sardines were

significantly different (P<0.05) in terms of the children's willingness to try these foods. No significant differences in willingness to try foods were found in any of the tuna or salmon-typical food pairs when McNemar tests were conducted for fish and non-fish pairs. Significant mackerel and sardine pairs with non-mackerel and non-sardine foods are recorded in Table 1. These results indicated that the willingness to try specific foods such as mackerel and sardines may be more of a factor than the typical and novel categories. These two fish are strong smelling foods which may have been an influencing factor in willingness to try them.

Overall, the children the majority of the children tried and rated most foods and beverages. This may have been due to the excitement of getting to participate in a new activity. Both the parents and the teachers expressed surprise that the children were so willing to try new foods and beverages. Children may be more willing to try foods than adults think (8).

Preference for Foods

The children were significantly (t = 2.635, *df* = 46, Sig. 1-tailed .0055, P<0.05) more likely to prefer typical foods (2.61, SD ± 0.50) than novel foods (2.39, SD ± 0.73). However, when the mackerel and sardine preference ratings were removed from the novel foods, there was no statistically significant difference (t= 1.245, *df* = 46, Sig. 1-tailed .1095, P<0.05) between the mean of the typical foods (2.61, SD ± 0.50) and the mean of novel foods (2.50, SD ± 0.72). The children were significantly (t = 4.612, *df* = 46, Sig. 1-tailed .000, P<0.05) more likely to prefer DHA fortified foods (2.63, SD ± 0.51) than DHA fish (2.05, SD ± 1.00). Preference scores are reported in Table 2.

These findings indicate that the children's preference seemed to be most favorable for fortified foods offered in this study over the fish offered and typical foods offered over novel foods offered. Novel fish was the least palatable to the children. These findings support other studies which have reported that US Midwesterners do not typically include fish in their diets, and it is difficult to get preschool children to try and prefer fish (20-21, 24).

Willingness to Try and Food Neophobia Relationship

The mean preschoolers' food neophobia score was 53.62, SD ± 14.34. A significant negative relationship (-.307, Sig. 1-tailed .018, $P<0.05$) was found between the mean of the children's willingness to try the novel foods and the mean preschoolers' food neophobia score. As the children's food neophobia scores increased, the children were less likely to try novel foods. This correlational finding supports other studies which have validated the use of the Food Neophobia Scale with observed taste test laboratory behavior (7, 10-11, 25). Other studies have indicated that food neophobic behaviors may be overcome with adult modeling as well as repeated exposure to the novel foods (26-29).

The scope of the study was limited to the Head Start/ExCITE US Midwestern population and limited by the available DHA fortified products on the market. The study was limited to one taste test session, but in order to increase food acceptance, children may need to try foods they dislike numerous times: this increased preference for foods with repeated exposure has been reported in other studies (26-30). The results of the fish preferences are supported by other regional studies, but further neophobia and preference

studies should be conducted to provide a better understanding of the long-term acceptance of DHA omega-3 foods and beverages into the Midwestern and nation-wide US diets.

CONCLUSIONS

This study provided new information to fill a gap in understanding US Midwestern preschool children's willingness to try and preference for DHA omega-3 foods and beverages. DHA fortified foods particularly those which are culturally typical may be the best way to increase US Midwestern preschool children's intake of this critically important fatty acid. The development of eating patterns which include DHA omega-3 fatty acids during the key preschool years may reduce these children's risks of developing obesity-related cardiovascular disease as adults.

TABLES

Table 1: McNemar Tests Significance for Willingness to Try Mackerel and Sardines

Food Tried	Mackerel n = 37 (78.7%)	Sardines n = 36 (76.6%)
2% Milk, n = 44 (93.6%)	**0.016***	**0.008***
Tuna, n = 41 (87.2%)	0.125	0.063
Banana Sweet Potato Yogurt, n = 44 (93.6%)	**0.016***	**0.008***
Blueberry Pomegranate Juice, n = 45 (95.7%)	**0.008***	**0.004***
Chocolate Milk, n = 46 (97.9%)	**0.004***	**0.002***
Hardboiled Egg, n = 42 (89.4%)	0.063	**0.031***
Margarine, n = 45 (95.7%)	**0.008***	**0.004***
Olive Oil, n = 44 (93.6%)	**0.016***	**0.008***
Plain Soymilk, n = 43 (91.5%)	**0.031***	**0.016***
Salmon, n = 42 (89.4%)	0.063	**0.031***
Strawberry Carrot Yogurt, n = 42 (89.4%)	0.063	**0.031***
Whole Milk, n = 46 (97.9%)	**0.004***	**0.002***

*Significant $P < 0.05$

Table 2: Willingness to Try and Preference for Typical and Novel Foods Rich in DHA

	Refused to Try		Yucky Rating		Neutral Rating		Yummy Rating		Mean Rating
	n	%	n	%	n	%	n	%	
Typical Foods									
Bread with Margarine Spread	2	4.3	7	14.9	0	0	38	80.9	2.69
Bread dipped in Olive Oil	3	6.4	14	29.8	2	4.3	28	59.6	2.32
2% Milk	3	6.4	4	8.5	0	0	44	93.6	2.82
Whole Milk	1	2.1	4	8.5	1	2.1	41	87.2	2.80
Chocolate Milk	1	2.1	0	0	1	2.1	45	95.7	2.98
Hardboiled Egg	5	10.6	7	14.9	3	6.4	32	68.1	2.60
Albacore Tuna	6	12.8	10	21.3	2	4.3	29	61.7	2.46
Novel Foods									
Plain Soymilk	4	8.5	8	17.0	0	0	35	74.5	2.63
Strawberry Carrot Yogurt	5	10.6	7	14.9	0	0	35	74.5	2.67
Banana Sweet Potato Yogurt	3	6.4	7	14.9	0	0	37	78.7	2.68
Blueberry Pomegranate Juice	2	4.3	0	0	0	0	45	95.7	3.00
Mackerel	10	21.3	14	29.8	1	2.1	22	46.8	2.22
Salmon	5	10.6	14	29.8	1	2.1	27	57.4	2.31
Sardines	11	23.4	16	34.0	1	2.1	19	40.4	2.08

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APPENDICIES

APPENDIX A

life'sDHA™ Omega-3 Fortified Foods & Omega-3 Eggs, DHA Content & Cost, and DHA Rich Canned Fish, DHA Content & Cost

Table A1: lifesDHA™ Omega-3 Fortified Foods & Omega-3 Eggs DHA Content & Cost

Fortified Food	Manufacturer	DHA/serving	Cost
LifeBalance Tortillas	Mission®	not stated	$2.50/pkg.
50% Reduced-fat Cheddar Cheese	Cabot®	32 mg/oz.	$3.09/6 oz.
Puritan Canola Oil	Crisco®	32 mg/serving	$2.88/ 16 oz.
Olive/Canola Oil Blend	Pompeian®	32 mg/1 Tbsp	$6.85/ 32 oz.
Plain Soymilk	Silk®	32 mg/8 oz. serving	$3.24/ 1/2 gal.
Little Blends Yogurt - Strawberry Carrot, Sweet Potato Banana, Apple Squash	Horizon Organic®	32 mg/4 oz. serving	$3.88/ 16 oz.
Milk 2%, Whole, 1% Chocolate	Horizon Organic®	32 mg/8 oz. serving	$4.50/ 1/2 gal.
Pomegranate Blueberry Juice	Minute Maid®	50 mg/ 8 oz. serving	$3.99/ 59 oz.
50/50 Blend Stick Butter	Smart Balance®	32 mg/1 Tbsp serving	$3.58/1 lb.
Tub Margarine	Smart Balance®	32 mg/1 Tbsp serving	$2.48/1 lb.
Omega-3 Eggs	Land O Lakes®	350 mg EPA & DHA/1 egg	$2.60/dzn.

Table A2: DHA Rich Canned Fish DHA Content & Cost

Fish	Manufacturer	DHA/serving	Cost
Tuna, Albacore Chunk White in Water	Chicken of the Sea®	535 mg/3 oz.	$2.50/ 5 oz.
Mackerel, Jack in Water	Chicken of the Sea®	677 mg/3 oz.	$1.50/ 15 oz.
Salmon Skinless, Boneless Pink in Water	Chicken of the Sea®	685 mg/3 oz.	$2.88/ 16 oz.
Sardines in Water	Chicken of the Sea®	468 mg/1 tin	$6.85/ 3.75 oz.

Values obtained at USDA National Nutrient Database. Available at: http://www.nal.usda.gov/fnic/foodcomp, Accessed March 5, 2011.

APPENDIX B

Lincoln Public Schools Permission Request Letter

University of Nebraska Lincoln
COLLEGE OF EDUCATION AND HUMAN SCIENCES
Department of Nutrition & Health Sciences

Bethany Murray
University of Nebraska-Lincoln
110 Ruth Lev. Hall
Lincoln, NE 68583-0806
(402) 472-3836

August 1, 2010

Dr. Leslie Lukin
Director of Assessment and Evaluation
Lincoln Public Schools
5901 O Street
Lincoln, NE 68510

Dear Dr. Lukin and Research Review Committee:

I am excited to propose the possibility of a *cutting-edge nutrition research collaboration* between myself, a doctoral candidate at the University of Nebraska-Lincoln Department of Nutrition, and the Lincoln Public Schools ExCITE/Head Start Program. The April meeting between Edith Zumwalt, Jessie Coffey, and myself was extremely valuable in helping me to reshape my proposed research to be an even better fit for the needs of LPS, and your interest was encouraging.

The purpose of this study will be to explore preschool children's willingness to try and preference for *chronic disease preventing* new and familiar foods. My particular interest is for foods rich in the docosahexaenoic acid (DHA) form of omega-3 fatty acids, a key nutrient in the preschool years, with known benefits for heart health, brain cell structure, and retinal development. Midwestern consumption of DHA is below recommended levels primarily because it is found naturally in fish, not a widely-accepted food in the Midwestern culture. However, a range of fortified omega-3 products are now available.

The proposed study will consist of the parents filling out a short check list and two questionnaires on their children's eating habits. In addition, the participating preschool children will participate in a fun food taste test assessment in the ExCITE classrooms. The check list and questionnaires will be sent to the parents via the children's take home folders. The taste test assessments will allow the children to try up to 14 foods rich in omega-3. Some of the foods will be new to the children and some will be familiar. The children's willingness to try the foods as well as their preference for the foods will be recorded. The children will rate the foods using a three cartoon face scale. Both portions of the study (the questionnaires and taste test assessment) should only take 20-30 minutes of parental time and 15-20 minutes of classroom time per child.

This study will require very little of the Head Start teachers and minimal classroom time per child. As a Registered Dietitian, I will make certain that all foods meet mandated guidelines for the program as well as food safety standards. Foods will be provided by UNL and prepared in an approved kitchen. Children with any known food allergies will be excluded from the study for safety concerns.

I believe the project is a good fit for the LPS ExCITE/Head Start Program for three reasons. First, I feel that this research project ties in nicely with the nutrition component of Head Start's mission. I believe that the results of this research will add to Head Start's community impact. Second, low-income children are at greatest risk for childhood obesity and related chronic diseases later in their lives. Preventative nutrition research is a priority in Nebraska and nationally. Third, this research will be helpful in the creation of a model for the introduction of new healthful foods to Head Start children.

I look forward to hearing your thoughts regarding a possible collaborative effort. I would be glad to answer any questions or concerns via phone or in person. My phone number is 417-718-6008. You can also contact me by e-mail bethanymurray@att.net.

I believe that the proposed study's findings could have paramount implications for guiding future research and knowledge of the relationship between children's development of eating patterns and chronic disease risk and prevention. I believe this study is a cutting-edge opportunity at a key time when chronic diseases are increasing in the United States. Thank you in advance for your consideration of this matter and for your valuable time.

Sincerely,

Bethany Murray, MS, RD, LD
Primary Investigator, University of Nebraska-Lincoln

Enclosure:

University of Nebraska-Lincoln Institutional Review Board Protocol & Instruments

APPENDIX C

Lincoln Public Schools Approval Letters

Lincoln Public Schools

5901 O Street • Box 82889 • Lincoln, NE 68501 • (402) 436-1790

RR 11-12

August 23, 2010

Bethany Murray
University of Nebraska-Lincoln
110 Ruth Lev. Hall
Lincoln, NE 68583-0806
bethanymurrary@att.net

RE: Request to Conduct Research in the Lincoln Public Schools

Dear Ms. Murray:

Your request to have Lincoln Public School students enrolled in the ExCITE/Head Start preschool programs participate a study entitled "Preschool children's willingness to try and peference for DHA Omaga-3 foods" is approved. Please contact Deila Steiner, the Director of Federal Programs, to secure her permission to proceed with the implementation of this study. Parental/guardian consent and student assent are required for this study. Please use the form and procedures submitted with your request.

Sincerely,

Leslie E. Lukin

Leslie E. Lukin, Ph.D.
Director of Assessment and Evaluation Services

cc: Edith Zumwalt, Director of Nutrition Services
Deila Steiner, Director of Federal Programs
Kay Byers, Supervisor of Elementary Personnel Service

Title of Research: Preschool children's willingness to try and peference for DHA Omaga-3 foods

LINCOLN PUBLIC SCHOOLS
Federal Programs
5901 O Street • P.O. Box 82889 • Lincoln, NE 68501-2889 • (402) 436-1997 Fax • (402) 436-1929

September 21, 2010

Rachel Wenzl
Research Compliance Services Specialist
Human Research Protection Program
312 N. 14th St., Ste 209, Alex West
Lincoln, NE 68588-0408

RE: Project #10810: A Preschool Children's Willingness to Try and Preference for DHA Omega-3 Foods Pilot Study A

Dear Ms. Wenzl:

Consultation was completed with Lincoln Public Schools early childhood leadership regarding *Project #10810: A Preschool Children's Willingness to Try and Preference for DHA Omega-3 Foods Pilot Study A* on September 21, 2010. Permission to proceed with the implementation of this study is approved.

Respectfully,

Deila J. Steiner

Dr. Deila J. Steiner
Director of Federal Programs

cc: Bethany Murray

APPENDIX D

Head Start/ExCITE Parental Recruitment Letter

University of Nebraska Lincoln
COLLEGE OF EDUCATION AND HUMAN SCIENCES
Department of Nutrition & Health Sciences

Dear Parent or Primary Caregiver:

I am excited to invite you to participate in a cutting-edge University of Nebraska research study on Preschool Children's Willingness to Try and Preference for DHA Omega-3 Foods. The information gathered from this study will be used to support parents in raising children with optimal eating habits. The long-term research goal is that by forming healthful habits, children will have a reduced risk for developing chronic diseases later in life.

You are being asked to participate in the study because your child is enrolling in the Lincoln Public Schools ExCITE/Head Start Program. The study will consist of your filling out a short check list and two questionnaires. In addition, your child may have fun participating in a taste test assessment. Children with known food allergies will not be eligible to participate. The check list and questionnaires will be completed in person or sent to you via your child's take home folder if you agree to participate. If you give consent for your child, he or she will be allowed to participate in the taste test assessment in his or her classroom. Both portions of the study (those completed by you and your child) should only take 20-30 minutes of your time.

Please be assured that your responses will be kept strictly confidential. Your responses and the data recorded for your child will be coded. There is no known risk for participating in this study. Participation is voluntary. You are free to decide not to participate in this study. You can also withdraw at any time without harming your relationship with the researchers or the University of Nebraska-Lincoln. If you have questions about the study, please do not hesitate to call me at (402) 472-3836 or e-mail me at bmurray15@huskers.unl.edu.

Your and your child's participation in the study will greatly help our efforts in understanding the development of preschool eating habits, positively contributing to the health of the next generation. I think your child will enjoy the taste test portion as well. Thank you in advance for your willingness to participate.

Sincerely,

Bethany Murray, MS, RD, LD, Principle Investigator

UNIVERSITY OF NEBRAKSA-LINCOLN

APPENDIX E

UNL Institutional Review Board Informed Consent Form

IRB# 20100810810EP
Date Approved: 10/06/2010
Valid Until: 08/26/2011

ID CODE_____

University of Nebraska Lincoln
COLLEGE OF EDUCATION AND HUMAN SCIENCES
Department of Nutrition & Health Sciences

Informed Consent Form

You and your preschool child enrolled in the Lincoln Public Schools ExCITE/Head Start Program are invited you to participate in a cutting-edge University of Nebraska research study entitled "**Preschool Children's Willingness to Try and Preference for DHA Omega-3 Foods**." Children with known food allergies will not be eligible to participate. The purpose of this study is to explore relationships between familial eating habits, reported child fear of trying new foods, and child willingness to try and preference for foods rich in DHA omega-3 fatty acids. The long-term goal of this study is to change the dietary eating habits of Midwestern low-income children to promote the acceptance of foods rich in DHA omega-3 fatty acids during the key preschool years in order to reduce their risks of developing obesity-related cardiovascular disease as adults. The results could be published in medical journals, books or presented at conferences.

The study will consist of your filling out a short check list and two questionnaires. In addition, your child will participate in a food taste test assessment. The check list and questionnaires will be sent to you via your child's take home folder if you agree to participate. If you give consent for your child, he or she will be allowed to participate in the taste test assessment in his or her classroom. The taste test assessment will allow the child to try up to 15 foods rich in omega-3. Some of the foods will be new to your child and some will be familiar. Your child's willingness to try the foods as well as his or her preference for the foods will be recorded. Your child will rate the foods using a three cartoon face scale. Both portions of the study (those completed by you and your child) should only take 20-30 minutes of your time.

Please be assured that your responses will be kept strictly confidential. Your responses and the data recorded for your child will be coded.

There is no known risk for participating in this study. Participation is voluntary. Your child will not in any way be forced to participate in the taste test assessment if he or she does not wish. You are free to decide not to participate in this study. You can also withdraw at any time without harming your relationship with the researchers or the University of Nebraska-Lincoln. The taste test should be an enjoyable experience for your child; however, no compensation will be provided for participation.

If you have questions about the study, please do not hesitate to call me at (402) 472-3836 or e-mail me at bmurray15@huskers.unl.edu. Sometimes study participants have questions or concerns about their rights. In that case you should call the University of Nebraska-Lincoln Institutional Review Board at (402) 472-6965.

ID CODE_____

After reading the above statements, my signature below indicates that I am willingly consenting to participate in this study AND consenting to my child _____'s participation in the taste test assessment. Furthermore my signature confirms that my child does **not** have any known food allergies.

Signature of Parent/Guardian: Relationship to Child:

_____ _____ _____

Signature of Parent/Guardian Date

Child Name: Age of Child:

_____ _____

Phone Number: Best Time to Reach:

_____ _____

You will be given a copy of this consent form to keep for your records.

Bethany Murray, MS, RD, LD, Principal Investigator Office: (402) 472-3836
Nancy M. Lewis, PhD, RD, LMNT, Secondary PI Office: (402) 472-4633

APPENDIX F

Eating Habits of Preschool Children Questionnaire

ID Code: _____

Eating Habits of Preschool Children

1. **Please check how often your *preschool child* eats or drinks the following foods and beverages.**

Food/Beverage	Never Tried	Less than once per month	Once per month	2-3 times per month	1-2 times per week	3-4 times per week	5-6 times per week	Once per day	More than once per day
Tub/Spread Margarine									
Olive Oil									
Cheddar Cheese									
2% Milk									
Whole Milk									
Chocolate Milk									
Plain Soymilk									
Yogurt									
Strawberry & Carrot Yogurt									
Banana & Sweet Potato Yogurt									
Non-Citrus Juice (ex. grape, apple, cranberry)									
Blueberry Pomegranate Juice									
White Bread									
Hard Boiled Eggs									
Mackerel									
Salmon									
Sardines									
Tuna									

ID Code: _____

2. Please check how often *you* eat or drink the following foods and beverages.

Food/Beverage	Never Tried	Less than once per month	Once per month	2-3 times per month	1-2 times per week	3-4 times per week	5-6 times per week	Once per day	More than once per day
Tub/Spread Margarine									
Olive Oil									
Cheddar Cheese									
2% Milk									
Whole Milk									
Chocolate Milk									
Plain Soymilk									
Yogurt									
Strawberry & Carrot Yogurt									
Banana & Sweet Potato Yogurt									
Non-Citrus Juice (ex. grape, apple, cranberry)									
Blueberry Pomegranate Juice									
White Bread									
Hard Boiled Eggs									
Mackerel									
Salmon									
Sardines									
Tuna									

3. **Has your child ever had any kind of fish?**
   - O Yes
   - O No

4. **Does your child have any known food allergies or food intolerances?**
   - O Yes
   - O No

   If yes, please check all that apply
   - O Dairy products (including milk, cheese, butter, and yogurt)
   - O Eggs
   - O Fish
   - O Soy
   - O Wheat
   - O Other, please specify _____

ID Code: _____

5. **Have you heard of omega-3 fatty acids?**

- O Yes
- O No

If yes, how knowledgeable would you say you are about omega-3 fatty acids?

- O Very knowledgeable
- O Somewhat knowledgeable
- O Not very knowledgeable
- O Not at all knowledgeable

6. **Have you heard of docosahexaenoic acid (DHA)?**

- O Yes
- O No

If yes, how knowledgeable would you say you are about DHA?

- O Very knowledgeable
- O Somewhat knowledgeable
- O Not very knowledgeable
- O Not at all knowledgeable

ID Code: _____

7. **Are you aware of any food or beverage products fortified with omega-3?**

   - O Yes
   - O No

   If yes, please list.

8. **Have you seen the life's DHA™ logo on any foods or beverages which you have purchased?**

   - O Yes
   - O No

   If yes, please list.

ID Code: _____

9. How many children do you have at home?

10. Please list the ages of the children in your household.

11. What is your race/ethnicity? Please check all that apply.

- O African American/Black
- O American Indian
- O Asian or Pacific Islander
- O Caucasian/White
- O Hispanic/Latino
- O Other_____

12. What is the highest level of education which you have completed?

- O Less than high school
- O High school graduate or GED
- O Some college
- O Associates degree
- O Bachelor's degree (4 year)
- O Graduate degree

APPENDIX G

Food Neophobia Scale Questionnaire

ID Code: _____

Family Eating Patterns*

Please check how much you agree or disagree with the following statements as it relates to ***your family eating patterns in general.***

	Agree Strongly	Agree Moderately	Agree	Neither Agree nor Disagree	Disagree	Disagree Moderately	Disagree Strongly
1. We are constantly sampling new and different foods.							
2. We don't trust new foods.							
3. If we don't know what is in a food, we won't try it.							
4. We like foods from different countries.							
5. Ethnic food looks too weird to eat.							
6. At dinner, we will try a new food.							
7. We are afraid to eat things we have never had before.							
8. We are very particular about the foods we will eat.							
9. We will eat almost anything.							
10. We like to try new ethnic restaurants.							

*Credit for scale to Pliner, P. (1994). Development of measures of food neophobia in children. *Appetite, 23*(2), 147-163.

ID Code: _____

Preschooler Eating Patterns*

Please check how much you agree or disagree with the following statements as it relates to your ***preschool child.***

	Agree Strongly	Agree Moderately	Agree	Neither Agree nor Disagree	Disagree	Disagree Moderately	Disagree Strongly
1. My child is constantly sampling new and different foods.							
2. My child doesn't trust new foods.							
3. If my child doesn't know what is in a food, he or she won't try it.							
4. My child likes foods from different countries.							
5. My child thinks ethnic food looks too weird to eat.							
6. At dinner, my child will try a new food.							
7. My child is afraid to eat things he or she has never had before.							
8. My child is very particular about the foods he or she will eat.							
9. My child will eat almost anything.							
10. My child likes to try new ethnic restaurants.							

*Credit for scale to Pliner, P. (1994). Development of measures of food neophobia in children. *Appetite, 23*(2), 147-163.

ID Code: _____

Preschooler Eating Patterns Continued*

Please check how much you agree or disagree with the following statements as it relates to your ***preschool child.***

	A Lot Like the Child	Usually Like the Child	Half the Time Like the Child	Rarely Like the Child	Not at All Like the Child
1. My child rarely takes new food without fussing.					
2. My child consistently dislikes many kinds of food.					
3. My child makes faces at new foods.					
4. Once my child decides he or she doesn't like something, there is no getting him or her to like it.					
5. My child has strong likes and dislikes in food.					

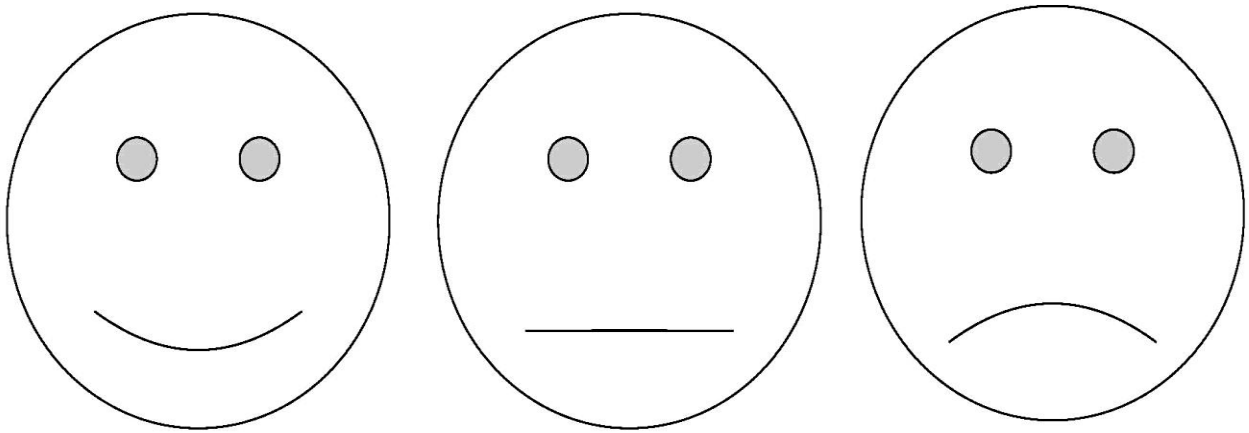
* Credit for scale to Rowe, D.C. & Plomin, R. (1977). Temperament in Early Childhood. *Journal of Personality Assessment, 41*(2), 150-156.

APPENDIX H

Three Face Hedonistic Scale

ID Code: _____

3 Point Hedonistic Face Scale



"Yummy" – 3

Foods:

"Just Okay" – 2

Foods:

"Yucky" – 1

Foods:

APPENDIX I

Frequency of Preschool and Parental Consumption of And Beverages Naturally Rich in DHA Omega-3 or those which have Fortified Counterparts

Table I1: Frequency of Preschool Consumption of Foods Naturally Rich in DHA Omega-3 or those which have Fortified Counterparts

	Never Tried		< 1 x/Month		1 x/ Month		2-3 x/Month		1-2 x/Week		3-4 x/Week		5-6 x/Week		1x/Day		> 1 x/Day	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Familiar Foods																		
Margarine Spread	0	0	5	10.6	2	4.3	10	21.3	17	36.2	6	12.8	4	8.5	2	4.3	1	2.1
Olive Oil	13	27.7	12	25.5	5	10.6	1	2.1	10	21.3	3	6.4	3	6.4	0	0	0	0
2% Milk	1	2.1	5	10.6	2	4.3	1	2.1	1	2.1	3	6.4	4	8.5	6	12.8	24	51.1
Whole Milk	9	19.1	18	38.3	2	4.3	2	4.3	2	4.3	6	12.8	1	2.1	2	4.3	5	10.6
Chocolate Milk	1	2.1	4	8.5	9	19.1	9	19.1	15	31.9	2	4.3	3	6.4	0	0	4	8.5
Hardboiled Egg	7	14.9	11	23.4	7	14.9	17	36.2	4	8.5	1	2.1	0	0	0	0	0	0
Albacore Tuna	9	19.1	10	21.3	14	29.8	8	17	4	8.5	1	2.1	1	2.1	0	0	0	0
Bread	2	4.3	7	14.9	5	10.6	6	12.8	9	19.1	7	14.9	8	17	2	4.3	1	2.1
Cheese*	0	0	2	4.3	1	2.1	3	6.4	17	36.2	17	36.2	6	12.8	1	2.1	0	0
Non-Citrus Juice*	1	2.1	1	2.1	4	8.5	7	14.9	5	10.6	16	34	7	14.9	3	6.4	3	6.4
Yogurt*	2	4.3	6	12.8	2	4.3	11	23.4	6	12.8	11	23.4	7	14.9	1	2.1	1	2.1
Novel Foods																		
Plain Soymilk	42	89.4	4	8.5	0	0	0	0	0	0	0	0	1	2.1	0	0	0	0
Strawberry Carrot Yogurt	39	83	1	2.1	3	6.4	0	0	3	6.4	1	2.1	0	0	0	0	0	0
Banana Sweet Potato Yogurt	39	83	4	8.5	0	0	2	4.3	1	2.1	1	2.1	0	0	0	0	0	0
Blueberry Pomegranate Juice	39	83	4	8.5	2	4.3	1	2.1	0	0	1	2.1	0	0	0	0	0	0
Mackerel	43	91.5	3	6.4	0	0	1	2.1	0	0	0	0	0	0	0	0	0	0
Salmon	33	70.2	8	17	4	8.5	2	4.3	0	0	0	0	0	0	0	0	0	0
Sardines	45	95.7	2	4.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table I2: Frequency of Parental Consumption of Foods Naturally Rich in DHA Omega-3 or those which have Fortified Counterparts

	Never Tried		< 1 x/Month		1 x/Month		2-3 x/Month		1-2 x/Week		3-4 x/Week		5-6 x/Week		1x/Day		> 1 x/Day	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Familiar Foods																		
Margarine Spread	0	0	2	4.3	5	10.6	7	14.9	14	29.8	10	21.3	3	6.4	5	10.6	1	2.1
Olive Oil	7	14.9	15	31.9	5	10.6	5	10.6	6	12.8	5	10.6	2	4.3	2	4.3	0	0
2% Milk	4	8.5	13	27.7	2	4.3	2	4.3	4	8.5	7	14.9	1	2.1	5	10.6	9	19.1
Whole Milk	7	14.9	28	59.6	2	4.3	2	4.3	0	0	4	8.5	1	2.1	1	2.1	2	4.3
Chocolate Milk	2	4.3	17	36.2	10	21.3	6	12.8	6	12.8	3	6.4	0	0	2	4.3	1	2.1
Hardboiled Egg	3	6.4	14	29.8	8	17	14	29.8	6	12.8	1	2.1	0	0	1	2.1	0	0
Albacore Tuna	4	8.5	11	23.4	8	17	12	25.5	7	14.9	4	8.5	1	2.1	0	0	0	0
Bread	1	2.1	13	27.7	2	4.3	5	10.6	6	12.8	8	17	5	10.6	4	8.5	3	6.4
Cheese*	0	0	4	8.5	2	4.3	3	6.4	12	25.5	19	40.4	6	12.8	1	2.1	0	0
Non-Citrus Juice*	4	8.5	6	12.8	5	10.6	5	10.6	10	21.3	10	21.3	2	4.3	4	8.5	1	2.1
Yogurt*	3	6.4	10	21.3	5	10.6	8	17	4	8.5	7	14.9	7	14.9	2	4.3	1	2.1
Novel Foods																		
Plain Soymilk	36	76.6	8	17	0	0	0	0	1	2.1	1	2.1	1	2.1	0	0	0	0
Strawberry Carrot Yogurt	44	93.6	1	2.1	1	2.1	1	2.1	0	0	0	0	0	0	0	0	0	0
Banana Sweet Potato Yogurt	45	95.7	1	2.1	0	0	1	2.1	0	0	0	0	0	0	0	0	0	0
Blueberry Pomegranate Juice	41	87.2	2	4.3	2	4.3	1	2.1	1	2.1	0	0	0	0	0	0	0	0
Mackerel	41	87.2	6	12.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salmon	20	42.6	17	36.2	5	10.6	5	10.6	0	0	0	0	0	0	0	0	0	0
Sardines	35	74.5	10	21.3	1	2.1	1	2.1	0	0	0	0	0	0	0	0	0	0