ASSOCIATION BETWEEN ESSENTIAL FATTY ACIDS IN GROWTH AND COGNITIVE FUNCTION IN GHANAIAN CHILDREN

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ABSTRACT

ASSOCIATION BETWEEN ESSENTIAL FATTY ACIDS IN GROWTH AND COGNITIVE FUNCTION IN GHANAIAN CHILDREN

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Background: There is a growing evidence from experimental and observational]studies on the importance of essential fatty acid (n-3 and n-6 fatty acids) in growth and cognitive development of children. The dietary intake of fats in most developing countries is usually lower than adequate levels. In Ghanaian children below five years of age, 19% are stunted, 5% are wasted and 11% are underweight. Stunting levels have decreased in the overall population, however, the situation still persist in Northern Ghana where 33% of all under-fives are stunted. The reason for this disparity is unknown, however, interventions have focused on vitamins, minerals and proteins, but none has focused fatty acids (FAs). Recent studies have utilized fat based supplements, however, none of the studies have assessed the FA status in Ghanaian children neither have the relationship between FA status and cognition been measured. Therefore the objective of this research was to measure whole blood levels of FA in Ghanaian children and to establish the association of the FA levels with growth and cognitive abilities.

Methods: The study was conducted in two populations in Ghana: Northern Ghana population - Savelugu-Nanton district (n=306) and Southern Ghana - Upper Manya Krobo district (n=209). A drop of whole blood was collected on an antioxidant treated card. The dried blood spot was analyzed for FA composition using gas chromatography. Weight

and height were measured, age and gender information were taken. Weight-for-height, height-for-age, body mass index-for-age and weight-for-length z-scores were calculated with the World Health Organization Anthro software suite. Executive function was assessed with dimensional change card sort (DCCS) task. Seeds, nuts and oils were collected from locations in Northern and Southern Ghana. Fats were extracted from these foods by acidified methanol. FA composition of extracts was measured using the DSQII quadruple GC/MS. Minerals in seeds were quantified with ICP emission spectroscopy.

Results: In the Northern Ghana population, 8.0% of children were essential FA deficient and 10.6% of the Southern Ghanaian children were essential FA deficient as defined by T/T ratio >0.02. In Northern Ghana: 29.7% of the children were stunted, n-6 FAs were inversely associated with stunting and n-3 FAs are positively associated with executive function. In Southern Ghana: 22.0% of children were stunted and no FAs was associated with stunting. When the FAs were compared for both regions, n-3 FAs levels were significantly higher in the Southern Ghana population and n-6 FAs level were significantly higher in the Northern Ghana population. Although these results are just associations, they indicate the possible role of n-6 FAs in growth and n-3 FAs in cognition. Melon seeds (agushie), soybeans, palm oil, fermented dawadawa, neri and cashew nuts were identified as good sources of EFA and minerals.

Conclusion: This dissertation demonstrates a strong association between whole blood essential fatty acids and growth and cognition. It further confirms the essentiality of n-6FAs in growth and gives insights on the relationship between n-3 FAs and cognition. Foods that contain EFAs in this population were also identified. These findings provide a basis of further research on how local foods can be used to eradicate malnutrition

This thesis is dedicated to my friend and partner the Holy Spirit.
You brought me this far

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KEY TO SYMBOLS AND ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha linolenic acid
BAZ	BMI for age z-score
DBS	Dried Blood spot
DCCS	Dimensional change card sort
DHA	Docoxahexanoic acid
EFA	Essential fatty acids
EFAD	Essential fatty acid deficiency
EPA	Eicosapentanoic acid
FA	Fatty acids
FADS	Fatty acid Desaturase
FAMES	Fatty acid methyl esters
GC-MS	Gas Chromatography - Mass Spectroscopy
HAZ	Height for age z-scores
LA	Linoleic acid
LCPUFA	Long Chain Poly Unsaturated Fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Poly Unsaturated fatty acid
WHO	World Health Organization

CHAPTER 1: RATIONALE

Background

During infancy and childhood, there is an increased need for nutrients in individuals because rapid growth and development occurs during this stage. This increased need causes infants and children to be susceptible to nutrient deficiencies. Nutrient deficiencies during this stage of life can have detrimental impacts among which is stunting. Stunting levels have declined in the past decades in Ghana but the situation still persist in Northern Ghana. Though the reasons for these regional differences in Ghana are not entirely clear, it is likely that location or local culture may govern limited access as well as cooking methods of the foods that could provide these dietary sources of fatty acids. The intake of fatty acids in most developing countries is low [1] and Ghana is not an exception and this dietary pattern could be leading to stunting and cognitive impairment. However, Ghana is home to seeds, nuts and oils that are high in fat but the EFA composition of some of these foods is unknown. This knowledge deficit may lead to under-utilization of such foods especially in child feeding. In an attempt to solve these problems in Ghana, a lot of interventions have been explored including the use of lipid based supplements [2], but the interventions do not assess the associations of various fatty acids in growth and cognitive function [3]. The supplements also utilizes only peanuts and soybean oil[2] but there are several seeds, nuts and oils in Ghana that could be explored and lead to diversification of the food supplementation. Apart from the supplements, the EFA rich foods identified can be included in child feeding. Considering the prevalence of stunting in Ghanaian children, the role of EFA in growth and cognitive function and the probable

under-utilization of EFA-rich seeds, nuts and oils, there is a need to know if there exist an association between EFA status and growth or cognitive function in children as well as the EFA composition of commonly consumed foods in Ghana.

The long term goal of this study is to determine the physiological role of EFA in growth and cognitive function in Ghanaian children 2 to 6 years of age and also to identify some Ghanaian foods that are rich in EFA that can be used for probable interventions especially in child feeding. The short term goal is to assess EFA status in Ghanaian children and to know their association with growth and cognitive function as well as quantify the EFA composition of commonly consumed seeds, nuts and oils in Ghana.

Our central hypothesis is that EFA levels is low in Ghanaian children between 2 to 6 years of age and EFA and will correlate with growth (as indicated by weight for age (underweight), weight for height (wasting), height for age (stunting) and BMI for age and cognitive abilities of these children. We will test the hypothesis using the following specific aims

Specific Aims

Specific Aim 1.Determine whole blood FA status in children 2-6 years of age from Northern and Southern Ghana and determine EFAD prevalence. Our central hypothesis is that there will be variations in EFA status in the different populations.

Specific Aim 2a. Establish the associations between FA status and weight for height (wasting), weight for age (underweight), and height for age (stunting) and BMI for age z-scores in these children. Our central hypothesis is that whole blood EFA and z-scores will be positively correlated. It is further hypothesized that mead acid content and T/T ratio will be negatively correlated with the z-scores.

Specific Aim 2b. Assess the relationship between FA status and cognitive abilities of these children. Our central hypothesis was that EPA and DHA will correlate positively with cognitive function. We also hypothesized that Mead acid and T/T ratio will correlate negatively with cognitive function.

Specific Aim 3. Identify oils, seeds and nuts used in this population and determine their FA and mineral content. Our central hypothesis is that some of the oil, seeds and nuts in Ghana have varying contents of EFA and minerals.

Significance and innovation

Though there have been studies in Ghana about EFA and child growth, these studies administered lipid based supplements without a detailed profiling of blood fatty acids [1], but this study will profile 24 fatty acids and know how the physiological changes of these fatty acids affect growth and cognitive function in children. The lipid based supplement adapted in a study in Ghana utilized only peanuts and soybean oil in its formulation [2], but there are seeds, nuts, and oils in Ghana whose EFA composition is unknown and the identification of such foods can lead to a diversification of the supplement formulation. Also the identification of these foods will be beneficial as these foods can be promoted to mothers so that they can be included in the foods for children. The EFA composition of the seeds, nuts and oils will be included in the West African food composition table.

Moreover, the assessment of fatty acids have been ignored in developing countries due to technical (FA oxidation) and logistical (storage/shipment) problems. The collaboration with Dr. Harris, a leading expert in blood fatty acid analysis and the utilization of blood spot card pre-treated with an anti-oxidant cocktail (OxystopTM) for use in this

study is very innovative. This treatment assures sample integrity at room temperature for up to two weeks during storage and shipment. This ensures that samples arrive in the United States for a detailed fatty acid analysis which would be difficult to be conducted in Ghana.

CHAPTER 2: LITERATURE REVIEW

Introduction

Stunting and cognitive impairment is a global public health problem because it prevents people from realizing their mental and physical potentials [3]. Apart from the funds that are drained from various national resources to alleviate the problem, undernutrition in early life can lead to neurological damage that can lower the wages of individuals because they never finish their schooling. Stunting, which can be a result of poor fetal growth and sustained poor growth in early life, followed by rapid weight gain in later years, may predispose an individual to the development of chronic diseases such as diabetes and cardiovascular complications[4].

The causes of stunting and cognitive impairment include social, economic and cultural factors, maternal and child care, health and sanitation, household food security and nutrient intake. Among these causes, nutrient intake is the immediate cause because there are certain critical and sensitive stages during which adequate nutrition is essential [5]. This means that the diet of individuals should comprise all nutrients in their right proportions: carbohydrates, proteins, fats, vitamins, and minerals.

The intake of fatty acids is important in growth and cognitive function in particular intakes of essential fatty acids (EFA) may prevent stunting and cognitive impairment. These EFAs include omega-3 fatty acids such as alpha linolenic acid and omega-6 fatty acids such as linoleic acid. Omega-3 fatty acids are found in foods such as flaxseed, soy, canola and walnut, while omega-6 fatty acid are found in foods such as sunflower and peanut oil.

These are referred as ‘essential’ because they cannot be synthesized in the body from metabolic precursors but need to be obtained from the diet [6].

EFAs (ALA and LA) and their derivatives (EPA, DHA and AA) have key roles in many developmental processes in humans. EFAs are responsible for myelination of brain neurons and the formation of synapses [7]. They are also known to have roles in stem cell proliferation and differentiation [8]. Supplementation of fish oils positively affects visual evoked potential [9], improves IQ maturation and visual acuity [10] in full term babies. EFA also improves verbal learning [11], short memory and linear growth in infants [12]. Progress in myelination and motor development [13] as well as faster processing of information [14] also occurs with intake of EFA in children. Improved problem solving [15] and better recognition memory [16] have also been documented in children who were supplemented with EFA. Additionally, maternal intake of EFAs is known to influence brain development of the fetus [17]. These are clear indications that EFAs can prevent stunting and cognitive impairment.

In Ghana, intake of fatty acids in infants is below adequate levels. This may make children vulnerable to essential fatty acid deficiency (EFAD), leading to growth deficits and cognitive impairment. The main cause of this problem is poverty and food insecurity. Therefore establishing the relationship between EFA and growth and cognitive function is important. Assessing EFA content in seeds, nuts and oils will set the foundation for intervention using locally available foods to curb the situation.

The Ghana story

Ghana is a small coastal country in West Africa with the majority of people residing the urban population. Like other developing countries, it has numerous natural resources but poverty still remains a problem especially in the north. Although agriculture in Ghana is a major contributor to the economy, it still remains at the traditional level where most farmers practice subsistence farming[18].

The Ghanaian diet is comprised of starchy roots (cassava, yam, and cocoyam), fruits (plantain, oranges) and cereals (maize, millet). Ghana is also the home of various seeds, nuts and oils that are rich in fatty acids. Among these are melon seeds, shea butter, tiger nuts, palm oil and coconut. The starches, cereals and fruits supply about 75% of dietary energy but proteins and lipids are below adequate levels. Due to urbanization, there has been a modification of the diet with an inclusion of wheat and rice but food insecurity remains a problem. [18]

Breastfeeding is a common practice in Ghana however only about 50% of children below 6 months are exclusively breastfed [18]. After 6 months of exclusive breastfeeding, complementary foods are introduced to children which are sometimes low in nutrients. For example, it is reported that among children between 6 and 24 months, only 36.6% have some fats added to their complementary food [19]. These feeding practices, in addition to the composition of the Ghanaian diet, are among the causes of stunted growth in Ghanaian children. For children below age 5, the prevalence of stunting, wasting, and underweight is 19%, 5% and 11% respectively. Stunting has declined nationwide but the situation persists in the Northern Region with 33.1% of all children below age 5 in the region are stunted [20]. There is a need to know the cause of these regional variations.

The UNICEF Global Damage Assessment Report states that some children might not be realizing their physical, mental and intellectual capabilities due to nutrient deficiencies [3]. Apart from stunting, cognitive impairment may also occur due to nutrient deficiencies. Nutrients such as iron and iodine have been associated with cognitive impairment and stunting [21] but there is no published work linking EFAD to cognitive function or growth in Ghana despite the known crucial role of EFA in development. In an attempt to solve these problems, currently, many interventions have been explored including the use of lipid-based supplements [2], but the interventions do not assess the associations between various fatty acids and growth and cognitive function [1]. There are also several seeds, nuts and oils locally grown in Ghana that are nutritionally dense and provides essential nutrients but have never been considered as components of the child's diet. These foods could be explored and included in child feeding as well as in food supplements. Considering the prevalence of stunting in Ghanaian children and its regional variation, there is a need to know the prevalence of EFA status of children in both regions, north and south, and how that relates to the prevalence of stunting. Also, given the role of EFA in growth and cognitive function it will be great to know the physiological role of individual fatty acids. Also, with the probable under-utilization of EFA-rich seeds, nuts and oils, EFA composition of commonly consumed foods in Ghana can be identified and assessed.

Fatty acids

Fatty acids play crucial roles in metabolism and these include their role as a major metabolic fuel (storage and transport of energy). They act as important components of

cell membranes in addition to their role in gene regulation. [22]. Beside these roles, some of them are closely associated with growth and cognitive development.

Dietary fatty acids comprise of all lipids that are abundant in animal and plant tissues and are eaten as food. The commonest fats have been grouped based on their degree of saturation: saturated fats have no double bonds, monounsaturated fatty acids (MUFA) have a single double bond and polyunsaturated fatty acids (PUFAs) have two or more double bonds. Both saturated fats and unsaturated fats are further classified based on their chain length[6]

Alpha linolenic acid (ALA) and linoleic acid (LA) are essential fatty acids, this means that, they cannot be synthesized in the body but must be included in the diet. EPA and DHA can be synthesized from ALA and AA can be synthesized from LA through desaturation, chain elongation and chain shortening (figure 1). The enzyme delta desaturase is an important enzyme in this. Both conversions from LA to AA and ALA to DHA compete for this enzyme and the affinity of the enzyme to substrate is in this order ALA>>LA>>OA. This has been used to develop biomarkers for EFAD[23].

Adequate intake of LA and ALA is based on the median intakes by different populations with diverse life stages and gender groups in the United States but there is insufficient data to set upper limits. Also, the term Adequate Intake (AI) is used because there is insufficient data to set RDAs. Consumption below the AI may lead to deficiencies in individuals and may have the capacity to cause chronic deficiencies[24].

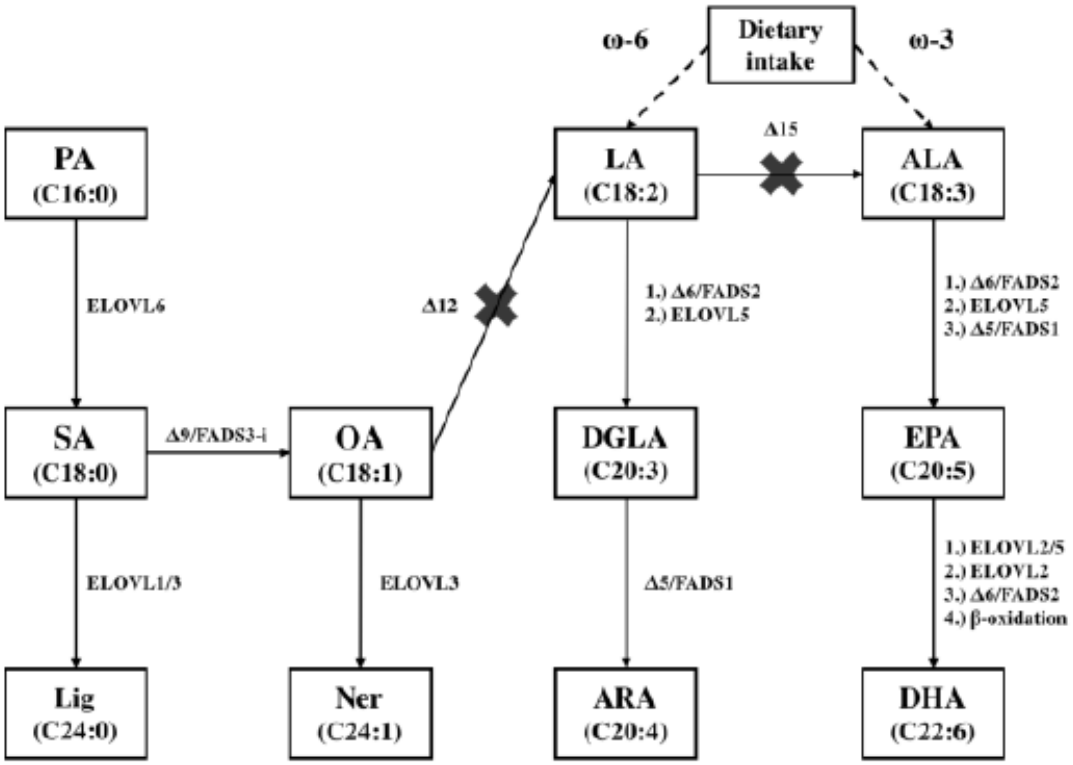

Figure 1: Fatty acid biosynthesis in humans

EFA in Cognitive function

It has been established that, EFAs play important roles in human development, both in fetal and neonatal development. Also the brain and retinal tissues have been shown to be highly dependent on EFAs, especially DHA for membrane fluidity and signal transduction. It has also been shown that, in children, EFAs contributes to cognitive development and may have a role in metabolic programming as well as in bone turnover and adipogenesis[25]. They also have roles in neurophysiology and disease prevention in adults [26] . These show that, throughout the life cycle, EFAs have crucial roles.

The period of growth and metabolic turnover in the entire human life cycle occurs during fetal developmental. It has been reported that, the average human brain exhibits a 60-fold increase in weight, from second trimester to two years; 20 to 1200g. Also, this is the period where neurons in the brain undergo rapid myelination and the synapses mature. It has been shown that ALA and LA levels decrease in the infant's brain during the third trimester of pregnancy, but the levels of DHA and AA increases about 30-fold. An increase in concentration may be the body's natural way of maintaining the right amounts for proper functioning. Among the functions of DHA and AA include the optimization of fluidity of cell membranes, modulation of neurotransmitter physiology including signal transduction by acetylcholine, dopamine and serotonin. They also have implications on cognition, vision and behavior. It has also been proven in randomized controlled trials in rats that, the groups fed with fish oils, soybean oil and safflower oil, had increased DHA and AA in the neuronal growth cone membrane. This suggests that, the maternal intake of dietary fatty acid may affect growth of fetal neurons and the biochemistry of neurotransmitters. Improved visual contrast discrimination, reversal task learning and psychomotor performance was enhanced in beagle puppies, which were fed with diets rich in DHA [22].

EFA in Growth

Although there is little literature about EFA and children above 2 years, it is known that, an adequate amount and balanced supply of DHA and AA are required for growth and cognitive development. It has also been shown that the supplementation of the diet of children aged 7 to 9 year with bread spread rich in omega-3 showed an improvement in

verbal and learning ability of the children. In addition, supplementation of fish oils for children aged 3 to 13 years showed improvements in cognitive function [22].

EFAs may have a role in growth and scientifically based evidence indicates that there is an association with omega-3 PUFA intake and weight gain as well as height [27]. Though there are fewer studies to show that essential fatty acid deficiency (EFAD) influences protein energy malnutrition (PEM), there exists an association between EFA status and PEM.

Though this claim has not been widely substantiated, both PEM and EFAD have similar symptoms, notably skin changes, impaired immunity, impaired and disturbed growth. It has been hypothesized that, a low fat intake can lead to impaired lipid digestion, absorption and transport and this can consequentially lead to desaturation and EFA beta oxidation and peroxidation. Prolonged EFAD may decrease lipid absorption hence worsens PEM by impairing nutrient absorption and dietary calorie utilization. Also dietary fats are known to provide energy for individuals, hence a diet that is deficient in fats can lead to PEM [28].

Biomarkers of Essential fatty acid deficiency

EFAD is prevalent in several populations in the world and it is the result of the deficiency of alpha linolenic acid and linoleic acid; fatty acids that belong to the omega-3 and omega-6 families. EFAD that is related to omega-3 deficiency is common and it can lead to impaired growth, skin lesions, infertility, kidney abnormalities, fatty liver disease, polydipsia and increased susceptibility to infections, as well as reduced learning and impaired vision. These symptoms may not be specific as they can be caused by other

clinical complications, hence an assessment of clinical symptoms as well as chemical assessment of imbalances between omega-3, omega-6, omega-7 and omega-9 fatty acids are used to diagnose EFAD [29].

Mead acid is regarded as the functional marker for EFAD. This is because, in the biochemical conversions of ALA to EPA and DHA, the enzyme responsible for the conversion faces competition from other substrates such as oleic acid and linoleic acid. The enzyme Δ- desaturase has an affinity to these substrates in this manner omega-3 >omega-6> oleic acid. Mead acid can only be formed from oleic acid when there is a deficiency of both omega-3 and omega-6 fatty acids. As a functional marker, it depicts the presence of physiological and nutritional deficiency of essential fatty acids. Conversely, mead acid can replace other PUFAs in the tissues and blood and can lead to platelet hyperactivity, vasoconstriction and altered cell to cell adhesion. Mead acid replacement consequentially lead to the production of pro-inflammatory marker lipoxygenase [29].

In the assessment of EFA status, the use of whole blood plasma may not be a preferred option because fatty acid profiles yields four different classes of fatty acids with different functions. Erythrocyte (RBC) fatty acid provides a more reliable parameter because it reflects bone marrow fatty acid content and plasma RBC phospholipid exchange processes from the preceding 2 – 3 months. In addition, RBC fatty acid that is derived from RBC plasma membrane has a full range of long chain polyunsaturated fatty acid and this gives an indication of the dietary consumption of fatty acids. On the other hand erythrocyte RBCs can be affected by age distribution[29].

Erythrocyte RBCs also reflect the fatty acid composition in the brain in humans: erythrocyte DHA is correlated with brain cortex DHA in a human study and this can indicate that erythrocyte DHA may be a valid marker of brain DHA in humans[30]. An earlier study in rats also demonstrates that red blood cell and neural membranes show similar composition of very-long-chain polyunsaturated fatty acids after dietary modification[31]. A transporter at the blood brain barrier, Mfsd2a, specifically transport DHA in the form of lysophosphatydyl choline-DHA from peripheral tissues into brain tissues[32], and the presence of DHA in the brain tissues enhances cognition.

In using the erythrocyte for the assessment of EFAD, there are some ratios that have been established that gives an indication of EFAD. Among these are the triene-tetraene (T/T) ratio, omega-3/omega-6 ratio, palmitoleic acid/linoleic acid ratio, and DHA/AA ratio. Among these ratios the gold standard and the most widely accepted to indicate EFAD is the T/T ratio[29].

Research gap

A recommendation ranging from 25-40% energy as total fat intake has been established [6]. However most people in developing countries have lower intakes of fats[33]. Specifically, Ghanaian diets are mainly carbohydrates with inadequate levels of proteins and fats, making the population susceptible to EFAD[34]. FA status is difficult to assess in many developing countries and few studies have been conducted on FA status and children. Recent studies in Ghana utilized FA based supplements[35, 36] but did not assess FA status in Ghanaian children, so this presents a very important research gap.

The prevalence of stunting in Ghana is 19% for children below 5 years of age. Although, there have been a reduction in stunting in the past decade, the situation still persist in Northern Ghana, where 33% of all children under 5 years are stunted[37]. Stunting is an indicator of chronic malnutrition which can be caused by inadequate dietary intake. Most of the interventions have focused on vitamin and mineral supplementation and fortification [3]in order to curb stunting, but the situation still persist. ALA and LA and their derivatives can be found in seeds, nuts, oils and animal sources such as fish and meat[33, 38], and some of these sources are uncommon in the diets of some Ghanaians[34] due to cost and this may be increasing the risk of EFAD in Ghana. The quest to find cheap alternative sources of EFA in Ghanaian seeds, nuts and oils is also a gap in literature.

In this research, we assessed the FA levels in children 2 to 6 years of age and related them to growth and cognitive function. This is important because there have not been any study in Ghana that has assessed the FA levels of children in this age group. Further, this age group was considered because, at this age, there is rapid growth and development and this is a critical period where interventions are likely to be effective. In addition, after two years of age, most children are weaned off breastmilk and they start consuming family foods which are usually low in fat intake, hence low FA status are expected for this age group.

CHAPTER 3: ASSOCIATION OF WHOLE BLOOD N-6 FATTY ACIDS WITH STUNTING IN 2-TO-6-YEAR-OLD NORTHERN GHANAIAN CHILDREN: A CROSS-SECTIONAL STUDY

Data in this chapter is published in Adjepong M, et al., (2018) Association of whole blood n-6 fatty acids with stunting in 2-to-6-year-old Northern Ghanaian children: A cross-sectional study. *PLoS ONE* 13(3)

Abstract

In Northern Ghana, 33% of children are stunted due to economic disparities. Dietary fatty acids (FA) are critical for growth, but whether blood FA levels are adequate in Ghanaian children is unknown. The objective of this study was to determine the association between whole blood FAs and growth parameters in Northern Ghanaian children 2-6 years of age. A drop of blood was collected on an antioxidant treated card and analyzed for FA composition. Weight and height were measured and z-scores were calculated. Relationships between FAs and growth parameters were analyzed by Spearman correlations, linear regressions, and factor analysis. Of the 307 children who participated, 29.7% were stunted and 8.0% were essential FA deficient (triene/tetraene ratio>0.02). Essential FA did not differ between stunted and non-stunted children and were not associated with height-for-age z-score (HAZ) or weight-for-age z-score (WAZ). In hemoglobin adjusted regression models, both HAZ and WAZ were positively associated with arachidonic acid ($p \leq 0.01$), dihomo-gamma-linolenic acid (DGLA, $p \leq 0.05$), docosatetraenoic acid ($p \leq 0.01$) and the ratio of DGLA/linoleic acid ($p \leq 0.01$). These data add to the growing body of evidence indicating n-6 FAs are critical in childhood linear

growth. Our findings provide new insights into the health status of an understudied Northern Ghanaian population.

Introduction

Childhood growth stunting, deemed stunting, is a major nutritional challenge that affects over 165 million children globally [39]. Continents with the highest prevalence of stunting include Africa, Asia, and South America, where stunted and underweight children have a threefold higher risk of mortality [40] compared to well-nourished children. On the African continent, the Sub-Saharan region is most affected by stunting [39]. Observational studies in African countries such as Ghana report roughly 20% of children are stunted, with regional differences in Northern Ghana experiencing stunting rates over 30% [20]. Several fatty acid (FA) supplementation trials in Ghana reported increases in hemoglobin (Hb) levels of pregnant women and may support growth spurts in children [20, 41], however, there is a dearth of research investigating the relationship between circulating FA levels and growth in Ghana. FAs are important for childhood growth and development, and have roles in energy utilization, brain myelination [35], hormone synthesis [41], and FAs serve as substrates for signaling molecules.

FAs are synthesized *de novo*, except for the two essential FAs (EFAs): linoleic acid (LA, an omega-6 [n-6]) and alpha-linolenic acid (ALA, an omega-3 [n-3]) [35, 42]. Humans lack the delta-12 and delta-15 desaturase enzymes required to synthesize LA and ALA, thus, dietary intake is the primary source of EFAs. LA and ALA are elongated and desaturated to produce long chain polyunsaturated fatty acids (LC-PUFAs), and LC-PUFAs serve as substrates for eicosanoids such as prostaglandins, which are involved in cell

differentiation and growth [43], and signal transduction [44]. The desaturation of LA and ALA is mediated by the delta-5 and delta-6 desaturase enzymes [45], while several elongases are involved in converting LA and ALA to LC-PUFAs.

The n-3 FAs and n-6 FAs have high affinity as substrates for elongase and desaturase enzymes but in their absence, omega-9 (n-9) FAs can serve as substrates for conversion to certain LC-PUFA species, notably the n-9 Mead acid [46, 47]. When an individual's diet is deficient of EFAs, oleic acid (C18:1n9), a non-essential n-9 FA, is converted to Mead acid (C20:3n9) [48]. Mead acid is then incorporated into phospholipids, cholesterol esters, triglycerides, and non-esterified free FAs [47, 49]. It is well accepted that those with EFA deficiency (EFAD), have higher levels of Mead acid and an elevated triene to tetraene ratio (T/T ratio), and the T/T ratio is defined as the ratio of Mead acid (3 double bonds, triene) to arachidonic acid (AA; 4 double bonds, tetraene) [50-53]. EFAD is defined by a T/T ratio > 0.02 in plasma samples [54, 55], and also established when Mead acid [55] levels are above 0.4% in red blood cells (RBCs) [53] and 0.21% in plasma [54].

Northern Ghanaian diets consist mainly of cereals and fruits, with intake of fats and proteins below adequate levels [34]. Additionally, only 36% of Ghanaian children between 6–36 month of age have fats added to their complementary food [37] . These low intakes of dietary fat may increase EFAD in infants and young children. In the Ghanaian diet, sources of LA include peanut and soybean oil, which are also high in ALA [56]. Fish, eggs, poultry, and whole grains are also good sources of EFAs [18, 19, 54]. In Ghana, malnutrition in infants and children below 5 years is mostly caused by inadequate complementary feeding practices[37] which can lead to insufficient EFA consumption .

Lipid-based supplementation has shown to increase linear growth in Ghanaian children[57]. However, blood assessments of FA levels are underreported in Ghanaian children and, consequently, EFAD prevalence is poorly characterized. Given the importance of FAs in growth and development, and the high prevalence of stunting in Northern Ghana, the objectives of this study were to assess blood FA levels in 2-to-6-year-old Northern Ghanaian children and their associations with growth, and to characterize blood FA profiles for this population to aid future research and intervention studies. It was hypothesized that whole blood EFA levels in Ghanaian children would positively correlate with the growth measures weight-for-age (WAZ), weight-for-height (WHZ), height-for-age (HAZ) and BMI-for-age z-scores (BAZ).

Subjects and methods

Study setting

The study was conducted in the Northern region of Ghana in the Savelugu-Nanton district [58]. The district covers 2022.6 sq. km with a population density of 68.9 persons per sq. km. The population of Savelugu-Nanton is 139,283 persons with 14,669 households. The average rainfall in the Savelugu-Nanton district is 600 mm. The district is also characterized by high temperatures with an average temperature of 34°C. The district is situated in the Savanna woodland that is capable of sustaining livestock, farming and the cultivation of crops such as rice, groundnuts, yams, cassava, maize, cowpea and sorghum. Over 80% of inhabitants are farmers. The main sources of water in the district are boreholes, rivers and streams, public taps, and pipe borne water. Though the primary source of water for taps and pipe borne water is the same, access to either categorizes

households under different income levels, perhaps reflecting differences in hygiene and even nutritional status. Thatch is the main roofing material for housing (50.9%). Illiteracy level is high with 69% of all inhabitants 11 years and above having no education. Some common diseases in the district include malaria, gastroenteritis, upper respiratory tract infection, diarrhea, anemia, and pneumonia. There are three operational community health post zones that deliver health services to the people[59]. The Savelugu-Nanton district was chosen for study as stunting levels are above the national average in the rural communities in this district [60] with overall district stunting level of 38.8% [61]. Additionally, it is one of the few areas in Northern Ghana with road access to rural communities.

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board at Michigan State University (IRB # 16-557) and the Committee on Human Research Publication and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (CHRPE/AP/236/16). The parent or caregiver of the participating child gave consent prior to the child's participation. A script of the written consent was read and translated in Dagbani to the parents or caregivers of the children. The parents or caregivers thumb printed the consent document to give consent. They were assured that participation was voluntary and confidential, and that their information would remain anonymous.

Sample size and subjects

Children (n=307) between 2 to 6 years of age residing in 5 communities in the Savelugu-Nanton district were recruited for the study. The communities were Janjorikukuo, Pong Tamale, Kparigilanyi, Morglaa and Fazhini. A power analysis was conducted from the results of an earlier study that measured maternal and infant erythrocyte fatty acid intake [38], and the fatty acid variation reported was utilized to run an a-priori sample size calculation for multiple regression based on an estimated medium effect size of 0.5 and significance level p=0.05. This indicated that 242 participants would yield statistical power of 80% [62]. 307 children were enrolled, raising the power to 90%. The exclusion criteria included sick and hospitalized children as well as children who were legally declared intellectually disabled. Data were collected in July 2016.

Anthropometric measurements

Height of all participants was measured to the nearest 0.1cm with a stadiometer (Seca, USA). Weight was measured using a digital bathroom scale to the nearest 0.1kg (Camry, model number: EB9003, China). All measurements were repeated and averages were reported. The date of birth was recorded from the child's health card or birth certificate. The sex of the child was also recorded.

Blood fatty acid assessment

Blood spots (40ul) were collected on a dried blood spot card (DBS) as previously described by Jumbe et al., 2016[63, 64]. A sterile single-use lancet was used in puncturing the tip of the middle finger to obtain drops of blood. The first drop of blood was wiped with

a sterilized dry pad. The drops of blood were then collected onto the DBS cards. The cards were stored in a dry, cool environment and shipped to the USA for FA analysis at OmegaQuant Analytics, LLC (Sioux Falls, SD). The average time between sample collection and arrival in the US lab was 8 days. Upon arrival in the US lab, the samples were stored at –80°C for 5 days and then analyzed as previously described [38, 63, 65]. Briefly, a punch from the DBS card was combined with the derivatizing reagent [boron trifluoride in methanol (14%), toluene, and methanol (35:30:35 parts)], shaken and heated at 100°C for 45 minutes. Forty parts of both hexane and distilled water were added after the mixture had cooled. After vortexing briefly, the samples were spun to separate layers and an aliquot of the hexane layer that contained the FA methyl esters was extracted. FA analysis was performed as previously described [66-68]. Unless otherwise stated, whole blood FA proportions are expressed as a percent of total identified FAs.

Hemoglobin and malaria status

Additional drops of blood from the same puncture site were used to assess hemoglobin concentration using a HemoCue photometer (HemoCue 301, Angelholm, Sweden), and malaria status using an antigen-based malaria rapid diagnostic test kit (Standard diagnostic Inc., Korea).

Data reduction and statistical analyses

Z-scores were calculated for the growth parameters HAZ, WAZ, and WHZ using WHO Anthro v3.2.2 igrowup package for R [69], to calculate z-scores for children < 5 years of

age, and WHO Anthro Plus [70] for children ≥ 5. Means and standard deviations were calculated for descriptive analysis. Stunting percentages were calculated based on the WHO standard population and definitions of moderate and severe stunting, wasting, and underweight [71]. FAs were expressed as percent composition of total blood FAs. Mean and standard deviations were calculated for blood FA composition. Total n-3 FA proportions were calculated as ∑ [ALA+ eicosapentaenoic acid (EPA) + docosapentaenoic n-3 (DPA n-3) + docosahexaenoic acid (DHA)]; total n-6 FA proportions were calculated as ∑ [LA + linoelaidic + eicosadienoic (EDA) + dihomo-gamma-linolenic (DGLA) + AA + docosatetraenoic (DTA) + docosapentaenoic n-6 (DPA n-6)]; total n-9 FA proportions were calculated as ∑ [oleic + elaidic + eicosenoic + Mead + nervonic]; total saturated FA proportions were calculated as ∑ [myristic + palmitic + stearic + arachidic + behenic + lignoceric]; total monounsaturated FA (MUFA) proportions were calculated as ∑ [palmitoleic + oleic + palmitelaidic + nervonic + elaidic + eicosenoic]; total polyunsaturated FA (PUFA) proportions were calculated as ∑ [total n-3 + total n-6]. T/T ratio was calculated from the ratio of Mead acid and AA [69]. Product-to-precursor ratios were calculated to estimate PUFA metabolism [72] as follows: EDA/LA to estimate elongase activity, GLA/LA and AA/DGLA to estimate desaturase activity, and DGLA/LA to estimate combined elongase and desaturase activity.

All statistical analyses were conducted using software R (R version 3.4.0). Correlations between participant characteristics, anthropometric measurements, and blood FAs were assessed using spearman correlations and graphically displayed using the R package *corrplot* [73]. Normal probability plots were assessed to verify the validity of regressions. Regression formulas consisted of either the dependent variable HAZ or WAZ, and models

were adjusted for each FA and Hb levels (i.e., HAZ = FA + Hb or WAZ = FA + Hb). Hemoglobin was selected as a covariate since it was significantly associated with HAZ and WAZ ($p \leq 0.01$). Regression models were adjusted for Hb and not adjusted for sex as there were few significantly different fatty acids (FAs) between sexes and regression values were unaffected when evaluated with sex adjustment. P-values were considered significant if $p \leq 0.05$. Exploratory factor analysis was carried out using the *psych* package [74]. Briefly, scree plot was used to determine four factors [29]. Palmitelaidic, linoelaidic, and elaidic acids were omitted from the analysis as they were not highly correlated with any other FAs ($r < 0.3$). Varimax rotation was used for orthogonal transformation of the factor loading matrix. FAs correlated with factors $r \geq 0.5$ were considered strongly correlated with the factor, regardless of sign. Factor loading scores were generated for each child and used to calculate regressions for each factor. The regressions were HAZ or WAZ = Hb + Factor.

Results

Subject characteristics

Demographic information is presented in Table 1. In this study, the median age of all 307 children was 46.8 months, with the youngest and oldest being 24.0 and 70.8 months, respectively. There were more males (52.1%) than females (47.9%) in the study. The median height of participants was 96.1 cm, and the median weight of participants was 13.5 kg. Hb levels ranged from 8.4 g/dl to 13.6 g/dl, malaria positivity was detected in 2.9% of the children, and all children were breastfed as infants with 94% of them having been breastfed until 20 months of age (data not shown). The median HAZ, WAZ, WHZ

and BAZ were -1.31, –1.16, -0.45, and –0.33, respectively. None of the participant characteristics differed by sex (Table 1). The standard deviations of the HAZ, WAZ, and WHZ distributions were relatively constant and close to the expected value of 1.0 (range: 0.78 – 1.14, data not shown). According to the WHO criteria [74], 29.3% of the children were stunted and 15% were underweight (Table 2). Approximately, 3.5% were categorized as wasting, or had a low BMI for their age.

Table 1: Sex differences are not associated with characteristics of participants

	Median (Q1, Q3)			
	Overall n=307	**Male n=160**	**Female n=147**	**p-value[a]**
Age (mo)	46.8 (37.2, 57.0)	48.0 (37.2, 57.6)	45.6 (37.2, 56.4)	0.73
Height (cm)	96.1 (80.0, 102)	96.6 (90.7, 103)	95.6 (89.1, 101)	0.13
Weight (kg)	13.5 (12.2, 15.5)	13.9 (12.6, 15.6)	13.3 (11.9, 15.3)	0.07
HAZ	-1.30 (-2.10, -0.69)	-1.32 (-2.10, -0.52)	-1.30 (-2.10, -0.85)	0.50
BAZ	-0.33 (-0.91, 0.11)	-0.24 (-0.96, 0.11)	-0.37 (-0.80, 0.09)	0.72
WAZ	-1.20 (-1.70, -0.52)	-1.20 (-1.70, -0.44)	-1.10 (-1.70, -0.58)	0.83
WHZ	-0.45 (-1.00, 0.02)	-0.39 (-1.10, 0.02)	-0.52 (-0.91, -0.05)	0.40
Hb (g/dL)	11.0 (10.3, 11.7)	11.0 (10.3, 11.7)	10.9 (10.4, 11.7)	0.60

The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data[69]. HAZ, height-for-age z-score; BAZ, BMI-for-age z-score; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score; Hb, hemoglobin.

[a]Wilcoxon-Mann-Whitney test was conducted to assess sex differences, p-values presented.

Table 2: Nutrition and growth status of the children

	Based on	**Severe (<-3SD)**	**Moderate (≤-2SD)**	**Unaffected**
Stunting	HAZ	6.07%	23.6%	70.3%
Malnutrition	BAZ	0.00%	3.19%	96.8%
Underweight	WAZ	1.60%	13.4%	85.0%
Wasting	WHZ	0.00%	3.50%	96.5%

The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data [69], SD, standard deviation; HAZ, height-for-age z-score; BAZ, BMI-for-age z-score; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score.

Fatty acid levels in whole blood

The median, first, and third quartiles comparing selected FAs of stunted (HAZ≤-2) and non-stunted children are shown in Table 3, and values for all FAs analyzed in our study are presented in Table S1. Approximately 8% of all children in the study had a whole blood T/T ratio greater than 0.02 and 6.8% of the study population had whole blood Mead acid levels above 0.21%. Oleic acid and total n-9 were significantly higher in stunted children, while DHA, the omega-3 index, and total n-3, and AA and DTA were all higher in non-stunted children. There was no significant difference for the T/T ratio or Mead acid between boys and girls.

Table 3: Median (Q1,Q3) of selected fatty acid proportions in whole blood[a]

Class	Fatty acid	Overall	Stunted	Non-stunted	p-value[b]
n-9	Oleic	20.8 (19.5, 22.6)	21.1 (20.1, 23.7)	20.6 (19.5, 22.2)	**≤0.05**
	Elaidic	0.17 (0.14, 0.23)	0.17 (0.14, 0.25)	0.17 (0.14, 0.23)	0.64
	Eicosenoic	0.31 (0.27, 0.39)	0.31 (0.27, 0.36)	0.31 (0.27, 0.40)	0.61
	Mead	0.13 (0.10, 0.16)	0.12 (0.10, 0.15)	0.13 (0.11, 0.17)	0.12
	Nervonic	0.72 (0.59, 0.91)	0.71 (0.57, 0.92)	0.73 (0.60, 0.90)	0.32
	Total n-9[c]	21.9 (20.7, 23.6)	22.2 (21.3, 24.5)	21.7 (20.5, 23.4)	**≤0.05**
n-3	ALA	0.16 (0.11, 0.21)	0.16 (0.11, 0.24)	0.15 (0.11, 0.21)	0.75
	EPA	0.18 (0.13, 0.24)	0.18 (0.13, 0.24)	0.18 (0.13, 0.24)	0.89
	DPA n-3	0.55 (0.47, 0.67)	0.54 (0.46, 0.67)	0.56 (0.47, 0.67)	0.72
	DHA	2.53 (2.18, 2.96)	2.42 (2.09, 2.76)	2.60 (2.24, 3.03)	**≤0.01**
	Total n-3[d]	3.47 (3.08, 3.95)	3.30 (2.98, 3.78)	3.51 (3.12, 4.00)	**≤0.05**
	O3I	2.70 (2.36, 3.17)	2.58 (2.29, 3.03)	2.74 (2.41, 3.20)	**≤0.01**
n-6	LA	20.7 (19.4, 21.7)	20.8 (19.7, 22.1)	20.6 (19.2, 21.5)	0.14
	GLA	0.15 (0.12, 0.20)	0.16 (0.12, 0.19)	0.15 (0.11, 0.20)	0.74
	EDA	0.29 (0.24, 0.33)	0.28 (0.24, 0.34)	0.29 (0.25, 0.33)	0.41
	DGLA	1.36 (1.18, 1.52)	1.35 (1.18, 1.49)	1.37 (1.19, 1.54)	0.32
	AA	11.0 (9.94, 11.9)	10.8 (9.60, 11.4)	11.2 (10.0, 11.9)	**≤0.01**
	DTA	1.68 (1.44, 1.92)	1.62 (1.36, 1.80)	1.72 (1.48, 1.95)	**≤0.01**
	DPA n-6	0.58 (0.47, 0.69)	0.54 (0.46, 0.68)	0.59 (0.48, 0.70)	0.06
	Total n-6[e]	36.0 (34.4, 37.4)	35.8 (34.5, 36.7)	36.1 (34.3, 37.6)	0.10
Ratios	GLA/LA	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	0.93
	EDA/LA	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.15
	DGLA/LA	0.07 (0.06, 0.08)	0.07 (0.06, 0.07)	0.07 (0.06, 0.08)	0.11
	AA/DGLA	8.05 (7.21, 8.99)	7.88 (7.04, 8.79)	8.11 (7.31, 9.08)	0.19

[a]Values represent blood fatty acid (FA) % composition. Stunted defined by height-for-age z-score (HAZ) ≤-2. n-9, omega-9; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; n-6, omega-6.

[b]P-value from Wilcoxon-Mann-Whitney test comparing stunted and non-stunted children.

[c]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic.

[d]Total n-3 includes ALA, EPA, DPA n-3, and DHA.

[e]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Correlations between fatty acids and growth parameters

Spearman correlations were calculated for participant characteristics, anthropometric measurements, and selected FAs (Fig. 2)**.** HAZ was positively correlated with AA (p≤0.01) and DTA (p≤0.01). HAZ was negatively correlated with total n-9 FAs (p≤0.05). WAZ was positively correlated with AA (p≤0.05) and DTA (p≤0.05). Interestingly, height and weight

were positively correlated with the ratio of DGLA/LA ($p \leq 0.05$), but were not significantly associated with either GLA/LA or EDA/LA. No significant associations were observed between the blood FA levels and BAZ or WHZ.

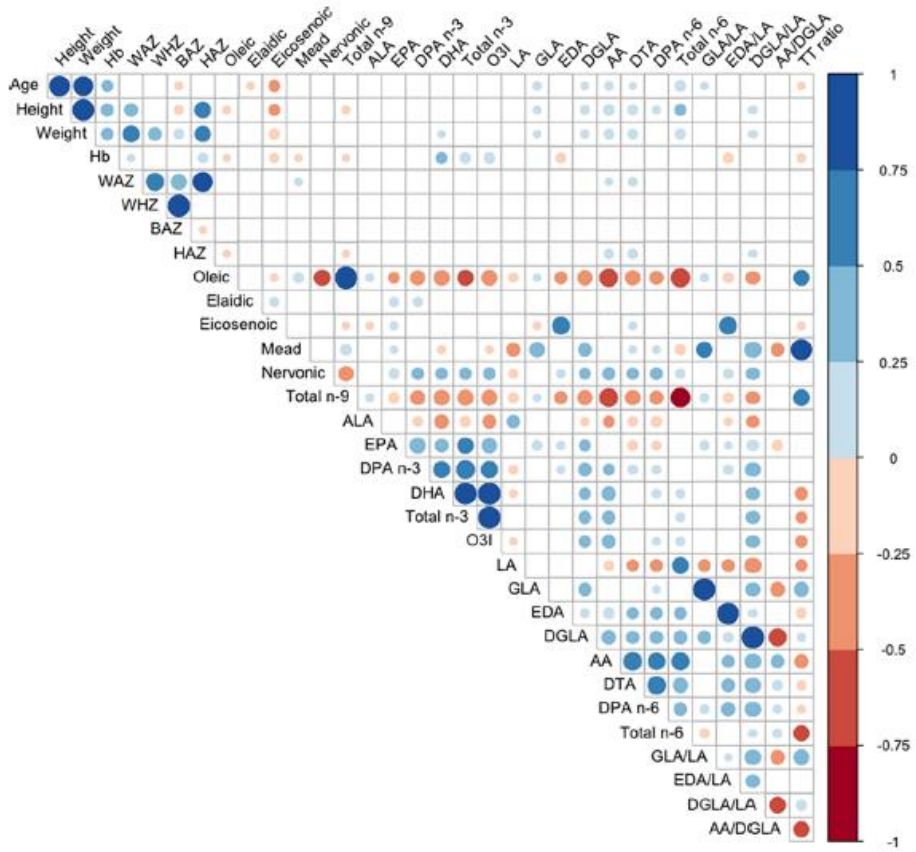

Figure 2: Spearman correlations between participant characteristics, anthropometric measurements, and selected blood FA levels.

[a]Spearman correlation matrix displays r correlation coefficients represented as circles, where large circles represent values closer toward 1 or -1 and smaller circles represent values closer toward 0.1 and -0.1. Blue shades denote positive r correlation coefficients, while red shades denote negative r correlation coefficients. Only results with $p \leq 0.05$ are displayed, thus, empty boxes were not significant. FA, fatty acid; Hb, hemoglobin; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score; BAZ, BMI-for-age z-score; HAZ, height-for-age z-score; n-9, omega-9; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; n-3, omega-3; O3I, omega-3 index; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; n-6, omega-6. Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic. Total n-3 includes ALA, EPA, DPA n-3, and DHA. Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Relationships or associations between fatty acids and growth parameters

Table 4 shows the results of the regression analysis for HAZ and WAZ by selected FAs. Since the results of models adjusted for Hb and Hb + Sex were similar, only Hb adjusted models are presented. There was a significant, positive relationship between HAZ and AA ($p \leq 0.01$), DGLA ($p \leq 0.05$), DTA ($p \leq 0.01$), and DGLA/LA ($p \leq 0.01$). In addition, WAZ was positively associated with stearic acid ($p \leq 0.05$), AA ($p \leq 0.01$), DGLA ($p \leq 0.05$), DTA ($p \leq 0.01$), and DGLA/LA ($p \leq 0.01$). Mead acid was positively associated with both HAZ and WAZ. The n-3 PUFAs were not associated with any of the growth parameters. No significant associations were observed between any of the FAs and WHZ or BAZ. A table of all analyzed FA regressions can be found in Supplementary Materials (Table S3)

Table 4: Regression results between HAZ, WAZ and selected fatty acids

		HAZ		WAZ	
Class	**Fatty acid**	**Beta ± SE**	**p-value**	**Beta ± SE**	**p-value**
n-9	Oleic	-0.04 ± 0.02	0.10	-0.03 ± 0.02	0.14
	Elaidic	-0.02 ± 0.33	0.94	-0.04 ± 0.25	0.87
	Eicosenoic	-0.35 ± 0.60	0.56	-0.01 ± 0.46	0.98
	Mead	2.48 ± 1.21	**≤0.05**	2.08 ± 0.93	**≤0.05**
	Nervonic	0.13 ± 0.29	0.65	0.14 ± 0.23	0.53
	Total n-9[b]	-0.04 ± 0.02	0.10	-0.03 ± 0.02	0.15
n-3	ALA	-0.67 ± 0.56	0.23	-0.32 ± 0.43	0.45
	EPA	0.07 ± 0.25	0.77	0.17 ± 0.19	0.36
	DPA n-3	0.03 ± 0.37	0.93	0.20 ± 0.28	0.48
	DHA	0.08 ± 0.10	0.43	0.09 ± 0.08	0.26
	Total n-3[c]	0.04 ± 0.07	0.62	0.06 ± 0.06	0.27
	O3I	0.06 ± 0.08	0.47	0.08 ± 0.06	0.23
n-6	LA	-0.06 ± 0.03	0.07	-0.04 ± 0.03	0.11
	GLA	-0.19 ± 0.95	0.84	-0.21 ± 0.73	0.78
	EDA	-1.48 ± 0.94	0.12	-0.80 ± 0.72	0.27
	DGLA	0.51 ± 0.25	**≤0.05**	0.39 ± 0.19	**≤0.05**
	AA	0.12 ± 0.04	**≤0.01**	0.08 ± 0.03	**≤0.01**
	DTA	0.50 ± 0.17	**≤0.01**	0.39 ± 0.13	**≤0.01**
	DPA n-6	0.56 ± 0.38	0.14	0.18 ± 0.29	0.54
	Total n-6[d]	0.03 ± 0.03	0.18	0.02 ± 0.02	0.26
Ratios	GLA/LA	4.19 ± 17.7	0.81	0.54 ± 13.6	0.97
	EDA/LA	-13.8 ± 18.3	0.45	-6.52 ± 14.0	0.64
	DGLA/LA	11.7 ± 4.39	**≤0.01**	8.34 ± 3.38	**≤0.01**
	AA/DGLA	0.02 ± 0.04	0.68	0.00 ± 0.03	0.92

[a]Models were adjusted for Hb. Beta ± standard error (SE) presented. HAZ, height-for-age z-score; WAZ, weight-for-age z-score; n-9, omega-9; n-3, omega-3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; O3I, omega-3 index; n-6, omega-6; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid.

[b]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic acid.

[c]Total n-3 includes ALA, EPA, DPA n-3, and DHA.

[d]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Factor analysis

Exploratory factor analysis was conducted to identify FA patterns that may be related to stunting. Scree Plot analysis indicated that four factors should be extracted. Factor loadings (Table 5) show the correlation between individual FAs and the respective factor. Individual factor loadings for each factor were regressed against HAZ and WAZ (Table 6). Factor 1 was significantly associated with HAZ and WAZ ($p \leq 0.01$). Several n-6 fatty acids, including AA, DGLA, and DTA were highly correlated with this factor. Factors 2-4 were not significantly associated with either HAZ or WAZ.

Table 5: HAZ and WAZ regressed on calculated factors

Fatty acid	Factor 1	Factor 2	Factor 3	Factor 4
AA	**0.89**	-0.09	0.08	0.14
DTA	**0.81**	0.07	0.13	-0.15
DPA n-6	**0.72**	0.27	0.09	-0.11
Stearic	**0.64**	-0.06	0.24	0.07
DGLA	**0.59**	0.31	-0.15	0.20
Palmitic	**-0.55**	0.27	-0.25	0.04
Oleic	**-0.69**	0.22	-0.08	-0.49
Palmitoleic	-0.23	**0.80**	0.10	0.12
Myristic	-0.38	**0.66**	0.04	0.09
GLA	0.05	**0.65**	-0.19	-0.06
Mead	0.18	**0.62**	-0.04	-0.16
LA	-0.19	**0.62**	-0.20	-0.02
Arachidic	-0.04	-0.17	**0.78**	-0.26
Eicosenoic	-0.05	0.13	**0.74**	0.18
Behenic	0.35	-0.29	**0.73**	-0.01
Lignoceric	0.49	-0.25	**0.54**	0.08
Nervonic	0.44	-0.05	**0.50**	0.21
DPA n-3	0.12	0.12	0.06	**0.87**
DHA	0.23	-0.07	-0.01	**0.82**
EPA	-0.22	-0.06	0.08	**0.81**
ALA	-0.35	-0.25	-0.23	-0.25
EDA	0.17	0.15	0.47	0.08

[a]Varimax rotated factor-loading matrix generated using the R-package psych. Factors named based on majority of highly correlated FAs. Numbers displayed represent each FA correlation with its respective factor. Correlations ≥0.50 are bolded. AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; DGLA, dihomo-gamma-linolenic acid; GLA, gamma-linolenic acid; LA, linoleic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ALA, alpha-linolenic acid; EDA, eicosadienoic acid.

Table 6: Factor analysis of fatty acids[a]

	HAZ		WAZ	
	Beta	**p-value**	**Beta**	**p-value**
Factor 1	0.18	**≤0.01**	0.13	**≤0.01**
Factor 2	0.03	0.60	0.01	0.82
Factor 3	-0.09	0.18	-0.01	0.91
Factor 4	0.01	0.86	0.02	0.65

Model: HAZ or WAZ=Factor+Hb

[a]HAZ, height for age z-score; WAZ, weight for age z-score; Hb, hemoglobin.

Discussion

The purpose of this study was to characterize the whole blood FA levels of Northern Ghanaian children and determine FA associations with growth parameters. Based on Mead acid levels and the T/T ratio, we found that EFAD was low in this population, however, the prevalence of stunting was high (29%). Furthermore, 6.7% of all participants had Mead acid levels above 0.21%, and 8.0% had a T/T ratio greater than 0.02, both lower than previously reported in Tanzania [63]. Interestingly, Mead acid was positively associated with both HAZ and WAZ despite low EFAD. In regression analyses, n-6 LC-PUFAs were inversely associated with stunting, and T/T ratio and total n-3 FAs were not significantly associated with any of the growth parameters. Taken together, our results indicate that whole blood n-6 LC-PUFAs levels are inversely associated with growth stunting, and even though EFAD was low, Mead acid levels were positively associated with growth parameters in this population of Northern Ghanaian children.

The levels of EFAs (i.e., ALA and LA) did not differ between stunted and non-stunted children, and were not associated with measures of linear growth. The n-3 LC-PUFAs were not associated with growth in regression analyses, although DHA, total n-3, and the omega-3 index were significantly lower in stunted children compared to non-stunted children. Although whole blood LA levels did not differ, n-6 LC-PUFAs such as AA, DTA, and DGLA were low in stunted children. As DGLA, AA, DTA, and total n-6 FA levels increased in the blood, there was an increase in HAZ, thus, a decrease in stunting. Overall these findings are consistent with previous reports in Tanzanian children [63] and support evidence that n-6 FAs are important in linear growth [75].

Dietary intake of PUFAs is reflective of whole blood PUFA levels [76], therefore, increasing dietary intake of PUFAs in these children may increase whole blood PUFA levels, improve growth parameters, and reduce stunting. AA status is positively correlated with first year growth in preterm infants [77], and AA can regulate gene expression related to cytokine production and osteoclast differentiation [76]. Dietary AA also positively correlates with plasma insulin-like growth factor-1 concentrations [76] which increases hypertrophic cell size and linear growth [78], and is a main predictor of height velocity in children. The mechanisms by which n-6 FAs affect growth and development include serving as substrates for the synthesis of ligands in signal transduction pathways[8]. For instance, AA-derived eicosanoids have key roles in normal growth and development, with prostaglandin E2 functioning in hormone regulation of bone development [75, 76, 79, 80].

Since the n-6 PUFAs DGLA, AA, and DTA were significantly associated with growth parameters, product-to-precursor ratios were assessed to investigate PUFA metabolism. As mentioned LA levels did not differ in regression or dichotomous analyses. The conversion of LA (C18:2 n-6) to DGLA (C20:3 n-6) occurs by: 1) LA elongation to form EDA, then EDA desaturation to form DGLA; or 2) LA desaturation to form GLA, then GLA is elongation to form DGLA [81]. The ratios of GLA/LA or EDA/LA were not significantly associated with growth parameters, however, the ratio of DGLA/LA was significantly associated with growth parameters. The *de novo* conversion of LA to DGLA utilizes the elongation of very long chain fatty acid protein 5 (ELOVL-5) and delta-6-desaturase enzyme, while the conversion of DGLA (C20:3) to AA (C20:4) utilizes the delta-5-desaturase enzyme. The ratio of AA/DGLA was not significantly associated with growth parameters. It is possible in this population of Northern Ghanaian children that PUFA

metabolism is altered through several enzymes involved in FA metabolism. What remains unclear is whether these observations are due to altered PUFA metabolism, lower dietary intakes of PUFA, or both.

Gene-diet interactions are known to potentially modulate enzyme activities of some desaturases that are involved in FA metabolism [82]. Fatty acid desaturase-1 (FADS1) and FADS2 genes that encode delta-5-desaturase and delta-6-desaturase, respectively [83], have been hypothesized to be under selective pressure by extreme PUFA diets. The hallmark paper by Fumagalli et al. (2015) reported that isolated populations with extreme diets in varying PUFA composition coped by physiologically adapting gene variants in FADS enzymes [84]. More interestingly, is these FADS gene variants were found to also significantly influence height and weight. We report Mead acid, a PUFA well recognized to accumulate under conditions of EFAD, was positively associated with HAZ and WAZ. This result was unexpected. Mead acid did not differ between stunted and non-stunted children, nor did LA or T/T ratio. One possible explanation is this rural population in Northern Ghana, over the millennia, may have physiologically adapted to EFAD and diets low in PUFAs, similar to the findings of Fumagalli et al. As previously mentioned we report that EFAD was low in this population, despite a prevalence of stunting at 29%. When individuals are deficient in dietary LA, oleic acid is desaturated by delta-6-desaturase, elongated by ELOVL5, and finally desaturated by delta-5-desaturase to form Mead acid [63]. We report the ratio of DGLA/LA was significantly associated with both HAZ and WAZ, and the *de novo* conversion of DGLA/LA is also dependent on delta-6-desaturase and ELOVL-5. Furthermore, in these children, Mead acid and the ratio of DGLA/LA were highly correlated (Fig. 2). Future research should investigate FADS gene variants in these

children to determine if our findings are due to altered metabolism at the biochemical level or due to altered dietary intake.

The purpose of this study was to assess blood FA levels in 2-to-6-year-old Northern Ghanaian children and associations with growth parameters. This study cannot be generalized to the entire Ghanaian population, since the study was performed in one village and dietary intake of foods can differ across Ghana. The authors acknowledge the children were not required to fast prior to whole blood collection, blood was collected throughout the day, and these factors may have added variability to our results. However, this variability is expected to be minimal since children in this village consume similar, low-fat meals compared to children in other populations. We did not measure indices of body fat such as mid-upper arm circumference or body composition. In addition, we do not have data on nutrient intake in this population. Nutritional deficiencies aside from EFAD can lead to poor growth in the children. For example, zinc can also affect FA metabolism, and we did not measure zinc. We acknowledge that product-to-precursor ratios provide an indirect estimation of enzyme activity and may not fully reflect biochemical activity. The authors speculate our Mead acid finding may be related to altered PUFA metabolism at the enzymatic level, and conducting this analysis was outside the scope of our current study. Therefore, future researchers should investigate FADS enzyme gene variants in this region of Northern Ghana.

To our knowledge this is the first study to assess whole blood FAs in Ghanaian children 2-6 year olds. This study utilized biomarkers of FA status rather than food intake questionnaires to study the associations between FA status and growth. The study was large enough to detect an association between growth metrics and FA levels. Additionally,

the use of a validated dried blood spot collection and blood transport system made the study logistically easier to conduct, and the method was also successfully used in a similar study in Tanzania [63]. Our findings add to the growing body of evidence indicating n-6 FAs play a crucial role in linear growth. These data provide new insights into the health of rural Northern Ghanaian children, and provide valuable information for potential intervention studies attempting to combat stunting via nutrient supplementation.

CHAPTER 4: WHOLE BLOOD N-3 FATTY ACIDS ARE ASSOCIATED WITH EXECUTIVE FUNCTION IN 2 TO 6-YEAR-OLD NORTHERN GHANAIAN CHILDREN.

Data this chapter is published in *Journal of Nutritional Biochemistry* by Adjepong et al., 2018.

Abstract

Several studies demonstrate the importance of essential fatty acids (EFAs), and the long chain polyunsaturated FA docosahexaenoic acid (DHA), on cognition and brain development. The objective of this study was to investigate the relationship between whole-blood FAs and executive function in children from Northern Ghana. A total of 307, 2-to-6-year-old children attempted the dimensional change card sort (DCCS) task to assess executive function, and dried blood spot samples were collected and analyzed for FA content. Significant differences in mean % total whole-blood fatty acids were observed between children who could not follow directions on the DCCS test (49.8% of the sample) and those who could (50.2% of the sample). Positive associations with DCCS performance were observed for DHA (β =0.25, p=0.06), total n-3 (β =0.17, p=0.06) and dihomo-gamma-linolenic acid (DGLA; β =0.60, p=0.06). Children with the highest levels of total n-3 and DHA were three and four times, respectively, more likely to pass at least one condition of the DCCS test of executive function than those with the lowest DHA levels. The results of this study indicate an association between n-3 FAs and high-level cognitive processes in children two to six years of age, providing impetus for further studies into possible interventions to improve EFA status of children in developing countries.

Introduction

Essential fatty acids (EFAs) and their long chain metabolites have crucial roles in human growth, both in fetal and neonatal development [85-87]. They accumulate in the fetus during pregnancy and during early childhood [85]. Long chain polyunsaturated fatty acids (LCPUFA) are also concentrated in the central nervous system [88] playing significant roles in neuronal growth and differentiation of cells and have been associated with cognitive abilities [88-90]. In addition, the brain and retinal function are highly dependent on EFAs, especially for membrane fluidity and signal transduction [26]. Due to these crucial roles of LCPUFAs, poor PUFA status may affect brain development and cognitive abilities in children [90]. There is rapid brain growth in infants and children as evidenced by the 60-fold increase in brain weight from the second trimester to two years of age: 20 to 1200g [85]. Maximal cerebral volume is achieved between 10-15 years of age, but 95% is reached by six years of age [91]. Thus, LCPUFA should be included in the diets of infants and children to ensure optimal brain development [92-94].

EFAs can be found in foods such as peanut and soybean oil, walnuts, fish, eggs, poultry and whole grains ([56, 95], but these are not affordable or available to a large proportion of the population in some developing countries. Specifically, the Ghanaian diet is carbohydrate and protein-rich, but fat-poor,[96] making the population susceptible to EFA deficiency. Several double-blind, randomized control studies in infants and children have established that supplementation with EFA [linoleic acid (LA) , alpha linolenic acid (ALA)] and/or their metabolites [DHA, eicosapentaenoic acid (EPA) and arachidonic acid (AA)] results in improved cognition as evidenced by improved visual acuity and IQ maturation [97], verbal learning, memory[11], progress in myelination, mental and motor

development [13] as well as influencing neurological development status[98]. Supplementation results in higher blood levels of EFAs and their metabolites as measured in these studies. Dalton et. al reported that higher supplementation of DHA and EPA-rich fish oil correlated with higher plasma levels of DHA and EPA in 7-9 year olds [11]. This suggests that high levels of circulating blood LCPUFAs can be induced by dietary supplementation and may improve cognitive function in children. Sheppard and Cheatham [99] concluded that LCPUFA influence the cognitive development of children especially with regard to planning and memory processing. A study conducted in Tanzania showed that whole blood FA status was associated with cognitive abilities in children 4-6 year old [64], however no studies of the association between whole blood FAs and cognitive function has yet been conducted in the Ghanaian population to the best of our knowledge.

Executive function, which involves inhibition, working memory and task switching [100] is the conscious control of thoughts and actions. It develops in children between the ages of two and ten years [100]. The frontal and temporal lobes of the brain controls executive function [99]. These two regions of the brain contain high amounts of AA and DHA and continue to develop after the second year of life [101]. The dimensional change card sorting (DCCS) task is a validated method commonly used to provide a unitary measure of executive function in young children [100] [102]. In this study, we utilized the DCCS task to assess executive function in Ghanaian children age 2-6. Little is known about cognitive function assessment in the Ghanaian population as well as their association with FAs. In this study, we assess the association between whole blood FA status and executive function in Ghanaian children using a DCCS test of executive function. We

hypothesized that whole blood levels of EPA, DHA, and both EFAs (ALA and LA) would be positively associated with performance on the DCCS test.

Methods

Study site

This study was conducted in Savelugu-Nanton; a 2023 sq.km district with a population density of 68.9 per sq. km. in the Northern region of Ghana. The district is situated in the Savanna woodland that is capable of sustaining livestock and many farming practices. The main sources of water in the district are boreholes, rivers and streams, public taps, and pipe borne water. Common diseases in Savelugu-Nanton include malaria, gastro enteritis, respiratory infections, diarrhea, and anemia. The district has three operational community health post (CHP) zones that deliver health services to the people [103].

Subjects and ethical approval

This study observed all ethical standards and was approved by the Institutional Review Board at Michigan State University (IRB # 16-557) and the Committee on Human Research Publication and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (CHRPE/AP/236/16). All the children in the village between 2 and 6 years of age invited to participate, and 313 healthy children were enrolled in this cross-sectional study in July 2016. All participants and their mothers/caregivers verbally consented to participate in the study.

Anthropometric measurements

Heights of all participants were measured to the nearest 0.1cm with a stadiometer (Seca, USA). Weight was measured using a digital bathroom scale to the nearest 0.1kg (Camry, model number: EB9003, China). The average of two height and weight measurements were recorded. The date of birth was recorded from the child's health card or birth certificate. The biological sex of the child was also recorded. Height, weight, date of birth and sex data were entered into World Health Organization (WHO) Anthro [104] and WHO AnthroPlus [72] software to calculate height-for-age (HAZ), weight-for-age (WAZ), and BMI-for-age (BAZ) z-scores.

Blood fatty acid assessment

Blood spots (40ul) were collected on a dried blood spot card (DBS) as previously described in Jumbe et al., 2016 [63]. The tip of the middle finger was punctured with a sterile single-use lancet to obtain drops of blood. A sterilized pad was used to wipe the first drop of blood. The drops of blood were then collected onto the DBS cards. The cards were stored in a dry, cool environment and then shipped to the USA for FA analysis at OmegaQuant Analytics, LLC (Sioux Falls, SD). The average time between sample collection and arrival in the US lab was 8 days. The samples were stored at –20 degrees Celsius and analyzed as previously described [66-68]. Concisely, the DBS card was punched and combined with the derivatizing reagent [boron trifluoride in methanol (14%), toluene, and methanol (35:30:35 parts)], shaken and heated at 100°C for 45 minutes. After cooling, forty parts of both hexane and distilled water were added and briefly vortexed. The samples were spun to separate layers and an aliquot of the hexane layer

that contained the FA methyl esters was extracted. FA analysis was performed as previously described [70, 71, 105]. Unless otherwise stated, whole blood FA proportions are expressed as a percent of total identified FAs.

Hemoglobin and Malaria status

To assess the hemoglobin levels of subjects, additional drops of blood from the same puncture site were used to assess hemoglobin concentration using a HemoCue photometer (HemoCue 301, Angelholm, Sweden). Malaria status was also assessed from a drop of blood using an antigen-based malaria rapid diagnostic test (RDT) kit (Standard Diagnostic Inc., Korea).

Cognitive assessment: Dimensional change card sort (DCCS)

The DCCS [100, 106] asks that the child sort a series of bivalent cards based on one of two instructed dimension (i.e., either color or shape). Following sorting an initial series of eight cards based upon color, the child is instructed to switch the categorization dimension and sort another series of eight cards based upon shape (see figure 3). This dimensional change in sorting behavior provides an index of executive function as the child must suppress their previously learned set of rules (i.e., sorting by color) and attentional inertia towards those attributes in order to flexibly adjust their behavioral actions and attention to sort the cards by a new set of rules (i.e., sorting by shape) [100, 107]. For each level of the DCCS test, the child was considered to have passed if he/she correctly sorted 6 of the 8 cards in both the pre- and post-switch phases of the task. Given

the population of interest and the large developmental spectrum assessed, four levels of the DCCS test were utilized to ensure a robust assessment of executive function. Children who passed the first (instructional) level were allowed to take other 3 levels. Children who failed (scored less than 6 out of 8) the first level were considered to not be able to follow instructions and not allowed to take other levels of the DCCS test. As prior research has demonstrated that children younger than 48 months of age particularly struggle to complete this task, an initial condition was performed to assess if the child's executive function was sufficiently developed to enable them to follow directions [64, 107, 108]. This condition utilized the same pre- and post-switch procedure as outlined above but utilized monovalent cards that only presented a singular dimension (i.e., either color or shape). If the child was able to pass this initial condition, they were then asked to attempt three additional conditions of the DCCS test. These conditions replicated the traditional DCCS test using bivalent cards, but manipulated the attentional characteristics of the cards by progressively integrating the color and shape attributes to reduce practice effects (Figure 3)[64]. The total number of DCCS test conditions passed was used as an index of executive function[64]. The mother or caregiver was present during all conditions of the DCCS test to observe the process and allow the child to feel comfortable and confident.

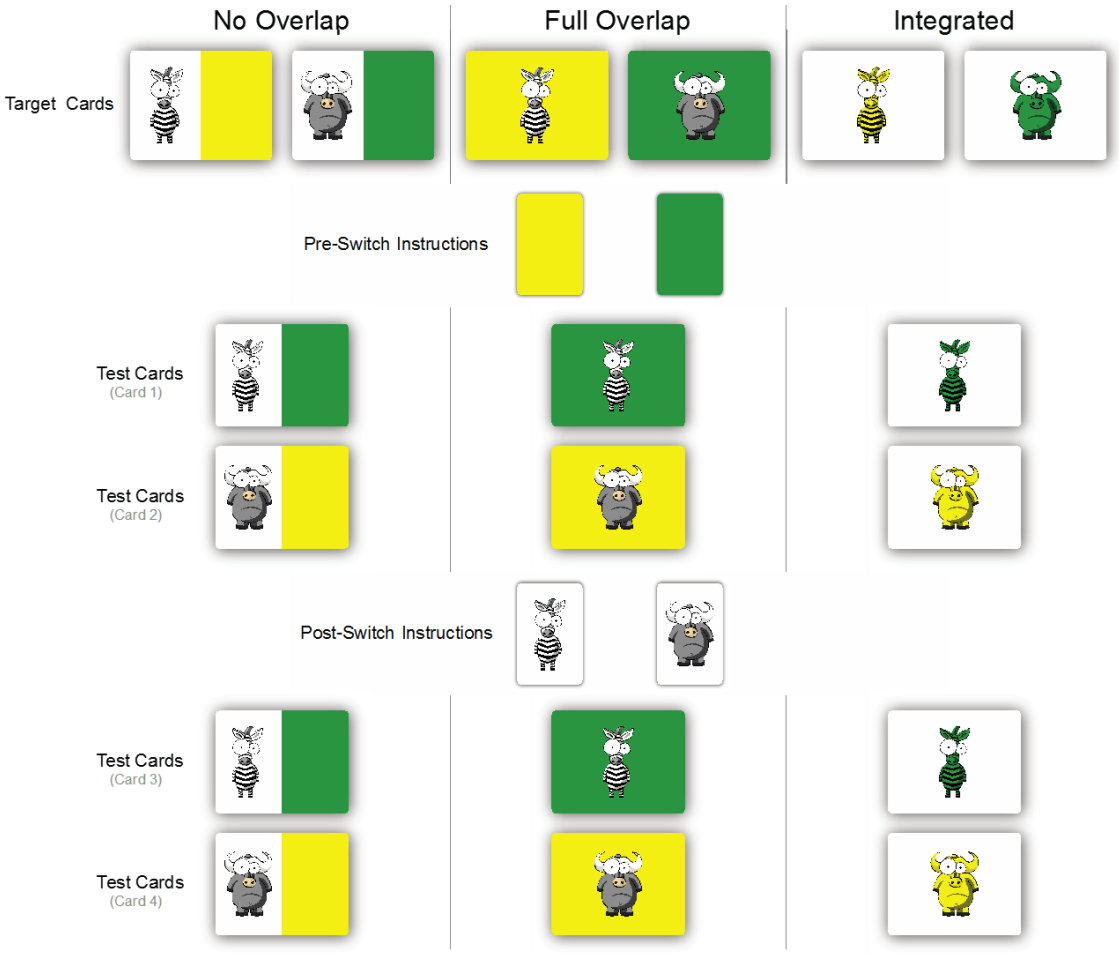

Figure 3: Illustration of the target cards and test cards used during the pre-switch and post switch

Data reduction and statistical analyses

A total of 313 children were recruited. A total of 6 children were removed from the analysis due to highly skewed fatty acid profiles failing the Grubs test for a number of fatty acids. The analyses presented herein are from the remaining 307 children. Although the level of fatty acid deficiency in Ghana is unknown, results from previous investigation in another African country [38] was used to calculate an *a priori* power analysis. Assuming a conservative effect size (f^2 = 0.05), a two-sided alpha of 0.05, and a beta of 0.20 (i.e.,

80% power) a sample of 242 participants was estimated to provide adequate power. A *post hoc* analysis of the statistical power using the method of Cohen & Jacob[109] was conducted. Using the data obtained from the analysis of linoleic acid, with 307 subjects and alpha set to 0.05, we had 90% power to detect an R-squared of 0.43. This was adjusted for 3 additional independent variables (Age, Hb and BAZ) with an R-squared of 0.37.

Descriptive analyses were conducted to obtain means and standard deviations for all participants. Means between groups (i.e. those who passed at least one DCCS task versus those who did not pass at least one DCCS task) were compared using t-tests (for continuous data) with R software and double-checked with SPSS. Models for linear regression included the FA of interest, and covariates hemoglobin, age and BMI-for-age (BAZ). Hemoglobin concentration was included in our model as a confounder because in similar populations it is a significant predictor of cognitive abilities [110]. Age and BAZ also showed significant association with the dependent variable (total passes). Malaria was not included as a covariate because only 2.93% of the children tested positive to malaria. Also, malaria was not significantly associated with DCCS performance and as such was not included in the model.

Factor analysis was conducted using SPSS version 24. Scree plot was used to identify four factors. Trans FAs palmitelaidic, linoelaidic, and elaidic acid were omitted from the analysis as they were not highly correlated with other FAs ($r<0.3$). Varimax rotation was used for orthogonal transformation of the factor loading matrix. FAs correlated with factors above $r=0.5$ were considered strongly correlated with the factor, regardless of sign. Factor loading scores generated for each subject were used to calculate regressions for each

factor to determine the associations between these factors and performance on the DCCS tasks. The regressions were Total pass = Factor + Age + Hb + BAZ. All statistical analyses were conducted using both SPSS and R software (R version 3.3.0). To determine associations between individual FA groups and executive function, we conducted binary logistic regressions for categorical variables using SAS version 9·4. In all cases, p-value < 0.05 was used to define statistical significance.

Results

Subject characteristics

In this study, 313 children between two and six years of age were enrolled. Six children with outlier myristic acid values were excluded from the study. Demographic information for all 307 participants are shown in table 7. In this study, the average age of all 307 participants was 46.5 months and there were more males (52.1%) in the study than females. The mean height was 96.2 cm and the mean weight was 13.9kg. The mean hemoglobin level of 11.5 was within the normal range [111]. Z-scores were used to calculate the prevalence of stunting (HAZ), malnutrition (BAZ) and wasting (WAZ). According to WHO standards,[112] 70%, 85% and 97% of the 307 participants had normal HAZ, WAZ and BAZ scores respectively. Children who passed the initial condition of the DCCS test were found to be older, taller, and had higher HB levels than children who failed the initial DCCS test (Table 8). Of the 307 children who attempted the DCCS task, 154 children (50.2%) were unable to follow directions as indicated by failing to pass the initial condition of the DCCS, 9 children (2.9%) passed the initial condition but not any

other DCCS conditions, 10 children (3.3%) passed two DCCS conditions, 57 children (18.6%) passed three DCCS conditions, and 77 children (25.1%) passed all four DCCS conditions. In comparing the FA levels between both groups, children who passed had significantly lower levels of total saturated fatty acids (p=0.02), but higher omega-6 DGLA (p=0.01) and arachidonic acid (p=0.01), as well as higher n-3 DHA (p=0.05).

Table 7: Characteristics of children who attempted the DCCS test

	Mean	SD	Range
Age (mo)	46.5	12.6	24.0-70.8
Sex (male)			
n	163		
%	52.1		
Height (cm)	96.2	8.72	72.9-119.9
Weight (kg)	13.9	2.41	8.40-21.20
Malaria (%)	1.97	0.17	1.00-2.00
HB (g/dL)	11.5	6.79	8.40-13.6
BAZ	-0.38	0.79	-2.82-1.83
HAZ	-1.34	1.13	-4.28-2.43
WAZ	-1.12	0.86	-3.40-1.60

HB, hemoglobin; BAZ, BMI-for-age z-score; HAZ, height-for-age z-score; WAZ, weight-for-age z-score. The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data [113]

Table 8: Characteristics of children stratified by dimensional change card sort performance for the initial condition (Mean values and standard deviations; numbers and percentages)

		Pass (n=153)	Fail (n=154)	
		Mean ± SE		P
Anthropometry	Age (mo)	53.6±0.81	39.5±0.88	**<0.001**
	Age range	24.0-70.8	24.0-69.6	
	Sex (male)			
	n	82	81	
	%	50.3%	49.7%	
	Height (cm)	100.8±0.62	91.5±0.54	**<0.001**
	Weight (kg)	15.0±0.20	12.8±0.20	**<0.001**
	Malaria (%)	1.97±0.01	1.97±0.01	0.736
	HB (g/dL)	11.2±1.07	10.8±0.93	**0.001**
	BAZ	-0.53±0.06	-0.23±0.06	**0.001**
	HAZ	-1.21±0.09	-1.47±0.09	**0.013**
	WAZ	-1.13±0.07	-1.11±0.07	0.681
SFAs	Myristic	0.18±0.01	0.22±0.01	**0.011**
	Palmitic	21.1±0.13	21.7±0.15	**0.001**
	Oleic	21.0±0.21	21.6±0.23	*0.086*
	Total SFA[1]	37.4±0.09	37.7±0.11	**0.018**
Omega-6 FAs	LA	20.7±0.14	20.5±0.16	0.313
	DGLA	1.40±0.0.02	1.32±0.0.02	**0.010**
	AA	11.0±0.13	10.5±0.13	**0.007**
	Total n-6[2]	36.1±0.18	35.2±0.22	**0.002**
Omega-3 FAs	ALA	0.19±0.01	0.18±0.01	0.259
	EPA	0.22±0.02	0.23±0.02	0.776
	DHA	2.69±0.05	2.54±0.05	**0.048**
	Total n-3[3]	3.67±0.09	3.54±0.08	0.196

HB, hemoglobin; BAZ, BMI-for-age z-score; HAZ, height-for-age z-score; WAZ, weight-for-age z-score. The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data [69].
[1]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric;
[2]Total n-6 includes linoleic, linolaidic, Y-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6;
[3]Total n-3 includes alpha linolenic, eicosapentaenoic, docosapentaeonic n-3, and docosahexaenoic;

FA levels in whole blood and regression analysis

Whole blood fatty acid levels of the 307 children whose data were analyzed are presented in Table 9. The mean levels of the essential fatty acids ALA and LA were, 0.18 and 20.56, respectively. The mean % of total FA in whole blood for DHA was 2.6, for EPA 0.22 and for the omega-3 index 4.5. Regression analysis between selected FAs and DCCS performance, adjusting for age, BAZ and Hb, is shown in Table 10. The n-3 FA DHA, as well as n-6 FA DGLA were positively associated with DCCS performance. To test the

hypothesis that whole blood levels of EPA, DHA, and both EFAs (ALA and LA) would be positively associated with executive control as indexed by performance on the DCCS tasks, multiple linear regression using EPA, DHA, ALA, LA, Hb, age, and BAZ was conducted. The model explained 38% of the variation (r^2=0.384; adjusted r^2=0.370, $p \leq 0.001$). DHA (β=0.40, p=0.02) and ALA (β=0.14, p=0.01) were significant contributors to the model, both being positively associated with performance on the DCCS test. A full model including all 25 single FAs as well as Hb concentrations, age, and BAZ was significant ($p<0.001$) and explained about 43% of the variance (r^2=0.429; adjusted r^2=0.374). In this full model, DHA (β=0.58, p=0.02) and ALA (β=2.75, p=0.007) were positively associated with DCCS performance. However, the effects of independent FAs and the covariates could not be determined due to high levels of collinearity leading to poor tolerance and variance inflation in the model.

Table 9: [1]Whole blood fatty acid proportions in Ghanaian children (Mean ± SE, n=307).

Class	Fatty acid	Mean ± SE	Range
SFA	Myristic	0.20±0.01	0.02-0.57
	Lignoceric	1.24±0.02	0.30-2.23
	Palmitelaidic	0.03±0.001	0.003-0.14
	Palmitic	21.4±0.10	17.0-28.0
	Behenic	0.86±0.01	0.33-1.44
	Arachidic	0.36±0.04	0.20-0.67
	Total SFA[2]	37.56±0.07	32.8-41.1
n-3 FA	Alpha-linolenic	0.18±0.01	0.04-0.78
	Eicosapentanoic	0.22±0.01	0.05-3.11
	Docosahexaenoic	2.62±0.04	1.35-6.12
	Omega-3 Index	4.55±0.52	2.97-11.74
	Total n-3[3]	3.61±0.05	2.02-11.1
n-6 FA	Linoleic	20.56±0.11	14.9-27.2
	Arachidonic acid	10.77±0.09	5.31-14.8
	GLA	0.17±0.004	0.04-0.49
	DGLA	1.36±0.01	0.69-2.44
	Docosatetraenoic	1.69±0.02	0.74-2.68
	Eicosadienoic	0.29±0.004	0.13-0.62
	Total n-6[4]	35.67±0.14	27.2-41.1
n-9 FA	Mead acid	0.14±0.003	0.04-0.39
	Oleic	21.3±0.16	15.7-31.2
	Eicosenoic	0.34±0.01	0.17-0.65
	Nervonic	0.75±0.01	0.22-1.34
	Total n-9[5]	22.4±0.15	17.4-31.8
Desaturases	SCD n-7	0.02±0.001	0.003-0.05
	SCD n-9	1.61±0.02	1.01-3.40
	D6d	0.067±0.001	0.03-0.12
	D5d	8.11±0.08	4.39-12.7
	Palmitoleic	0.367±0.01	0.06-1.40
	Total MUFA	23.0±0.15	17.7-32.4
	Total PUFA	39.3±0.16	29.7-45.0
	T/T ratio	0.013±0.00	0.003-0.04

[1]Expressed as FA % proportion (n=307);
[2]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric;
[3]Total n-3 includes alpha linolenic, eicosapentaenoic, docosapentaenoic n-3, and docosahexaenoic;
[4]Total n-6 includes linoleic, linolaidic, Y-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6;
[5]Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic SFA, saturated fatty acid; SCD n-7, stearoyl CoA desaturase n-7; [8]SCD n-9, stearoyl CoA desaturase n-9; D6d, delta-9-desaturase; D5d, delta-5-desaturase; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; T/T, triene-to-tetraene

Table 10: Regression results for performance on the dimensional change card sort test and selected fatty acids (FA).

Class	Fatty acid	Regression results for total pass (n=307)	
		B ± SE	P
SFA	Myristic	-0.18 ± 0.72	0.80
	Lignoceric	-0.17±0.26	0.50
	Palmitelaidic	-5.38±3.13	**0.09**
	Palmitic	-0.03±0.05	0.53
	Behenic	-0.43±0.44	0.33
	Total SFA[a]	0.00±0.07	0.96
n-3 FA	Alpha-linolenic	1.04±0.70	0.14
	Eicosapentanoic	0.27±0.32	0.40
	Docosahexaenoic	0.25±0.13	**0.06**
	Omega-3 Index	0.17±0.09	**0.07**
	Total n-3[b]	0.17±0.09	**0.07**
n-6 FA	Linoleic	-0.02±0.04	0.60
	Arachidonic	0.02±0.05	0.63
	GLA	0.94±1.21	0.44
	DGLA	0.60±0.32	**0.06**
	Docosatetraenoic	0.11±0.22	0.61
	Total n-6[c]	0.01±0.03	0.84
n-9 FA	Mead acid	-0.81±1.55	0.60
	Oleic	-0.02±0.03	0.55
	Eicosenoic	0.11±0.79	0.89
	Nervonic	-0.15±0.37	0.69
	Total n-9[d]	-0.02±0.03	0.44
Desaturases	SCD n-7	-0.46±9.39	0.96
	SCD n-9	-0.21±0.23	0.35
	D6D	10.04±5.62	**0.08**
	D5D	-0.09±0.06	0.10
Other	Palmitoleic	-0.02±0.41	0.96
	Total MUFA	-0.02±0.03	0.44
	Total PUFA	0.02±0.03	0.43
	T/T ratio	-9.76±15.4	0.53

Model: Total pass= Fatty acid of All Children + Age + BAZ + Hemoglobin)
[a]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric.
[b]Total n-3 includes alpha-linolenic, EPA, DPA n-3, and DHA.
[c]Total n-6 includes linoleic, linolaidic, GLA, eicosadienoic, DGLA, arachidonic, DTA, DPA n-6. [d]Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic.
SFA, saturated fatty acid; SCD n-7, stearoyl CoA desaturase n-7; SCD n-9, stearoyl CoA desaturase n-9; D6d, delta-6-desaturase; D5d, delta-5-desaturase.

Polytomous logistic regression

To determine the association between individual fatty acids and DCCS performance, a binary logistic regression analysis was performed to assess the relationship between the children who passed the initial phase of the DCCS test versus those who failed, for every

unit increase in the fatty acid of interest. Since a histogram plot shows a skewed data, all fatty acids of interest were categorized into three groups based on biological significance, instead of tertiles, and results shown in Table 11. Children with highest DHA levels per the category (DHA >4.0%) were four times more likely to pass the initial phase of the DCCS than children with the lowest DHA levels (DHA <2.0%) (OR: 3·5; 95%CI: 1.3·9.2 – 35·3; p=0·02).

Table 11: Associations of significant fatty acids, as tertiles, with dimensional change card sort test performance.

	Test for exposure	*Test for trend*
FA[a]	OR[95% CI][b]	OR (p trend)[c]
DHA (%)		**1.91**
≤ 2.0	1	**(0.008)**
>2.0 to ≤ 3.0	1.61 [0.71, 3.69]	
> 3.0	**3.50 [1.33, 9.24]**	
omega-3 index		**1.52**
≤ 4.0	1	***(0.047)***
>4.0 to ≤ 5.0	1.65 [0.82, 3.31]	
> 5.0	**2.31 [1.01, 5.29]**	
Total n-3 (%)		**1.72**
≤ 3.0	1	**(0.02)**
>3.0 to ≤ 4.0	1.08 [0.52, 2.26]	
> 4.0	**2.91 [1.20, 7.06]**	

[a] Whole blood FAs were separated into tertiles.
[b] Test for exposure was conducted to determine if increases in FA tertiles, compared to the lowest tertile, were associated with passing the DCCS test compared to failing. Odds ratio (OR) [95% Confidence Interval (CI)] are displayed.
[c] Test for trend was conducted to determine if increases in FA tertiles were associated with passing the DCCS test compared to failing. Odds ratio (p-value) are displayed.
[b,c] Polytomous logistic regression was used to regress BMI category on FA tertiles. All data are referenced against children who failed the first DCCS test. Both test for trend and test for exposure were adjusted for age, HB, malaria and BAZ. P-values bolded if p≤0.05 or italicized is p≤0.09 and >0.05.

Factor analysis

Factor analysis was conducted to identify group effects and to bypass the problems of collinearity. When factor analysis was used to determine how combinations of the FAs might be associated with performance on the DCCS tasks, four main factors emerged.

The factor loading matrix is shown in Table 12. Multiple linear regression using the four factors showed no factor to be significantly associated with performance on the DCCS tasks (Table 13). When combined with Hb concentrations, age, and BAZ, these parameters explained 37% of the variance (r^2=0.369; adjusted r^2=0.354) in the performance on the DCCS tasks

Table 12: Factor analysis of fatty acids.[a]

Fatty acid	Factor 1	Factor 2	Factor 3	Factor 4
AA	**0.89**	0.12	-0.07	0.13
DTA	**0.80**	0.16	0.09	-0.15
DPAn6	**0.71**	0.11	0.29	-0.11
Stearic	**0.65**	0.25	-0.05	0.06
DGLA	**0.58**	-0.10	0.33	0.20
Oleic	**-0.70**	-0.12	0.21	-0.47
Palmitic	**-0.56**	-0.25	0.26	0.05
ALA	-0.34	-0.24	-0.25	-0.26
Elaidic	-0.18	-0.03	0.07	0.17
Behenic	0.31	**0.76**	-0.28	-0.02
Arachidic	-0.07	**0.76**	-0.17	-0.26
Eicosenoic	-0.07	**0.70**	0.11	0.19
Lignoceric	0.45	**0.60**	-0.24	0.05
Nervonic	0.39	**0.57**	-0.05	0.19
Eicosadienoic	0.16	**0.46**	0.14	0.08
Linoelaidic	0.03	0.08	0.02	-0.06
Palmitoleic	-0.25	-0.10	**0.79**	0.15
GLA	0.03	-0.17	**0.66**	-0.06
Myristic	-0.39	0.01	**0.64**	0.12
Mead	0.15	0.00	**0.63**	-0.17
LA	-0.15	-0.23	**-0.62**	-0.02
DPAn-3	0.13	0.07	0.10	**0.87**
DHA	0.24	0.02	-0.09	**0.80**
EPA	-0.21	0.08	-0.09	**0.80**
Palmitelaidic	0.13	-0.10	0.21	0.24

[a]Varimax rotated factor-loading matrix generated using the R-package psych. Factors named based on majority of highly correlated FAs. Numbers displayed represent each FA correlation with its respective factor. Correlations >0.40 are bolded.

Table 13: Regression† results for performance on the dimensional change card sort test and fatty acid (FA) factors

Factor	Parameter estimate	Standardized parameter estimate	p-value
Factor 1	0.07	0.04	0.41
Factor 2	0.002	0.001	0.98
Factor 3	-0.09	-0.05	0.27
Factor 4	0.08	0.05	0.33
Age	0.08	0.56	**<0.01***
HB	0.02	0.01	0.78
BMI-for-age (BAZ)	-0.22	-0.10	**0.04***

*All significant associations (P<0·05). † Model: total passes= factor 1 + factor 2 + factor 3 + factor 4 + Age + Hb concentration +BMI-for-age z-score; model P value, P<0·001; r^2 0.369; adjusted r^2 0.354.

Discussion

Our data generally support the hypothesis that children with higher whole blood levels of EFAs (LA and ALA) as well as DHA and EPA were more likely to pass the DCCS test, an indicator of executive function. The multiple linear regression model using EPA, DHA, ALA, LA, Hb, age, and BAZ was significant and explained 38% of the variation in DCCS performance. However, when utilizing individual regression analysis, the essential fatty acids ALA and LA were not significantly associated with DCCS performance, nor in the factor analysis. Yet, regardless of the type of analysis herein, we show that children with higher whole blood levels of DHA, total n-3 and the omega-3 index calculation were more likely to pass the DCCS test. Consistent with this observation, children with higher blood n-3 FA levels were more likely to pass the DCCS test.

When blood levels of DHA and total n-3 FAs increase, children exhibited improved cognition. This is consistent in a number of randomized, controlled, human supplementation studies which have shown that when full term infants were

supplemented with LCPUFA that contained DHA, AA and LA, there was an improvement in visual acuity and IQ maturation[97]. Supplementation of children 3 -10 year olds with fish oils that contained DHA, eicosapentaenoic acid (EPA) and gamma linolenic acid (GLA) also resulted in improved non-verbal cognitive development [114]. Also, there was an improvement in short term memory when children 6 to 10 years old were supplemented with ALA, DHA, LA and micronutrients [12]. In addition, maternal supplementation of DHA and EPA was beneficial in cognitive function of their offspring, suggesting that there are long term effects of the supplementation on offspring [115]. Although the amount of DHA in the brain is variable depending on dietary intake, DHA and AA are the most highly concentrated PUFA in neural phospholipids including subcellular membranes [116]. The mechanisms by which DHA and n-3 FA affects cognition include myelination of axons [13].

DHA and n-3 FAs influences membrane fluidity, neurotransmitter receptor activity and nutrition of nerve cells [42, 117], as well as disruption of lipid rafts that affect signal transduction pathways [118]. Specifically, the cell membrane is a phospholipid bilayer, which contains lipid rafts. Lipid rafts are specialized lipid domains that differ in lipid composition by their cholesterol and sphingolipid content. They serve as a center for assembling signaling molecules, and their domains are disrupted under certain stimuli optimizing plasma membrane function and influencing membrane fluidity. DHA and n-3 FA acyl chains can exhibit conformational changes eliciting a stimulus that can disrupt the domains of the lipid raft. When DHA/EPA are incorporated into the rafts, the cholesterol molecules are redistributed to non-rafts leading to declusteration of the raft system. The non-raft proteins are sequestered into declustered rafts and this triggers a

downstream signaling activating cell receptor membranes for communication between cells. This communication between cells can enhance cognitive function. Further, the nerve cell membrane determines the amount of nutrient that can pass through the cell [118]. Rigid membranes does not allow adequate nutrients to get into the cells and the arrangement of the cellular domains is dependent of the presence of double bonds [119]. The presence of numerous double bonds in the DHA and n-3 FA molecular structure increases the fluidity of the membrane allowing nutrients to get into the cells [119], hence n-3 FAs help in the nourishment of cells making cells healthy and less prone to injury. Additionally, the nerve cell membrane contains proteins that act as receptors for some neurotransmitters, transmitting signals across a synapse. Also, fluidity of membranes allows receptors to recognize neurotransmitter and sends the message they contain. These factors provide evidence that n-3 FAs and DHA have significant roles in cognitive function.

The omega-3 index (EPA+DHA), a measure of n-3 FAs in red blood cells, influences cardiovascular health and has also been associated with cognitive abilities. Together, DHA and EPA are involved in many aspects of brain function including blood-brain barrier integrity and brain blood flow [116]. This study demonstrated that the omega-3 index was positively associated with performance of the DCCS test. The direction of association was consistent with studies conducted by van der Wurff et al, 2015 [120], which reported that there was a strong positive association between omega -3 index and letter digit substitution test, indicating that children with higher omega-3 index may have a faster information processing speed and less impulsivity. In addition, in this study, low n-3 intake was associated with a decrease in DCCS test performance. Consistent with previous

studies by Sheppard and Cheatham, 2017 [121], children with higher n-6s and lower n-3s performed poorly on the DCCS test. Sheppard and Cheatham, 2017 [121] reported that plasma n-6 and n-3 FA levels predicted and affected performance on working memory and planning tasks. In general, the hippocampus and frontal cortex of the brain is responsible for memory and executive function [121].These parts of the brain are sensitive to changes in n-3 FAs because of the role n-3 FAs play in neurotransmitter concentration, receptor density and function and neuronal growth [121]. In addition, a study in a mouse model indicated that supplementation with only DHA, (an n-3 FA) or AA (an n-6 FA) was insufficient for optimal development [122] supporting the essentiality of both n-6 and n-3 FAs in growth and development. Omega-3 and n-6 FA levels do change with age; omega-3 levels increasing over a lifetime while omega-6 levels decrease [123]. While these results reflect changes in FA levels over decades, there was no significant difference in FA levels for different age categories within a small range for our study. In another study comparing FA changes in 3-8-year old European children, Wolters et. al reported increasing n-6 FA, LA, with age and no significant association with n-3 FA DHA [124].

This study has a number of strengths. This study utilized an objective biomarker to assess dietary fatty acid intake other than conventional and less precise methods such as food frequency questionnaire or the diet history techniques. Food frequency questionnaires are not highly accurate at estimating circulating blood levels of LCPUFAs [125]. Also, the study includes a diverse panel of FAs and estimated desaturase activities, first of its kind in the Ghanaian population. In addition, in this study we culturally adapted a standard DCCS test to suit this population and the overall observed performance is consistent with

previous investigations conducted in the Tanzania[64], USA [126] and Scotland [107]. This investigation builds off our prior work in the Tanzanian population [64], to demonstrate these relationships despite potential genetic variation in FA biosynthesis across these populations.

With regards to limitations, this study was a cross sectional study and all associations that are reported are correlative rather than causative. It was conducted in the Savelugu-Nanton municipality and hence the results cannot be generalized to the entire Ghanaian population or other areas in the world. The collection of blood for this study was done throughout the day with no fasting required and this may increase variability in the whole blood FA measurements. However, variation in this setting are likely to be minimal as children from the village consume relatively similar and low-fat meals compared to children in other settings. Whole blood samples for lipids were analyzed and are not able to differentiate amongst the source compartment of the lipid. Another limitation to this study is that, the children in this population may have other nutritional deficiencies or diseases/infections that may cause poor cognition, however, the study accounted for at least two factors that are known to be linked to cognition: malaria and low hemoglobin levels. Finally, although the authors did not collect socio-economic data from the parents/caregivers in this population, there is likely to be little variability in these factors since the population is relatively homogeneous [103]. In summary, whole blood n-3 FA levels, in particular n-3 fatty acids, are associated with executive function in this cohort of Ghanaian children. Whether n-3 FA supplementation earlier in life would improve cognitive performance in these children would need to be examined in a randomized trial.

CHAPTER 5: QUANTIFICATION OF FATTY ACID AND MINERAL LEVELS OF SELECTED SEEDS, NUTS AND OILS IN NORTHERN GHANA

Data in this chapter is under review in the *Journal of Food Science Technology* by *Adjepong et al., 2018*

Abstract

Fatty acids (FAs) and micronutrients are required for growth and development of children. The objective of this study was to determine the mineral and FA composition of Northern Ghanaian foods. Seven seeds and three oils were collected from a local market. Freeze-dried seeds and food grade oils were packaged and shipped to the US. FAs were quantified by GC/MS. ANOVAs were conducted on FA concentrations and Tukey's post hoc test was used to compare foods. Palm oil contained significantly higher amounts of the saturated fatty acid (SFA) palmitic acid (293.1 mg/g; $p<.001$). Shea butter (292.0 mg/g) and palm oil (246.5 mg/g) were highest in oleic acid ($p<.001$). Soybean was significantly higher in alpha-linolenic acid (ALA) (2.98 mg/g; $p<.01$). Neri (68.4mg/g) and fermented dawadawa (56.3mg/g) had significant amounts of total PUFAs ($p<.0001$). Iron levels in soybean (353 mg/kg), neri (282 mg/kg) and fermented dawadawa (165 mg/kg) were relatively high. The identification of the nutrient content of these foods can increase its utilization in homes and in industrial settings especially in the formulation of complementary foods and new recipes. Together, these foods may be useful for future intervention to curb stunting and potential cognitive impairment in this population.

Introduction

Essential fatty acids (EFAs) and minerals are important dietary components required in human growth and development. They are key in cell membrane formation and proper development of brain and nerve cells. FAs also serve as major dietary energy source playing crucial roles in cell differentiation and metabolism [8, 127]. Although FAs are important in the human diet, their intakes in African diets are low [33]. Specifically, the dietary composition of Ghanaians is mainly starchy roots and cereals with levels of protein and fat below adequate levels. Though the dietary supply meets population energy requirements, the low dietary diversity in the population may be causing a deficiency in proteins and fats [18].

Although Ghana has achieved its goal according to the Millennium Development Goal 2 (MDG), uneven wealth distribution and poverty still prevails in the Northern sector of the country. In addition, only 36.6% of Ghanaian children from 6 to 24 months old have fats added to complementary foods [19]. Low dietary diversity in some parts of Northern Ghana, especially in food insecure households, has led to low dietary intake of fats [128] and may contribute to EFA deficiency in the population. The prevalence of mineral deficiencies is high in Ghana as well [129], with iron-deficiency anemia more prevalent in Northern Ghana than other regions [20]. The national stunting levels among Ghanaian children below five years old is 19%. However, in Northern Ghana, it stands at 33% [20]. Whole blood FAs are associated with stunting [63] and cognition [64]; hence, the availability and inclusion of adequate amounts of FAs and minerals in the diet is important. EFAs cannot be synthesized in the body and must be provided by the diet. EFAs are abundant in foods such as soybean oil, canola oil, flaxseed oil, nuts, and animal products.

Linoleic acid (LA), an omega-6 (n-6) FA, and alpha-linolenic acid (ALA), an omega-3 (n-3) FA, are EFAs. Arachidonic acid (AA), is a downstream metabolite derived from LA, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are metabolites derived from ALA. EFAs and their long-chain metabolites are important for cognition, growth and immune function [33, 130]. Seafood, especially fish, is a good source of EPA and DHA. However, fish is expensive and not sufficiently available to Ghanaians, evidenced by the fact that the amount of fish required to meet the nutritional needs of Ghanaians exceeds current fish production (marine, inland, and aquaculture) [131]. It has further been reported that the distribution and consumption of fish in Northern Ghana is low [132], leading to a high probability of low EFA intake in the population. The need for the exploration of alternative, available and affordable sources of EFAs is important.

Mineral deficiencies are prevalent in developing countries [129, 133, 134]. Although the prevalence of anemia in Ghanaian children 6 to 59 months old is 66%, the Northern region reports 82.1%, with iron deficiency as a major contributor [20]. Mineral deficiencies are also associated with growth stunting and cognitive impairment [135, 136] because some minerals such as iron and zinc are cofactors needed in EFA metabolism. For example, zinc deficiency impairs the conversion of LA to AA and ALA to EPA and DHA [137]. The evaluation of the mineral composition of seeds and nuts has been conducted in some studies, though the available data are highly variant due to environmental factors such as soil type, agronomic practices and climate [138, 139]. Geographical differences can directly affect the concentration of minerals in crop plants, and dietary mineral content, impairing EFA metabolism and potentially leading to growth stunting and cognitive impairment in the population.

The West African food composition (WAFC) table is a nutrient database which publishes an estimation of nutrients such as proteins, carbohydrates, total fat, vitamins, and minerals in food. [140] However, it contains many weaknesses: 1) The composition table contains the total fat composition of foods with no information on individual FA contents. 2) There is little information on the origin of the foods analyzed; 3) The data represent average values derived from compositional data of 8 countries, not just Ghana; 4) Most of the mineral and vitamin data in the table are based on information obtained from several non-African countries. Due to these limitations, the nutrient content listed in the WAFC table does not represent the foods in Ghana, and analyses that quantify individual FAs, especially EFAs, and mineral content of local foods in Ghana are needed. This study quantified the amount of EFAs, very long chain PUFAs (VLCPUFAs) and minerals in local foods available at a market in Northern Ghana. Children in this population have high levels of stunting which is associated with a potential EFA deficiency. Therefore, identifying FAs in local foods can be a step to increase utilization of such foods in homes and in industrial settings especially in the formulation of complementary foods and new recipes.

Materials and Methods

Preparation of selected seeds, oils, and nuts in Ghana

Seeds, nuts, and oils (Table 14) were purchased at a local market in Tamale, Northern Ghana. Oils obtained from the markets were transferred to opaque dark plastic containers to prevent lipid oxidation during transport and storage to the US for fatty acid analysis. Local seeds and nuts collected from the market were transported to a lab where they were crushed, freeze-dried and stored in containers for transport to the US for fatty acid

analysis to prevent lipid oxidation. All samples were shipped to the Michigan State University Food Science and Human Nutrition laboratory. Once received, the samples were purged with high purity nitrogen and stored at -20°C until analysis. Freeze drying technique was chosen because of shipping duration and power outages in this region. The technique also ensured sample integrity. The extraction yield of freeze-dried samples are comparable to air-dried samples. [141]. In addition, the quantity of LA and ALA from freeze-dried samples is at least as abundant as air-drying methods [142].

Crude seed oil extraction

All glassware used was washed with high performance liquid chromatography (HPLC) grade organic solvents to remove mineral residues and FA contaminants. Lipids were extracted from seed material as previously described [143], but modified as specified [144]. In brief, a total of 400 mg freeze-dried seed material was incubated for 2h at RT in 12 mL of a 2:1 (v/v) mixture of HPLC grade chloroform (Avantor Performance Materials, Inc., Center Valley, PA) and methanol containing 100 µg butylated hydroxytoluene (BHT)/mL (Sigma–Aldrich, St. Louis, MO) to release lipids into solution. Extracted seed samples were filtered using lipid-free filters (FGE Healthcare UK Limited, Buckinghamshire, UK) into glass tubes containing 2.5 mL of 0.88% (w/v) aqueous KCl (J.T. Baker, Phillipsburg, NJ) to separate aqueous and organic layers. The tubes were centrifuged and the bottom organic layer was transferred to a new tube and dried under high-purity nitrogen. The total crude seed oil was weighed.

Methylation of oils to FAMEs, neutralization, and FAME isolation

The samples were resuspended in chloroform/methanol (2:1 v/v, 100 µg BHT/mL) to obtain a final lipid concentration of 20 mg/mL. The resuspended oils were prepared for methylation as described by Cequier-Sanchez et al. [143]. In brief, 100 µL of lipid extract solution was transferred to clean 16×100 mm Teflon-lined screw-capped glass tubes. To each sample, 200 µg of the internal standard, Stearic acid *d35* (Sigma - Aldrich, Lot #TP1700V) suspended in HPLC-chloroform, was added. The resultant mixture was dried under high-purity nitrogen at RT. The samples were methylated with 2% acidified methanol as described by Agren et al. [145] and modified by Pickens et al. [70]. The mixture was neutralized and isolated as previously described. [146]. Briefly, the mixture was neutralized with salt buffer and FAMEs were extracted with hexane.

FAME identification, analysis, and data Processing

Resuspended FAMEs were transferred to GC vials with glass inserts for analysis. Prior to analysis, the sample injection order was randomized. FAMEs were identified and quantified (in triplicate) using a dual stage quadrupole (DSQ)II quadrupole GC/MS (Thermo Scientific, Waltham, MA) equipped with a DB-23, 30-m column (0.25 mm id; Agilent Technologies, Santa Clara, CA) using helium as a carrier gas. GC/MS temperature profile and selective ion monitoring (SIM) were performed as previously described [144]. Identification and quantification of individual FAMEs were done with standard FAME mixture (Part# CRM47885; Lot# LC06601V; Supelco, Bellefonte, PA) and standard curves were prepared as previously described [144]. Detected FAME concentrations below the lower limit of quantification (LLOQ) are defined for each FA in

Tables 2-4. DHA, EPA, and linoelaidic acid were below the LLOQ in all samples analyzed and were excluded from the tables. FAME peak integration and quantification was performed using TargetLynx V4.1 (Waters, Milford, MA) based on the FAME standard's retention time and SIM ions and ratios. The concentrations of re-suspended FAMEs were normalized to the amount of food-grade oil (i.e. palm oil) or crude seed oil and total seed material for the seed samples (i.e. neri seed).

Mineral analysis

Freeze-dried samples were analyzed for their mineral content. The samples DAW, FDD, NER, SAM, SOY, PNT and BAB were analyzed by a third-party contractor. The minerals that were analyzed include zinc, iron, potassium, phosphorus, sodium, magnesium, manganese and calcium. Identification and quantification of minerals was performed using ICP emission spectroscopy (ICP_S: 28, AOAC International no. 984.27, 985.01, and 2011.14) [144]. For each sample, concentrations of minerals are expressed as mg mineral/kg sample.

Statistical Analyses

Mean fatty acid concentrations (mg/g) and standard deviation values are presented in Tables 2-4. Parametric one-way ANOVA was conducted for each FA and p-values are given. Concentration values below the LLOQ were excluded from all analyses. Tukey's honest significant difference (HSD) post-hoc test was used for multiple comparisons of significant models. Statistical analyses were conducted using R (R version 3.3.0).

Results

FA composition of the seeds, nuts, and food-grade oils are reported as the concentration of each FA in mg per gram of food sample (Table 2–4). Table 14 describes the FAs and oils that were analyzed. Samples were found to contain varying levels of several FAs, as shown in the data.

Table 14: Foods analyzed

Food	Abbreviation	General	Description
Baobab seed	BAB	*Adansonia sp.*	Seeds of an ancient tree, whose fruits possess a velvety shell
Dawadawa	DAW	*Parkia sp.*	Seeds of a perennial tree, African locust bean plant
Fermented dawadawa	FDD	*Parkia sp.*	Fermented seeds of the African locust bean plant
Neri seed	NER	*Cucumropsis sp.*	Climbing vine, flattened seeds, seeds were freeze dried
Soy bean	SOY	*Glycine sp*	A legume, bean seeds
Peanut	PNT	*Arachis sp.*	A root of a tropical legume
Sesame seed	SAM	*Sesamum sp.*	Seeds from a domesticated oil seed plant
Shea butter (oil)	SHB	*Vitellaria sp.*	An ivory-coloured fat extracted from African shea nut
Palm oil	PAL	*Elaeis sp*	Crude oil processed locally, red-orange, high in beta carotene
Palm kernel oil	PKO	*Elaeis sp.*	Oil derived from the kernel of the oil palm

Saturated fatty acids

All samples analyzed were found to contain saturated FAs. Food-grade oils were more abundant in saturated fats than seeds (Table 15). SHB, PAL, and PKO were high in total saturated FAs (343.4 mg/g, 330.8 mg/g, and 153.3 mg/g, respectively). PAL was significantly higher in palmitic acid than all other foods (293.1 mg/g; $p<.001$). SHB was highest in stearic acid content (313.8 mg/g; $p<.001$). PKO contained the highest amount of myristic acid (82.8 mg/g; $p<.001$). SAM (27.8 mg/g), SOY (6.36 mg/g), DAW (17.3 mg/g)

Table 15: Saturated fatty acids expressed in mg FA/g crude oil or mg FA/g seed (Mean ± SD).

Sample ID	Sample Type	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Arachidic C20:0	Lignoceric C24:0	Total Saturated FAs
Baobab seed	Seed	0.15±0.02^{B}	12.0±1.44^{B}	3.28±0.42^{D}	0.77±0.10^{D}	0.17±0.03^{E}	16.4±1.93^{C}
Dawadawa	Seed	<LLOQ	5.15±1.09^{B}	8.28±1.44^{CD}	2.74±0.87^{C}	1.09±0.24^{C}	17.3±1.43^{C}
Fermented dawadawa	Seed	<LLOQ	14.7±1.85^{B}	21.9±2.88^{BC}	5.49±0.73^{B}	2.42±0.36^{A}	44.5±5.79^{C}
Neri seed	Seed	<LLOQ	24.4±1.04^{B}	13.0±1.22^{CD}	0.78±0.05^{D}	0.19±0.01^{E}	38.4±1.87^{C}
Soy bean	Seed	0.05±0.03^{B}	4.53±0.22^{B}	1.49±0.21^{D}	0.19±0.07^{D}	0.10±0.02^{E}	6.36±0.32^{C}
Peanut	Nut	<LLOQ	19.4±0.46^{B}	6.03±0.26^{D}	2.50±0.06^{C}	1.97±0.08^{B}	29.9±0.72^{C}
Sesame seed	Seed	<LLOQ	16.3±3.86^{B}	10.4±2.12^{CD}	0.99±0.21^{D}	0.15±0.01^{E}	27.8±6.18^{C}
Shea butter	Oil	<LLOQ	20.7±0.29^{B}	314±14.83^{A}	8.20±0.28^{A}	0.64±0.03^{D}	343±15.1^{A}
Palm oil	Oil	6.16±1.26^{B}	293±56.5^{A}	28.7±5.48^{B}	2.24±0.36^{C}	0.70±0.05^{CD}	331±63.7^{A}
Palm kernel oil	Oil	82.8±3.97^{A}	54.2±2.82^{B}	15.2±0.96^{BCD}	<LLOQ	<LLOQ	153±7.82^{B}
p-val		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

^{A,B,C,D,E,F}Denote Tukey HSD comparison at .05 significance level. Any value stated as <LLOQ was either below the limit of quantification, or below the lowest point on the standard curve. LLOQ, lower limit of quantification.

Monounsaturated fatty acids

Food-grade oils were more abundant in MUFAs compared to seeds (Table 16). The MUFAs quantified were palmitoleic (C16:1), oleic (C18:1) and eicosaenoic (C20:1). SHB (292.0 mg/g) and PAL (246.5 mg/g) were significantly higher in oleic acid than all other foods ($p<.001$). Levels of eicosaenoic and palmitoleic acid were low in all samples.

Polyunsaturated fatty acids

The PUFAs considered in this study include ALA (C18:3n3), LA (C18:2n6) and docosadienoic (C22:2) acid (Table 17). SOY was significantly higher in ALA (2.98 mg/g; $p<.01$) than all other foods. NER was significantly higher in LA (68.2 mg/g; $p<.01$) than all foods except FDD ($p=.13$).

Table 16: Monounsaturated fatty acids expressed in mg FA/g crude oil, mg FA/g nut or mg FA/g seed (Mean ± SD)

Sample ID	Sample Type	Palmitoleic C16:1	Oleic C18:1	Eicosaenoic C20:1	Total MUFAs
Baobab seed	Seed	0.25±0.25^{B}	15.2±1.62^{CD}	0.20±0.03^{D}	15.6±1.68^{CDE}
Dawadawa	Seed	0.03±0.01^{C}	9.20±2.35^{D}	0.43±0.12^{D}	9.68±2.21^{E}
Fermented dawadawa	Seed	<LLOQ	24.8±2.75^{CD}	0.97±0.14^{C}	25.9±2.90^{CDE}
Neri seed	Seed	0.11±0.01^{BC}	12.6±1.05^{CD}	0.28±0.02^{D}	13.0±1.05^{CDE}
Soy bean	Seed	0.04±0.02^{C}	11.8±2.53^{D}	0.19±0.05^{D}	12.1±2.47^{DE}
Peanut	Nut	<LLOQ	55.0±6.91^{BCD}	2.28±0.09^{A}	57.3±6.99^{BCD}
Sesame seed	Seed	0.15±0.04^{BC}	58.7±14.0^{BC}	0.49±0.05^{D}	59.3±13.9^{BC}
Shea butter (oil)	Oil	<LLOQ	292±11.0^{A}	2.20±0.09^{A}	294±11.0^{A}
Palm oil	Oil	0.68±0.15^{A}	247±46.7^{A}	1.57±0.29^{B}	249±47.2^{A}
Palm kernel oil	Oil	<LLOQ	91.5±0.82^{B}	0.85±0.03^{C}	92.4±0.80^{B}
p-value		<.0001	<.0001	<.0001	<.0001

^{A,B,C,D,E,F}Denote Tukey HSD comparison at .05 significance level. Any value stated as <LLOQ was either below the limit of quantification, or below the lowest point on the standard curve. LLOQ, lower limit of quantification.

Table 17: Polyunsaturated fatty acids expressed in mg FA/g crude oil or mg FA/g seed (Mean ± SD).

Sample ID	Sample Type	α-Linolenic (ALA) C18:3n3	Linoleic C18:2n6	Docosadienoic C22:2	Total PUFAs
Baobab seed	Seed	0.14±0.02^{c}	7.41±0.89^{d}	0.38±0.04	7.93±0.96^{f}
Dawadawa	Seed	0.34±0.05^{c}	20.8±4.15^{d}	<LLOQ	21.1±4.10^{ef}
Fermented dawadawa	Seed	0.94±0.03^{bc}	55.3±8.38^{ab}	<LLOQ	56.3±8.40^{ab}
Neri seed	Seed	0.20±0.01^{c}	68.2±3.78^{a}	<LLOQ	68.4±3.78^{a}
Soy bean	Seed	2.98±0.85^{a}	20.3±2.68^{d}	<LLOQ	23.3±3.46^{de}
Peanut	Nut	0.11±0.02^{c}	37.5±4.75^{c}	<LLOQ	37.7±4.75^{cd}
Sesame seed	Seed	0.48±0.01^{c}	47.2±10.8^{bc}	<LLOQ	47.7±10.8^{bc}
Shea butter (oil)	Oil	0.82±0.09^{c}	39.5±1.47^{c}	<LLOQ	40.3±1.56^{c}
Palm oil	Oil	1.79±0.31^{b}	47.3±3.79^{bc}	<LLOQ	49.0±3.76^{bc}
Palm kernel oil	Oil	<lloq	15.3±1.01^{d}	<LLOQ	15.3±1.01^{ef}
p-value		<.0001	<.0001	-	<.0001

^{a,b,c,d,e,f}Denote Tukey HSD comparison at .05 significance level. Any value stated as <LLOQ was either below the limit of quantification, or below the lowest point on the standard curve. LLOQ, lower limit of quantification.

Minerals

Minerals were abundant in seeds and nuts (Table 18). Iron levels in SOY (353 mg/kg), NER (282 mg/kg) and FDD (165 mg/kg) were relatively high, while FDD (55 mg/kg), SAM (51 mg/kg), SOY (45 mg/kg) and NER (34 mg/kg) contained high levels of zinc compared to other foods analyzed. High amounts of calcium were found in SAM (8767 mg/kg), FDD (4500 mg/kg) and SOY (3223 mg/kg). All samples analyzed had varying amounts of phosphorus (2980 mg/kg to 5500 mg/kg) and potassium (3007 mg/kg to 15733 mg/kg) and lower amounts of sodium (<300 mg/kg) and copper (9.1 mg/kg to 18.8 mg/kg). A high amount of manganese was found in FDD (145 mg/kg) and SOY (79 mg/kg).

Table 18: Mineral levels of seeds and nuts expressed as mg/kg

Sample ID	Calcium	Copper	Iron	Magnesium	Manganese	Phosphorus	Potassium	Sodium	Zinc
BAB	2070	9.1	120	3700	13.9	5500	12000	<197	30.2
DAW	5100	10	127	3257	73.7	2980	11867	301.33	40.33
FDD	4500	18.5	165	3240	145	4000	3007	<200	55
Neri	490	13.1	282	2127	22.2	4633	3500	<200	34.3
SOY	3223	11.43	353	2410	79	4733	15733	<194	44.7
PNUT	553	9.87	30	1987	16	3400	6900	<194	30.6
SAM	8767	18.8	111	2587	23.6	4767	4400	<197	51.3

Discussion

This study reports the FA and mineral composition of some seeds and food grade oils in Northern Ghana. The study shows that SOY and PAL contained levels of ALA, but SOY had significantly higher amounts of ALA ($p<0.001$). NER had significantly higher amounts of LA ($p<0.001$) than all foods with the exception of FDD ($p<0.153$). SHB and PAL contained significantly higher amounts of oleic acid than all the other foods analyzed ($p<0.001$). SHB, PAL, and PKO contained higher amounts of saturated fats while SAM. SOY, NER and FDD had low amounts of saturated fats. Mineral compositions of foods were as follows: SOY, NER and FDD, contained the highest amounts of iron; FDD, SAM, and SOY contained the highest levels of zinc and calcium. All the samples analyzed contained similarly high amounts of phosphorus and potassium.

Some of the foods identified and analyzed could be incorporated into diets to improve their nutritional quality. SOY, PAL, NER, FDD, and SAM are good sources of EFAs; hence, their inclusion in the diet may alleviate stunting in this population. PAL and SHB are reported to contain high amounts of oleic acid. However, they also have high amounts of saturated FAs. While oleic acid has crucial roles in human development, especially in immunity and in the myelination of brain neurons, saturated FAs have been associated with compromised cognitive flexibility in children [144]. Although SHB and PAL may be useful in diet to help in immune and cognitive function development, their high levels of saturated FAs may counteract or offset these benefits. Therefore, some processing techniques to eliminate saturated fats from SHB and PAL should be explored.

Furthermore, some minerals such as iron and zinc serve as cofactors in FA metabolism [137]. Calcium, a nutrient that is mainly supplied through the diet, is a primary bone

forming mineral. An increase in dietary calcium increases mineralization of the skeleton, especially during growing years [147]. This study shows that SOY, NERI, FDD, and SAM contain high amounts of zinc, calcium and iron. Dietary intake of these minerals may promote growth in individuals. However, the bioavailability of these minerals in foods can be impaired by high phytate levels, the storage form of elemental phosphorus. Since all the seeds contain phosphorus, wet processing methods such as soaking in water, boiling, fermentation and/or germination treatments may reduce phytic acid levels, increasing the bioavailability of nutrients [148]. The women in these communities can be taught such techniques to achieve full nutritional benefits of the available foods.

Some foods identified to be of high nutritional quality in this study are under-utilized both at household levels and in industrial settings. According to the results obtained from this study, NER, SOY, and SAM have a higher nutritional quality (FAs and minerals) than PNT. However, there have been numerous food supplements that incorporate PNT only, so it may be of interest to explore and utilize NER, SOY, and SAMs in nutrient supplements and also in complementary foods. NER, SOY, and SAM are less commonly consumed compared to other foods that are available in markets in Northern Ghana. Sesame seed is a cash crop and is relatively expensive because a bulk of what is produced is sold for income and not for domestic consumption. Through women cooperatives, the nutritional benefits of sesame seeds can be taught to the inhabitants so that they can prioritize feeding it to their children. Similarly, household consumption of NER and SOY can be increased through nutrition education and promotion, and help prevent stunting in the community.

We compared the results of our study to two renowned food composition databases: the West African Food Composition table and the USDA food composition database. Our mineral concentrations were comparable to concentrations reported in the WAFC table [149]. We acknowledge there is also some variability when we compared FA levels with the USDA food composition database [150]. The USDA food composition databases derive individual FA values based on others' results of total fat content, and this may contribute to the variability observed as our data were directly calculated and was not normalized to mean fats based on other researchers' findings. Further, the USDA does not report any statistics of variability.

To our knowledge, this study is the first to extensively characterize the fatty acid and mineral composition of local seeds, nuts, and oils in Northern Ghana. This study reveals the potential of SOY, SAM, FDD and NER from Northern Ghana to increase dietary EFA and mineral sources in the population. Additionally, the mineral composition of NER, SAM, and FDD in the WAFC is incomplete and our study adds to that body of knowledge. Despite these strengths, we acknowledge that this study has some limitations. First, the data from this study cannot be generalized to the entire population in Northern Ghana, hence future studies should investigate FA and mineral profiles from several markets in Northern Ghana. Moreover, the foods reported on were collected from markets rather than household collections, and there may exist variation between what is consumed in households compared to that obtained from the market. Another limitation of this study is that we do not have details regarding the processing methods of food-grade oils in Ghana. Additionally, the cooking time for the various foods analyzed is different, and the cooking time of oils are known to affects its nutritional quality. Future studies should investigate

the impact of local cooking methods on EFA degradation and measure antioxidant vitamins that may protect EFAs during cooking and processing. Due to these limitations, the results of this study should be interpreted with caution.

Conclusions

In conclusion, FDD, NER, SOY, and SAM are important sources of EFAs and minerals available in markets in Northern Ghana due to their high content of ALA and LA as well as zinc, calcium and iron. Processing methods to remove saturated FAs from PAL and SHB could be adopted due to their high content of saturated fats which could counteract the nutritional benefits in these oils It is widely known that EFAD and mineral deficiencies are associated with stunting and cognitive impairment. NER, SOY, and SAM could be promoted to inhabitants in the community to increase consumption. The foods could also be incorporated into diets and dietary supplements to potentially help prevent stunting and cognitive impairment in Northern Ghana. Future studies should investigate the incorporation of these foods in the diets of children in Northern Ghana to reduce the prevalence of iron-deficiency anemia and stunting.

CHAPTER 6: ASSOCIATION OF WHOLE BLOOD FATTY ACIDS AND GROWTH IN GHANAIAN CHILDREN 2-6 YEARS OF AGE

Abstract

In Ghana, stunting rates in children below 5 years of age vary regionally between Northern and Southern sectors. Dietary fatty acids (FAs) are crucial for linear growth. This study determined the association between whole blood FAs and growth parameters in Southern Ghanaian children 2-6 years of age. A drop of blood was collected on an antioxidant treated card and analyzed for FA composition. Anthropometric measurements were taken and z-scores calculated. Relationships between FAs and growth parameters were analyzed. Of the 209 subjects, 22% were stunted and 10.6% were essential FA deficient (triene/tetraene ratio>0.02). None of FAs were associated with growth parameters except total saturated fats and linoleic acid which showed trending negative significance. When the data were compared to previously reported data from Northern Ghana, the analysis showed that most n-3 FA levels were significantly higher and n-6 FA levels lower in the Southern Ghana population ($p<0.001$). Fish and seafood consumption in this Southern cohort is high and could account for low essential FA deficiency and lower stunting rates.

Introduction

Growth stunting, a condition of impaired development, is a strong indicator of chronic malnutrition and a major global nutritional challenge in Ghana [20]. Growth stunting affects over 165 million children globally, with Africa, Asia and South America reporting a higher prevalence [151]. Several countries in Sub-Saharan Africa are mostly affected

by stunting [151]. Studies in Ghana as reported by the Ghana demographic health survey (GDHS) states that 19% of Ghanaian children under five years of age are stunted [20]. Stunting typically becomes permanent once established and may be caused by poor maternal health, frequent childhood infections and inadequate nutrient intake [5]. There have been numerous interventions implemented to curb stunting including dietary supplementation and fortification of vitamins and minerals. Specifically, recent fatty acid (FA) supplementation studies in Ghana demonstrate that, lipid-based supplements increased hemoglobin (Hb) levels of pregnant women and growth spurts in children [35, 41]. However, these studies do not characterize the FA content in whole blood. Further, research investigating the relationship between circulating FA levels and growth is scarce in this population.

FAs have numerous physiological functions in human growth and development. For example, polyunsaturated FA (PUFA)-derived eicosanoids can activate transcriptional factors to influence stem cell proliferation and differentiation [8], and essential FAs (EFAs) help build structural barriers to prevent energy loss by the accumulation of LA into the stratum corneum [82]. These FAs can be metabolized into molecules with specificity for receptors whose signal transduction pathway results in changes to linear growth [82]. EFAs are those FAs which cannot be produced in the body because the human body lacks specific enzymes required for their *de novo* biosynthesis. Linoleic acid (C18:2n6, LA) and alpha-linolenic acid (C18:3n3, ALA) are the two main EFAs in the human diet [43, 44]. These omega-6 (n-6) and n-3 FAs are substrates for the desaturases (delta-5 and delta-6 desaturase) and elongases that produce the long chain (LC) PUFAs such as docosahexaenoic acid (DHA), arachidonic acid (AA) among others [48]. The elongase

enzymes usually prefer n-6 and n-3 FAs as substrates, but in their absence, they convert n-9 FAs into some LC-PUFA such as Mead acid, the appearance of which is a hallmark of EFA deficiency [47, 49]. More specifically, when an individual's diet is deficient of EFAs, oleic acid (C18:1n9), a non-essential n-9 FA, is converted to Mead acid (C20:3n9)[50]. Mead acid is then incorporated into phospholipids, cholesterol esters, triglycerides and non-esterified free FAs [51, 52]. Therefore, in EFA deficiency (EFAD), there are elevated levels of Mead acid with a decrease in the production of other EFA metabolites such as arachidonic acid. The ratio of Mead acid (3 double bonds, triene) and AA (4 double bonds, tetraene), termed as triene to tetraene ratio (T/T ratio), is a functional biomarker for EFAD [53-55]. EFAD is defined by a T/T ratio > 0.02 in plasma samples [53, 54], and also established when Mead acid [47] levels are above 0.4% in red blood cells (RBCs) [23] and 0.21% in plasma [54].

Child undernutrition is prevalent in Ghana due to low dietary diversity and poor infant and young child feeding (IYCF). Specifically, only 15% of breastfed Ghanaian children met minimum standards of IYCF practices with respect to both dietary diversity and feeding frequency[20]. Additionally, Ghanaian children 6-23 months are also affected by poor feeding practices such as inadequate complementary feeding. Sometimes, the complementary foods are deficient in essential nutrients such as proteins and fats [58]. Further, anemia, which is often described as an indicator of poor nutrition and poor health, is also prevalent in many parts of Ghana, with all the regions having a prevalence greater than the 40% World Health Organization (WHO) cut off [19]. These dietary intake patterns coupled with poor infant feeding practices could increase EFAD in infants and young children. Apart from fish, eggs, poultry and whole grains are also good sources of EFAs

[150, 152]. The dietary sources of the parent EFAs in Ghanaian diets are peanut (both ALA and LA-rich), melon seeds (LA-rich) and soy bean (ALA-rich) [56]. Insufficient consumption of foods which are good sources of EFAs may be leading to growth impairment.

Recently, some small-scale studies in Ghana using lipid-based supplementation have showed an increase in linear growth in Ghanaian children [35, 41], however, blood assessment of FA levels are typically not reported in Ghanaian children and the prevalence of EFAD has been poorly documented. Considering the significance of FAs in growth and development, the objective of this study was to assess blood FA levels in 2-to-6-year-old Ghanaian children and their association with growth.

Materials and Methods

Study setting

The study was conducted in the Upper-Manya Krobo district whose district capital is Asesewa located in eastern region of Ghana. The district covers 859.1 sq. km with a population of 72,092. The district comprises of 13,111 households with an average household size of 4.6 persons per household. The rainfall ranges from 900mm to 1500mm with temperature ranging from 26°C to 32°C. The district lies within the semi-deciduous forest and savanna zone. Palm, dawadawa, mango, neem and acacia interspersed with shrubs are the major vegetation in the district. Agriculture, forestry and fishing constitute the largest industry in the locality employing over 72% of the workforce aged 15 years and above. Boreholes, tubewell, pumps, rivers and streams constitute the sources of water supply of household for domestic purposes. Metal sheet is the main

roofing material for housing (87.9%). Illiteracy level is high with 33.3% of all inhabitants 11 years and above having no education. Like other communities in Ghana, the inhabitants are at risk of diseases and other contagious illnesses. The community has one hospital, 3 maternity homes, 4 health centers and 15 Community Health Posts[153]

Sample size and subjects

Children (n=209) between 2 to 6 years of age residing in communities in the Upper Manya Krobo district, Ghana were recruited for the study. A sub sample from a larger cohort, recruited as previously described [154] were enrolled in this study. Communities that were inaccessible for more than two weeks during a given period were also excluded. At household level, a household with a target child who had a medical/birth defect that affect eating and normal growth was excluded (eg. cerebral palsy). Data were collected from March to July 2017. An a-priori power analysis was conducted from the results of an earlier study that measured maternal and infant erythrocyte FA levels [38].

Ethical Standards Disclosure

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Ethics review board at McGill University Canada(IRB#180-1013), the Institutional Review Board at Michigan State University (IRB # 16-557) and the Nogouchi Memorial Institute for Medical Research ethics committee (IRB # 027/13-14). Written informed consent was obtained from all subjects/patients. A script of the written consent was read and translated in Twi, Krobo and Ewe to the parents or caregivers of the children. The parent or caregiver

of the participating child gave consent prior to the child's participation. The parents or caregivers thumb printed the consent document to give consent. They were assured that participation was voluntary and confidential, and that their information would remain anonymous.

Anthropometric measurements

Heights of all participants were measured to the nearest 0.1cm with a Shorrboard stadiometer (Weigh and Measure LLC, USA). Weight was measured using a digital bathroom scale to the nearest 0.1kg (Tanita BMB-800, Japan). The average of two height and weight measurements were recorded. The date of birth and gender was recorded from the child's health card or birth certificate. Height, weight, date of birth and sex data were entered into World Health Organization (WHO) Anthro [69] and WHO AnthroPlus [72] software to calculate height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), and BMI-for-age (BAZ) z-scores.

Blood fatty acid assessment

Capillary blood sample (40ul) was obtained by puncturing the middle finger using a sterile single-use lancet as previously described by Jumbe et al. [63, 65]. The first drop of blood was wiped with a sterilized dry pad. The drops of blood were then collected onto the dried blood spot cards, which are pre-treated with anti-oxidant cocktail. The cards were stored in a dry, cool environment and shipped to the USA for FA analysis at OmegaQuant Analytics, LLC (Sioux Falls, SD). On average, the time from sample collection time to

arrival to the US was 8 days. The samples were stored at –80°C till they were analyzed as previously described [66-68]. Briefly, the cards were punched and combined with the derivatizing reagent [boron trifluoride in methanol (14%), toluene, and methanol (35:30:35 parts)]. The mixture was shaken and heated at 100°C for 45 minutes. Forty parts of both hexane and distilled water were added after the mixture had cooled. The mixture was vortexed and then separated into distinct layers. An aliquot of the hexane layer that contained the FA methyl esters was extracted. FA analysis was performed as previously described [70, 71, 105]. Unless otherwise stated, whole blood FA proportions are expressed as a percent of total identified FAs.

Hemoglobin and malaria status

A hemocue photometer (HemoCue 301, Angelholm, Sweden) was used to determine the hemoglobin concentration. The malaria status was determined using an antigen-based malaria rapid diagnostic test (RDT) kit (Standard diagnostic Inc., Korea). These tests were conducted using additional drops of blood from the same punch.

Dietary intake assessment

Using a structured questionnaire, the intake of various foods consumed by subjects within 24 hours prior to blood sample collection was assessed. The foods that were of interest include fish and seafood, diary, meats and fruits.

Data reduction and statistical analyses

Z-scores for the growth parameters HAZ, WAZ, BAZ and WHZ were calculated using WHO Anthro [69]. Means and standard deviations were calculated for descriptive analysis. Based on the WHO standard population and definitions of moderate and severe stunting, wasting, and underweight percentages were calculated [74]. The FA values presented here are expressed as percent composition of total blood FAs. Total n-3 FA proportions were calculated as ∑ [alpha-linolenic + eicosapentaenoic acid (EPA) + docosapentaenoic n-3 + docosahexaenoic acid (DHA)]; total n-6 FA proportions were calculated as ∑ [linoleic + linoelaidic + eicosadienoic + dihomo-gamma-linolenic + arachidonic + docosatetraenoic + docosapentaenoic n-6]; total n-9 FA proportions were calculated as ∑ [oleic + elaidic + eicosenoic + Mead + nervonic]; total saturated FA proportions were calculated as ∑ [myristic + palmitic + stearic + arachidic + behenic + lignoceric]; total MUFA proportions were calculated as ∑ [palmitoleic + oleic + palmitelaidic + nervonic + elaidic + eicosenoic]. T/T ratio was calculated from the ratio of Mead acid and AA[29]. FA product/precursor ratio was used to estimate the desaturase activity [155] as follows: D5D= AA/DGLA; D6D= DGLA/LA.

FA composition of children who were stunted and those who were not, as well as FA levels of northern vs southern Ghana children, were compared using two sample t-test. Normal probability plots were assessed to verify the validity of regressions. The regression model for HAZ, BAZ, WHZ or WAZ was adjusted for Hb and malaria. Hemoglobin was used as a covariate as it was significantly associated with HAZ and WAZ ($p \leq .01$). Malaria was also adjusted in the model as a significant percentage (11%) of the children tested positive to RDT/malaria test. Regression formulas consisted of either the

dependent variable HAZ, WAZ, WHZ, or BAZ, and models were adjusted for each FA and Hb levels (e.g., HAZ = FA + Hb + malaria). Regression models were not adjusted for sex as there were few significantly different FAs between sexes and regression values were unaffected when evaluated with sex adjustment. P-values were considered significant if p≤ 0.05. All statistical analyses were conducted using software R (R version 3.3.0) and verified with SPSS version 24 (IBM).

Regression models were not adjusted for sex as there were few significantly different fatty acids (FAs) between sexes and regression values were unaffected when evaluated with sex adjustment. P-values were considered significant if p≤ 0.05. All statistical analyses were conducted using software R (R version 3.3.0) and verified with SPSS version 24 (IBM).

Results

Subject characteristics

The demographic information for the subjects are presented in Table 19. The mean age of all 209 subjects was 38.31±9.98 months. There were more males (51.7%) than females (48.3%) in the study. The average height of participants was 91.45±7.10 cm, and the average weight of participants was 12.78±2.13 kg. The average hemoglobin level was 10.90g/dl. A positive malaria test was detected in 11.00% of the children. The mean HAZ, WAZ, WHZ and BAZ were -1.35, -1.04, -0.41 and -0.27 respectively. The standard deviations of the HAZ, WAZ, and WHZ distributions were relatively constant and close to the expected value of 1.0 (range: 0.91 – 1.01). Using the WHO guidelines and criteria[74],

22% of the children were stunted, 12.9% were underweight and 3.4% were wasted. (Table 20).

Table 19:Demographic characteristics of participants

	Mean ± SD		
	Overall	Male	Female
	(n=209)	(n=108)	(n=101)
Age (mo)	38.31±9.98	38.46±9.95	38.15±10.06
Height (cm)	91.45±7.10	91.92±7.04	90.96±7.16
Weight (kg)	12.78±2.13	13.08±1.99	12.47±2.24
HAZ	-1.35±0.91	-1.40±0.88	-1.28±0.94
BAZ	-0.27±1.01	-0.15±0.88	-0.39±1.12
WAZ	-1.04±0.97	-1.00±0.86	-1.08±1.08
WHZ	-0.41±1.01	-0.32±0.89	-0.49±1.12
Hb (g/dL)	10.90±1.36	10.8±1.45	11.00±1.25

HAZ, height-for-age z-score; BAZ, BMI-for-age z-score; WAZ, weight-for-age z-score; WHZ, weight-for-height z score; Hb, hemoglobin.The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data[69].

Fatty acid levels in whole blood

The mean fatty acid levels for overall, stunted and non-stunted children in Southern Ghana are shown in Table 21. Approximately 10.5% of all children in the study had whole blood T/T ratio greater than 0.02 and 4.5% had Mead acid levels above 0.21%. For all 209 children enrolled in the study, the average percent whole blood measured was 40.2% saturated FAs, 23.3% total monounsaturated FAs, 29.1% total omega-6 and 7.2% total omega-3 FAs. The major component components of omega-6 and omega-3 FAs were LA

Table 20: Nutrition and growth status of children.

	Based on	**Severe**	**Moderate**	**Unaffected**
		(<-3SD)	**(≤-2SD)**	
Stunting	HAZ	4.30%	17.70%	78.00%
Malnutrition	BAZ	1.00%	1.90%	97.10%
Underweight	WAZ	2.40%	10.50%	87.10%
Wasting	WHZ	1.00%	2.40%	96.60%

HAZ, height-for-age z-score; BAZ, BMI-for-age z-score; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score;
[a]The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data[69].

and DHA, accounting for 16.7% and 5.1% of whole blood respectively. Overall, the mean blood FAs % of total did not differ between stunted and non-stunted children. Of interest, there was no significant difference between Mead Acid when comparing stunted vs non-stunted children. Although not significant, children who were stunted had lower blood levels of DHA (4.95%) than those who were not stunted (5.13%) or the overall group (5.09%). Children who were stunted has significantly higher ratios of delta-5 (p=0.054) and delta-6 (p=0.027) desaturase activity. However, no significant difference was found between T/T ratio of both groups.

Table 21: Mean fatty acid levels for overall, stunted and non-stunted children in Southern Ghana (Values expressed as mean ± standard deviation)

Class	Fatty Acid	Overall (n=209)	Stunted (n=46)	Not Stunted (n=163)	P Value
Saturated FAs	Myristic	0.82±0.59	0.76±0.41	0.84±0.63	0.46
	Palmitic	25.7±1.62	25.8±1.53	25.6±1.65	0.592
	Stearic	11.9±1.16	12.1±1.14	11.9±1.16	0.209
	Arachidic	9.18±1.56	0.31±0.06	0.31±0.06	0.752
	Behenic	0.84±0.41	0.59±0.16	0.56±0.15	0.215
	Lignoceric	0.90±0.34	0.95±0.39	0.88±0.32	0.251
	Total sat	40.2±1.54	40.5±1.46	40.1±1.56	0.108
MUFAs	Palmitoleic	0.84±0.41	0.88±0.50	0.82±0.39	0.411
	Oleic Acid	21.0±2.68	20.8±2.56	21.0±2.72	0.596
	Eicosenoic	0.34±0.14	0.37±0.13	0.33±0.14	0.156
	Nervonic	0.83±0.30	0.87±0.30	0.82±0.29	0.248
	Total MUFA	23.3±2.70	23.2±2.62	23.3±2.73	0.818
n-3FAs	ALA	0.25±0.10	0.27±0.12	0.25±0.09	0.645
	EPA	0.80±0.35	0.74±0.23	0.81±0.38	0.22
	DPA	1.01±0.21	1.04±0.21	1.00±0.21	0.364
	DHA	5.09±0.98	4.95±0.95	5.13±0.98	0.263
	Total n-3	7.15±1.34	7.00±1.19	7.19±1.37	0.379
	Omega-3 Index	8.03±1.37	7.80±1.23	8.09±1.40	0.204
n-6 FAs	LA	16.7±1.92	16.6±2.43	16.7±1.76	0.589
	GLA	0.21±0.09	0.20±0.10	0.21±0.08	0.61
	EDA	0.03±0.05	0.25±0.06	0.25±0.05	0.156
	Eicosatrienoic	1.24±0.23	1.30±0.25	1.22±0.22	0.048
	AA	9.18±1.56	9.09±1.29	9.21±1.64	0.645
	DTA	1.03±0.25	1.09±0.22	1.02±0.26	0.118
	DPA	0.45±0.13	1.02±0.26	0.44±0.13	0.262
	Total n-6	29.1±2.60	29.0±2.71	29.1±2.57	0.779
Ratios	Mead acid	0.09±0.01	0.09±0.07	0.09±0.06	0.920
	n-6/ n-3 ratio	4.19±0.81	4.26±0.84	4.18±0.08	0.561
	Delta-6-Desaturase	0.08±0.02	0.08±0.02	0.07±0.02	0.027
	Delta-5-Desaturase	7.61±1.68	7.22±1.48	7.72±1.72	0.054
	T/T ratio	7.61±1.68	7.22±1.48	7.72±1.72	0.054

[a]Values represent blood fatty acid (FA) % composition. Stunted defined by height-for-age z-score (HAZ)≤-2. n-9, omega-9; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; n-6, omega-6.

[b]P-value from Wilcoxon-Mann-Whitney test comparing stunted and non-stunted children.

[c]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic.

[d]Total n-3 includes ALA, EPA, DPA n-3, and DHA.

[e]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Table 22: Regression results between HAZ, WAZ, and selected fatty acids

	Fatty acid	HAZ		WAZ	
		B±SE	p-value	B±SE	p-value
Saturated fats	Myristic Acid	-0.09±0.11	0.38	-0.10±0.12	0.39
	Palmitic Acid	-0.04±0.04	0.38	0.00±0.04	0.83
	Stearic Acid	-0.04±0.06	0.50	-0.01±0.06	0.92
	Total sat	-0.07±0.04	**0.08**	-0.03±0.05	0.56
MUFAs	Palmitoleic Acid	-0.08±0.15	0.61	0.01±0.16	0.96
	Oleic Acid	-0.00±0.02	0.92	0.01±0.03	0.81
	Eicosenoic Acid	-0.78±0.46	0.89	-0.48±0.49	0.32
	Nervonic Acid	0.04±0.22	0.86	0.14±0.23	0.53
	Total MUFA	-0.01±0.02	0.80	-0.01±0.03	0.78
n-3FAs	ALA	-0.57±0.66	0.39	0.32±0.70	0.65
	EPA	0.00±0.18	1.00	0.04±0.19	0.85
	DPA	-0.20±0.31	0.51	0.20±0.33	0.53
	DHA	0.06±0.07	0.39	0.03±0.07	0.72
	Total n-3	0.02±0.05	0.65	0.02±0.05	0.68
n-6FAs	LA	0.04±0.03	0.22	-0.02±0.04	0.68
	GLA	0.77±0.68	0.25	0.82±0.72	0.26
	EDA	0.50±1.25	0.69	0.75±1.33	0.57
	Eicosatrienoic	0.05±0.28	0.87	0.31±0.30	0.30
	AA	0.01±0.04	0.75	-0.01±0.05	0.90
	DTA	-0.27±0.25	0.29	0.02±0.27	0.95
	DPA	-0.13±0.48	0.78	0.40±0.51	0.43
	Total n6	0.03±0.03	0.32	-0.01±0.03	0.83
Ratios	n-6/n-3 ratio	0.02±0.08	0.77	-0.07±0.08	0.42
	Omega-3 Index	0.03±0.05	0.48	0.02±0.05	0.48
	T/T ratio	5.51±9.21	0.55	-4.53±9.83	0.65

Model: HAZ=fatty acid + hemoglobin+malaria; WAZ= fatty acid + hemoglobin+malaria).
[a]Values represent blood fatty acid (FA) % composition. Stunted defined by height-for-age z-score (HAZ)≤-2. n-9, omega-9; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; n-6, omega-6.
[b]P-value from Wilcoxon-Mann-Whitney test comparing stunted and non-stunted children.
[c]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic.
[d]Total n-3 includes ALA, EPA, DPA n-3, and DHA.
[e]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Relationships or associations between fatty acids and growth parameters

Table 22 shows the results of the regression analysis between HAZ and WAZ for selected FAs. There was no significant association between FAs and WAZ and HAZ except for total saturated fats (p=0.08) which was associated with HAZ. Table 23 shows a regression

between WHZ and BAZ and some selected fatty acids. With the exception of LA which was related with WHZ (p=0.08) and BAZ (p=0.07) all other FAs were not associated with any of the growth parameters.

Table 23: Regression results between WHZ, BAZ, and selected fatty acids (model: WHZ=fatty acid + hemoglobin + malaria; BAZ= fatty acid + hemoglobin + malaria)

		WHZ		BAZ	
	Fatty acid	**B±SE**	**p-value**	**B±SE**	**p-value**
Saturated fats	Myristic	-0.10±0.12	0.41	-0.07±0.12	0.55
	Palmitic	0.02±0.05	0.65	0.03±0.05	0.55
	Stearic	0.03±0.06	0.59	0.03±0.06	0.63
	Total sat	0.03±0.05	0.5	0.03±0.05	0.50
MUFA	Palmitoleic	0.12± 0.17	0.50	0.10±0.17	0.56
	Oleic	0.02±0.03	0.56	0.02±0.03	0.57
	Eicosenoic	0.02±0.51	0.97	0.11±0.51	0.83
	Nervonic	0.19±0.24	0.43	0.19±0.24	0.43
	Total MUFA	0.01±0.03	0.43	0.02±0.03	0.44
n-3 FAs	ALA	0.88±0.73	0.23	1.06±0.73	0.15
	EPA	0.03±0.20	0.87	0.03±0.20	0.87
	DPA	0.46 ±0.34	0.18	0.49±0.34	0.15
	DHA	-0.02±0.07	0.80	-0.03±0.07	0.72
	Total n-3	0.01±0.05	0.89	0.01±0.05	0.91
n-6 FAs	LA	-0.07±0.04	**0.08**	-0.07±0.04	**0.07**
	GLA	0.78±0.75	0.30	0.54±0.75	0.48
	Eicosadienoic	0.50±1.39	0.67	0.51±0.39	0.72
	Eicosatrienoic Acid	0.52±0.39	0.09	0.52±0.31	0.10
	AA	-0.03±0.05	0.56	-0.03±0.05	0.53
	DTA	0.27±0.28	0.33	0.28±0.28	0.29
	DPA	0.66±0.53	0.22	0.72±0.53	0.16
	Total n6	-0.04±0.03	0.19	-0.04±0.03	0.17
Ratios	n-6/Total n-3 ratio	-0.12±0.09	0.18	-0.12±0.09	0.18
	Omega-3 Index	-0.01±0.05	0.86	-0.01±0.05	0.81
	T/T ratio	-8.27±10.2	0.42	11.4±10.2	0.27

[a]Values represent blood fatty acid (FA) % composition. Stunted defined by height-for-age z-score (HAZ)≤-2. n-9, omega-9; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; n-6, omega-6.
[b]P-value from Wilcoxon-Mann-Whitney test comparing stunted and non-stunted children.
[c]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic.
[d]Total n-3 includes ALA, EPA, DPA n-3, and DHA.
[e]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Mean fatty acid levels for Northern and Southern Ghana, compared

Next, we compared the mean fatty acid levels between our previously published data from Northern Ghanaian children [156] to these data from Southern Ghanaian children. Interestingly, there were highly significant differences in mean whole blood % for most of the fatty acids ($p<0.001$; Table 23). Specifically, the mean whole blood levels of n-3 FAs such as ALA, EPA, DHA, DPA n-3, and omega–3 index were significantly higher ($p<0.001$) in the Southern Ghana population than in the Northern Ghana population. Mean level of DHA and the omega-3 index, for example, were 2.62% and 8.03% respectively in the Northern Ghana population compared to 5.09% and 4.55% in the Southern Ghana population. Further, the mean whole blood n-6 FAs LA, AA. DTA, DPA n-6 and were higher in the Northern Ghana population ($p<0.001$). The T/T ratio and Mead acid were higher in Northern Ghana population than the in Southern Ghana population ($p<0.001$; table 24). This indicates a higher rate of EFA in the Northern Ghanaian population.

Table 24: Mean FA levels for Northern and Southern Ghana, expressed as mean ± standard deviation.

Class	Fatty acid (% of total)	Southern Ghana N=209	Northern Ghana N=307	P-Value
n-3 FAs	ALA	0.25±0.10	0.18±0.12	<0.001
	EPA	0.80±0.35	0.22±0.26	<0.001
	DPA	1.01±0.21	0.58±0.18	<0.001
	DHA	5.09±0.98	2.62±0.64	<0.001
	Total n-3[1]	7.15±1.34	3.61±0.91	<0.001
	Omega-3 Index[2]	8.03±1.37	4.55±0.92	<0.001
n-6 FAs	LA	16.7±1.92	20.6±1.85	<0.001
	GLA	0.21±0.09	0.17±0.07	<0.001
	Eicosadienoic	0.25±0.05	0.29±0.07	<0.001
	Eicosatrienoic	1.24±0.23	1.36±0.26	<0.001
	AA	9.18±1.56	10.8±1.61	<0.001
	DTA	1.03±0.25	1.69±0.37	<0.001
	DPA	0.45±0.13	0.59±0.17	<0.001
	Total n-6[3]	29.1±2.60	35.7±2.53	<0.001
ratios	n-6/n-3 ratio	4.20±0.81	10.3±2.00	<0.001
	TT Ratio	0.010±0.007	0.013±0.005	<0.001
	Mead acid	0.09±0.06	0.14±0.05	<0.001

[1]Total n-3 includes alpha linolenic, eicosapentaenoic, docosapentaenoic n-3, and docosahexaenoic;
[2]EPA+DHA
[3]Total n-6 includes linoleic, linolaidic, Ɣ-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6;
T/T, triene-to-tetraene:ALA, alha linolenic acid; EPA, Eicosapentaenoic acid; DHA, Docosqahexaenoic acid; LA, linoleic acid, GLA, Gamma linoleic acid, ; DTA, docosatetraenoic acid, docosapentaenoic acid

Dietary intake of proteins in Southern Ghana

Protein intake in the population is summarized in table 25. The records of protein intake shows that 92.82% consumed fish or sea food 24 hours prior to data collection. The records also show that 23.92%, 18.18% and 17.22% of the children consumed meat from poultry, eggs and dairy products respectively.

Discussion

The objective of this study was to describe the whole blood FA levels of Southern Ghanaian children and to determine the association between FAs and growth parameters. The prevalence of stunting in in our sample was 22% which is higher than the 17% stunting rate reported by the GDHS in the Eastern region of Ghana. However, the stunting rate in this population is lower than what is reported in the Northern Ghana [157]. The prevalence of EFAD was 4.64% and 10.6% based on the Mead acid levels and T/T ratio respectively. These values are both lower than what was previously reported in Tanzania [63]. With the exception of total saturated FAs and LA which showed a trending significance with some growth parameters, none of FAs were associated with growth parameters. When the blood levels of FAs from this study were compared to previously reported data from Northern Ghana, most n-3 FAs levels were significantly higher in the Southern Ghana population ($p<0.001$) and most n-6 FAs level were significantly higher in the Northern Ghana population ($p<0.001$).

Mean omega-3 index in the Southern Ghana population is significantly higher ($p<0.001$) than what was reported in an earlier study in Northern Ghana [156]. Whole blood levels of DHA and EPA are highly dependent on dietary intake. Although, there is conversion of ALA to its metabolites including EPA and DHA, there is limited conversion of ALA to DHA in mammalian species [158]. It is therefore likely that the high EPA and DHA levels were obtained from the diet and contribute to the omega-3 index. While the intake of carbohydrates are similar across populations in Ghana[159], it is likely that location or local culture may contribute to limited access of foods rich in DHA and EPA. Over 90% of the subjects consumed fish and sea food in the 24 hours prior to data collection. This consumption pattern is higher when compared to data collected by the GDHS that states that for children between the ages of 6-23 months, only 47.6% of them consumed meat, fish and poultry 24 hours prior to data collection [37]. As stated earlier, apart from agriculture and forestry, fishing is one economic activity that is common among inhabitants of Upper Manya Krobo district [153]. As a primary economic activity in the communities, fishing can increase accessibility of fish hence increase consumption compared to the Northern region where the inhabitants are predominantly farmers and traders living in mostly landlocked communities [59]. Geographic difference between northern and southern Ghana contributes to the kinds of food being grown, harvested, and hence dietary composition of the foods being consumed [18]. These geographic differences could explain the regional variations of omega-3 index across the two populations. The omega-3 index for Southern Ghanaian children are also higher than values reported for Australian school aged children [160], UK children aged 7-9 years [161], Tanzanian children 2-6 years [64] and in European children 3-8 years [162]. The

reasons for these variations is likely attributable to the dietary variation in the various populations.

Omega-6 FAs have crucial roles in growth. A previous study from our group reported an association of n-6 PUFAs and linear growth in Tanzanian children [63]. LC n-6 PUFAs such as AA modulate varied physiological responses such as cell growth and differentiation and in conditions that involve altered cellular proliferation [82]. Although, this population had a lower stunting levels, most of the n-6 FAs were significantly lower than those reported in a population whose stunting rates are higher. The significant difference in delta-5 and delta-6 desaturase activity between stunted and non-stunted groups can be attributed to differing levels of DGLA among both groups. As much as a decrease in dietary consumption of n-6 precursor LA in this population may lead to a decrease in n-6 FA metabolites, which could further lead to the suppression of the n-6 FA pathways by the high amounts of dietary n-3 FAs. These findings support previous observations that, the n-3 and n-6 FA pathways are competitive and an increase in dietary intake of one could suppress the other [122].

The exact mechanism of n-3 FAs in growth remains unclear, however their roles in growth is undisputed [163]. Omega-3 FAs have beneficial roles in bone metabolism, by increasing bone formation that affects peak bone mass and reducing bone loss [25]. This is because n-3 FAs reduce inflammatory cytokines, increases calcium resorption and enhances calcium levels [25]; roles that contribute to growth in children. The southern Ghanaian children who participated in this study were reported to have high fish and sea food consumption. Fish and seafood are superior sources of EPA and DHA. They also contain high quality proteins, amino acids, fiber, vitamins and minerals [164, 165]. In

addition, fish and seafood have high amounts of essential amino acids such as leucine, lysine and tryptophan [166] which would lead to lower levels of protein energy malnutrition. A recent study by Semba et al. reported that child stunting is associated with circulating amino acids and also reported that stunted children have a limited amount of tryptophan and lysine in their diet. [167]. This study population did not record high levels of stunting. The low level of stunting could possibly be due to high amounts of essential amino acids from high dietary intake of fish and seafood in the population since the children were recruited from fishing communities [153]. The high intake of proteins in this population can rule out protein energy malnutrition which leads to growth stunting. It is also likely that the high intake of proteins in this population may enhance lipid synthesis and further promote linear growth.

This is the first study to assess whole blood FAs in Southern Ghanaian children 2-6-year olds. This study utilized whole blood FA biomarkers and the association with growth. The use of a validated dried blood spot collection and blood transport system made the study logistically easier to conduct. This method was also successfully used in a similar study in Northern Ghana [156] and in Tanzania [63]. Aside from these strengths, this study also has limitations that should be noted. We did not collect information on extensive dietary intake hence we are unable to account for the role of other nutrients on growth parameters. Iodine, zinc and other vitamins and minerals have roles to play in growth, but the study could not account for all these nutrients. The collection of blood samples took place throughout the day and the subjects were not required to fast. Since the FA intake across this population is similar, this will not have any effect on the variability of the whole blood FA measurements. Finally, this study cannot be generalized to the whole Ghanaian

population because the study was performed in one area in Southern Ghana and dietary intake of foods may differ across locations in Ghana.

Conclusions

This study assessed whole blood levels of EFAs and their relationship with growth parameters in Southern Ghanaian children 2-6 years of age. EFAD was observed in 10.64% of children while stunting was observed in 22% of all children, yet the was no association observed with whole blood FA and growth parameters. These data suggest that other factors other than EFAD may influence stunting in this population. There is therefore the need for further studies to explore other nutrients and other factors that could be related to stunting in this population. Further, over 90% of all subjects reported dietary intake of fish and seafood prior to blood collection and this could decrease the risk of protein-energy malnutrition and EFAD as a potential cause of stunting in this population. Interestingly, the whole blood n-3 FAs are much higher in this population than that reported in children in other geographical areas. The intake of fish in this population could account for low stunting levels in this population consistent with previous studies demonstrating that increasing consumption of fish and fish oil intake can increase in linear growth in children [165, 167]. There is a need for similar randomized studies, in other populations in Ghana with both high and low fish consumptions**.**

CHAPTER 7: ASSOCIATION OF WHOLE BLOOD FATTY ACIDS WITH COGNITIVE FUNCTION IN 2 TO 6-YEAR-OLD SOUTHERN GHANAIAN CHILDREN.

Abstract

The role of essential fatty acids (EFAs) and the long chain polyunsaturated FA docosahexaenoic acid (DHA), on cognition and brain development have been demonstrated in a number of studies. The objective of this study was to investigate the relationship between whole-blood FAs and executive function in children from Southern Ghana. A total of 209, 2-to-6-year-old children attempted the dimensional change card sort (DCCS) task to assess executive function, and dried blood spot samples were collected and analyzed for FA content. Of the 209 participants only 25 of them were able to complete the instructional phase. Twenty children were randomly selected again from the same population for re-testing. Among the children who attempted the DCCS test, whole blood levels of linoleic acid was positively associated with executive function. The analysis further showed that, children with low levels of myristic acid ($p=0.024$) and total saturated fats ($p=0.049$) performed better in the initial phase of the DCCS test. Children who were picked up from private schools performed better than those in public schools. Children who were picked up at home performed poorly on all phases of the DCCS test. Our study supports that lower levels of whole blood saturated fats may improve cognitive function. Also children who have been exposed to education may perform better at the DCCS test, hence future studies can probe to know the effect of education in DCCS in children in developing countries.

Introduction

Human growth, both in the fetus and neonates are greatly enhanced by essential fatty acids (EFAs) and their long chain metabolites [85-87]. During pregnancy and early childhood, they accumulate brain and neonatal tissues. [85]. Long chain polyunsaturated fatty acids (LCPUFA) are also concentrated in the central nervous system [88]. They have significant roles in neuronal growth and differentiation of cells and have been associated with cognitive abilities [88-90]. Further, retinal tissues and the brain depend on EFAs, especially for membrane fluidity and signal transduction [26]. LCPUFAs have critical roles in brain development therefore poor PUFA status may affect brain development and cognitive abilities in children [90]. LCPUFA could be included in the diets of infants and children to ensure optimal brain development [92-94].

The diets in most developing countries lack animal foods because majority of inhabitants in such populations are unable to afford diets rich in animal foods [33, 90]. Lack of animal foods may lead to PUFA deficiency[90]. Precisely, the Ghanaian diet are high in carbohydrates but low in fats and proteins[96]. This dietary pattern pre-disposes a section of the population to EFA deficiency. Numerous studies on EFAs have been limited to the use of supplements comprising of EFA [linoleic acid (LA), alpha linolenic acid (ALA)] and/or their metabolites [DHA, eicosapentaenoic acid (EPA) and arachidonic acid (AA)]. The results of these studies have established the roles of EFAs in improved cognition [11, 13, 97, 98]. A study conducted in Tanzania showed that whole blood FA status was associated with cognitive abilities in children 4-6 year old [64]. Similar unpublished studies have been conducted in a population in Northern Ghana, a population with low fish intake.

However no study of the association between whole blood FAs and cognitive function has yet been conducted a population with high fish intake.

Executive function, the conscious control of thoughts and action involves inhibition, working memory and task switching [100]. Executive function develops in children between the ages of two and ten years [100] and it is controlled by the frontal and temporal lobes of the brain [99]. The frontal and temporal lobes of the brain have high amounts of AA and DHA and they continue to develop after the second year of life [101]. Executive function can be assessed by the dimensional change card sorting (DCCS) task, which is a validated method used to assess cognitive function [100] [102]. This study utilized the DCCS task to assess executive function in Ghanaian children age 2-6. In the Ghanaian population, little is known about cognitive function assessment their association with FAs. We hypothesized that whole blood levels of EPA, DHA, and both EFAs (ALA and LA) would be positively associated with performance on the DCCS test.

Methods

Study setting

The study was conducted in a rural village in the eastern region of Ghana, specifically, in the Upper-Manya Krobo district whose district capital is Asesewa. The entire district has a population of 72,092 and covers 859.1 sq. km. The district comprises of 13,111 households with an average household size of 4.6 persons per household. The temperature ranges from 26°C to 32°C with rainfall ranging from 900mm to 1500mm. The district lies within the semi-deciduous forest and savanna zone. Agriculture, forestry and

fishing employs 72% of the workforce aged 15 years and above, constituting the largest industry in the locality. Household water supply is usually from boreholes, tube well, pumps, rivers. Metal sheet is the main roofing material for housing (87.9%). Illiteracy level is high with 33.3% of all inhabitants 11 years and above having no education. The inhabitants in Asesewa, like any community in Ghana are at risk of diseases and other contagious illnesses. The community has one hospital, 3 maternity homes, 4 health centers and 15 Community Health Posts[153]

Sample size and subjects

Children (n=242) between 2 to 6 years of age residing in communities in the Upper Manya Krobo district, Ghana were recruited for the study. A sub-sample from a larger cohort, recruited as previously described [154] were enrolled in this study. At the community level, the urban town of Asesewa, the district capital was exempted from the study. Further, communities that were inaccessible for more than two weeks during a given period were also excluded. At household level, a household with a target child who had a medical/birth defect that affect eating and normal growth was excluded (eg. cerebral palsy). Data were collected from March to July 2017.

Ethical Standards Disclosure

All procedures involving human subjects/patients were approved by the Ethics review board at McGill University Canada (IRB#180-1013), the Institutional Review Board at Michigan State University (IRB # 16-557) and the Nogouchi Memorial Institute for Medical

Research ethics committee (IRB # 027/13-14). Written informed consent was obtained from all subjects/patients. A script of the written consent was read and translated in Twi, Krobo and Ewe to the parents or caregivers of the children. The parent or caregiver of the participating child gave consent prior to the child's participation. They were assured that participation was voluntary and confidential, and that their information would remain anonymous.

Dietary intake assessment

The intake of various foods consumed by subjects within 24 hours of blood sample collection was assessed using a structured questionnaire. The foods that were of interest include fish and seafood, diary, meats and fruits.

Anthropometric measurements

Anthropometric measurements were taken using averages of the records taken. With a Shorrboard stadiometer (Weigh and Measure LLC, USA), heights of all participants were measured to the nearest 0.1cm. Weight was measured using a digital bathroom scale to the nearest 0.1kg (Tanita BMB-800, Japan). The average of two height and weight measurements were recorded. The date of birth was recorded from the child's health card or birth certificate. The gender of the child was also recorded. Height, weight, date of birth and sex data were entered into World Health Organization (WHO) Anthro [69] and WHO AnthroPlus [72] software to calculate height-for-age (HAZ), weight-for-age (WAZ), weight-for-height (WHZ), and BMI-for-age (BAZ) z-scores.

Blood fatty acid assessment

Using a sterile single-use lancet, capillary blood sample (40ul) was obtained by puncturing the middle finger as previously described by Jumbe et al., 2016 [63, 65]. The first drop of blood was wiped with a sterilized dry pad. The drops of blood were then collected onto the DBS cards, cards which are pre-treated with anti-oxidant cocktail. The cards were stored in a dry, cool environment and shipped to the USA for FA analysis at OmegaQuant Analytics, LLC (Sioux Falls, SD). The cards are kept averagely for 8 days, thus from, the sample collection time and time of arrival to the US lab. The samples were stored at –80°C till they were analyzed as previously described [66-68]. Briefly, the cards were punched and combined with the derivatizing reagent [boron trifluoride in methanol (14%), toluene, and methanol (35:30:35 parts)]. The mixture was shaken and heated at 100°C for 45 minutes. Forty parts of both hexane and distilled water were added after the mixture had cooled. The mixture was vortexed and then separated into distinct layers. An aliquot of the hexane layer that contained the FA methyl esters was extracted. FA analysis was performed as previously described [70, 71, 105]. Unless otherwise stated, whole blood FA proportions are expressed as a percent of total identified FAs.

Hemoglobin and malaria status

Using a hemocue photometer (HemoCue 301, Angelholm, Sweden) hemoglobin concentration was determined. The malaria status was determined using an antigen-based malaria rapid diagnostic test (RDT) kit (Standard diagnostic Inc., Korea). These tests were conducted using additional drops of blood from the same punch.

Cognitive assessment: Dimensional change card sort (DCCS)

The DCCS [100, 106] is a cognitive function assessment tool that requires the child sort a series of bivalent cards based on one of two instructed dimension (i.e., either color or shape).Initially, the child is asked to sort a series of eight cards based on colour. The child is instructed to switch the categorization dimension and sort another series of eight cards based upon shape (figure 3), after sorting an initial series of eight cards based upon color, This dimensional change in sorting behavior offers an index of executive function as the child must subdue their previously learned set of rules (i.e., sorting by color) and attentional inertia towards those attributes in order to flexibly adjust their behavioral actions and attention to sort the cards by a new set of rules (i.e., sorting by shape) [100, 107]. The child was considered to have passed if he/she correctly sorted 6 of the 8 cards in both the pre- and post-switch phases of the task for each level of the DCCS test. Considering the population of interest and the large developmental spectrum assessed, four levels of the DCCS test were utilized to ensure a vigorous assessment of executive function. While children who passed the first (instructional) level were allowed to take other 3 levels, children who failed (scored less than 6 out of 8) the first level were considered to not be able to follow instructions and not allowed to take other levels of the DCCS test. An initial condition was performed to assess if the child's executive function was sufficiently developed to enable them to follow directions, this was important because previous research has demonstrated that children younger than 48 months of age particularly struggle to complete this task,[64, 107, 108]. This condition utilized the same pre- and post-switch procedure as outlined above but utilized monovalent cards that only presented a singular dimension (i.e., either color or shape). If the child was able to pass

this initial condition, they were then asked to attempt three additional conditions of the DCCS test. These conditions replicated the traditional DCCS test using bivalent cards, but manipulated the attentional characteristics of the cards by progressively integrating the color and shape attributes to reduce practice effects (see Figure 1)[64]. The total number of DCCS test conditions passed was used as an index of executive function[64]. The mother or caregiver was present during all conditions of the DCCS test to observe the process and allow the child to feel comfortable and confident.

Of the 242 children who were recruited, only 25 of them were able to complete the instructional test. This was unjustified because most of the children (n=149) were aged 3 years and above at the time of the testing. A number of reasons were speculated for the poor performance of the DCCS test. Among the reasons included inconsistencies on how the DCCS test was administered. In view of this, 20 subjects were randomly selected and the test was administered again.

Data reduction and statistical analyses

Z-scores for the growth parameters HAZ, WAZ, BAZ and WHZ were calculated using the WHO Anthro [69]. Means and standard deviations were calculated for descriptive analysis. Based on the WHO standard population and definitions of moderate and severe stunting, wasting, and underweight [74], stunting percentages were calculated. The FA values presented here are expressed as percent composition of total blood FAs. Total n-3 FA proportions were calculated as ∑ [alpha-linolenic + eicosapentaenoic acid (EPA) + docosapentaenoic n-3 + docosahexaenoic acid (DHA)]; total n-6 FA proportions were

calculated as ∑ [linoleic + linoelaidic + eicosadienoic + dihomo-gamma-linolenic + arachidonic + docosatetraenoic + docosapentaenoic n-6]; total n-9 FA proportions were calculated as ∑ [oleic + elaidic + eicosenoic + Mead + nervonic]; total saturated FA proportions were calculated as ∑ [myristic + palmitic + stearic + arachidic + behenic + lignoceric]; total MUFA proportions were calculated as ∑ [palmitoleic + oleic + palmitelaidic + nervonic + elaidic + eicosenoic]. T/T ratio was calculated from the ratio of Mead acid and AA[29]. FA product/precursor ratio was used to estimate the desaturase activity [155] as follows: D5D= AA/DGLA; D6D= DGLA/LA.

The analyses presented here are for the 25 participants who were able to complete the instructional phase at the first testing and the 20 subjects who were re-tested.

Descriptive analyses were conducted to obtain means and standard deviations for all participants. Means between groups (i.e. those who passed the initial condition of the DCCS test versus those who did not pass were compared using t-tests (for continuous data).

Models for linear regression included the FA of interest, and covariates hemoglobin, age, malaria and BMI-for-age (BAZ). Hemoglobin concentration was included in our model as a confounder because in similar populations it is a significant predictor of cognitive abilities [110]. Age, malaria and BAZ also showed significant association with the dependent variable (total passes).

Pearson and Spearman correlations were performed on the data. SPSS version 24 were used for data analysis.

Results

Subject characteristics

The data collection for this study was done twice due to the reasons stated below.

Cognitive function assessment with the DCCS showed that 25 out of the 209 children (12%) were able to pass the initial condition. This means that majority of the children (88%) were unable to proceed to the next phases of the test, based to the conditions of the testing. This was lower than expected. A number of reasons were speculated, and the most probable one was attributed to the way the cognitive function test was administered. The team decided to re-test some of the children to test the new hypothesis: whether the children's inability to complete the DCCS test was due to the way the test was administered. The second testing showed that, of 18 children who were unable to do the test during the first testing, 7 (38.8%) of them were able to complete the initial phase, while 11 (61.2%) were unable to complete the initial phase, hence couldn't complete the test.

Due to the reasons stated above, the results presented in this section can be grouped into three:

- The subjects who passed the initial phase at the first testing, n=24.
- Merged data of subjects who passed the initial phase and those who did not pass, n=209
- Subjects who passed the initial phase at the second testing, n=20

- Merged data of subjects who passed the tests at both testing, n=38 (Some of the children, (n=3), participated in both testing and there was missing data for 2 participants.

Table 25 presents subject characteristics for all children who passed the initial instruction. The average age of all the children who passed the initial phase is 45.6 months with a range of 38.0-51.0 months. The mean hemoglobin concentration is 11.0 and was within the normal range [111]. The mean weight and height is 14.2 kg and 97.2cm respectively. Z-scores were used to calculate the prevalence of stunting (HAZ), malnutrition (BAZ) and wasting (WAZ). According to WHO standards,[112]. Over 90% of the 24 participants had normal HAZ, WAZ and BAZ scores.

Table 25: Characteristics of children who passed the initial instruction (n=24)

	Mean	SD	Range
Age (mo)	45.9	10.14	38.0-51.0
Sex (male)			
n	10		
%	41.7		
Malaria (%)	4.2		
Height (cm)	97.2	4.5	87.9-105.5
Weight (kg)	14.2	1.6	10.5-17.7
HB (g/dL)	11.0	1.2	6.9-12.4
BAZ	-0.30	0.94	-2.05-1.83
HAZ	-1.07	0.88	-2.2-1.87
WAZ	-0.88	0.82	-2.8-0.85

HB, hemoglobin; BAZ, BMI-for-age z-score; HAZ, height-for-age z-score; WAZ, weight-for-age z-score. The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data [168]

Children who passed the initial condition of the DCCS test were found to be older, taller and heavier than children who failed the initial DCCS test (Table 26). Of the 209 children who attempted the DCCS task, 24 children (11.5%) were able to follow directions as indicated, and passed the initial condition of the DCCS. Five (5) children (2.4%) passed the initial condition but not any other DCCS conditions, 6 children (2.9%) passed two DCCS conditions, 6 children (2.9%) passed three DCCS conditions, and 7 children (3.3%) passed all four DCCS conditions. When the FA levels between the two groups were compared, children who passed the initial conditions reported significantly lower levels of myristic acid (p=0.024), ALA (p=0.01) nervonic acid (p=0.04) and total saturated FAs (p=0.049).

Table 26: Characteristics of children stratified by dimensional change card sort performance for the initial condition (Mean values and standard deviations; numbers and percentages)

		Pass (n=24)	Fail (n=185)	
		Mean ± SE		P
Anthropometry	Age (mo)	45.92±0.67	37.32±0.75	**<0.001**
	Age range	38.0-51.0	13.0-52.0-69.6	
	Sex (male)			
	n	10	87	
	%	41.6%	47.0%	
	Height (cm)	97.3±0.88	90.69±0.0.52	**0.001**
	Weight (kg)	14.12±0.34	12.6±0.16	**<0.001**
	Malaria (%)	1.96±0.04	4.96±1.24	0.386
	HB (g/dL)	10.96±0.24	10.88±0.10	0.789
	BAZ	-0.40±0.20	-0.25±0.07	0.505
	HAZ	-1.06±0.17	-1.38±0.07	0.089
	WAZ	-0.93±0.17	-1.05±0.07	0.566
SFAs	Myristic	0.56±0.05	0.85±0.05	**0.024**
	Palmitic	25.57±0.38	25.66±0.11	0.805
	Total SFA[1]	39.56±0.24	40.22±0.11	0.049
Omega-6 FAs	LA	17.11±0.31	16.63±0.14	0.246
	DGLA	1.19±0.0.05	1.24±0.02	0.239
	AA	9.04±0.36	9.20±0.0.11	0.653
	Total n-6[2]	29.29±0.56	26.06±0.19	0.668
Omega-3 FAs	ALA	0.20±0.02	0.26±0.01	**0.011**
	EPA	0.71±0.07	0.81±0.03	0.218
	DHA	5.03±0.24	5.10±0.06	0.770
	Total n-3[3]	6.90±0.33	7.18±0.09	0.337
Others	n-6/n-3	4.46±0.21	5.10±0.07	0.09
	Nervonic acid	0.71±0.07	0.84±0.02	**0.04**
	Eicosadienoic	0.23±0.01	0.25±0.01	**0.06**
	Oleic	21.89±0.70	20.85±0.19	0.074

HB, hemoglobin; BAZ, BMI-for-age z-score; HAZ, height-for-age z-score; WAZ, weight-for-age z-score. The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data [72].
[1]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric;
[2]Total n-6 includes linoleic, linolaidic, Ƴ-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6;
[3]Total n-3 includes alpha linolenic, eicosapentaenoic, docosapentaeonic n-3, and docosahexaenoic;

Regression between fatty acids and executive function measures

Regression analysis between selected FAs and DCCS performance, (n=209) adjusting for age, malaria, BAZ and Hb, is shown in Table 27. Oleic acid (p=0.05) and LA (p=0.03) were positively associated with DCCS performance. To test the hypothesis that whole blood levels of EPA, DHA, and both EFAs (ALA and LA) would be positively associated with executive control as indexed by performance on the DCCS tasks, multiple linear regression using EPA, DHA, ALA, LA, Hb, malaria age, and BAZ was conducted. The model explained 11% of the variation (r^2=0.112; adjusted r^2=0.077, $p \leq 0.002$). LA (β=0.069, p=0.034) was the only significant contributors to the model. A full model including all 25 single FAs as well as Hb concentrations, malaria, age, and BAZ was significant (p=0.003) and explained about 24% of the variance (r^2=0.239; adjusted r^2=0.121). In the full model, none of the FAs were associated with DCCS performance.

Table 27: Regression results for performance on the dimensional change card sort test and selected fatty acids (FA). (Model: Total pass= Fatty acid of All Children + Age + BAZ + Hemoglobin+malaria)

Class	Fatty acid	Regression results for all subjects (n=209)	
		B ± SE	P
SFA	Myristic	-0.14 ± 0.11	0.20
	Palimitic	-0.00±0.04	0.98
	Stearic acid	-0.10±0.05	**0.06**
	Behenic	-0.69±0.41	0.09
	Lignoceric	-0.33±0.18	0.07
	Arachidic	-0.16±0.95	0.86
	Total SFA[a]	-0.10±0.04	**0.01**
n-3 FA	Alpha-linolenic	1.03±0.63	0.10
	Eicosapentanoic	-0.09±0.17	0.61
	Docosahexaenoic	-0.04±0.06	0.48
	DPA	-0.63±0.29	**0.03**
	Omega-3 Index	-0.03±0.05	0.47
	Total n-3[b]	-0.05±0.05	0.27
n-6 FA	Linoleic	0.07±0.03	**0.03**
	Arachidonic	0.03±0.04	0.45
	GLA	-1.29±0.66	**0.05**
	DGLA	-0.58±0.27	**0.03**
	Docosatetraenoic	-0.12±0.24	0.61
	Total n-6[c]	0.02±0.02	0.40
n-9 FA	Mead acid	-1.25±1.1	0.26
	Oleic	0.04±0.02	**0.05**
	Eicosenoic	0.58±0.44	0.18
	Nervonic	-0.41±0.20	0.05
	Elaidic	0.47±0.496	0.34
	Total n-9[d]	0.04±0.02	0.08
Desaturases	SCD n-7	-10.38±3.86	**<0.01**
	SCD n-9	0.35±0.16	**0.03**
	D6D	-10.95±3.59	**<0.01**
	D5D	0.04±0.04	0.27
Others	Total MUFA	0.03±0.02	0.22
	Total PUFA	0.00±0.02	0.84
	T/T ratio	-5.82±9.31	0.53
	n-6/n-3 ratio	0.17±0.07	0.02
	Palmitoleic	-0.375±0.14	0.01
	Eicosadienoic	-2.22±1.17	0.06
	Palmitelaidic	-1.87±1.26	0.14

[a]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric.
[b]Total n-3 includes alpha-linolenic, EPA, DPA n-3, and DHA.
[c]Total n-6 includes linoleic, linolaidic, GLA, eicosadienoic, DGLA, arachidonic, DTA, DPA n-6.

Table 27 (cont'd)

[d]Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic. SFA, saturated fatty acid; SCD n-7, stearoyl CoA desaturase n-7; SCD n-9, stearoyl CoA desaturase n-9; D6d, delta-6-desaturase; D5d, delta-5-desaturase.

Regression analysis between selected FAs and DCCS performance, (n=24) adjusting for age, malaria, BAZ, Hb is shown in table 28. Total MUFAs and total n-9 were positively associated with DCCS and they were trending towards statistical significance.

Table 28: Regression results for performance on the dimensional change card sort test and selected fatty acids (FA). (Model: Total pass= Fatty acid of All Children + Age + BAZ + Hemoglobin+malaria)

Class	Fatty acid	Regression results for total pass (n=24)	
		B ± SE	P
SFA	Myristic	-0.76 ± 0.88	0.40
	Palimitic	0.14±0.13	0.31
	Palmitelaidic	-2.35±4.67	0.62
	Stearic acid	-0.10±0.21	0.61
	Elaidic	1.34±1.74	0.45
	Behenic	-0.91±2.28	0.69
	Arachidic	2.00±4.65	0.67
	Total SFA[a]	-0.18±0.21	0.41
n-3 FA	ALA	-0.45±3.50	0.90
	EPA	-0.26±0.73	0.73
	DHA	-0.23±0.18	0.23
	DPA	-1.27±0.93	0.18
	Omega-3 Index	-0.16±0.14	0.27
	Total n-3[b]	-0.17±14	0.24
n-6 FA	LA	-0.14±0.17	0.41
	AA	-0.23.±0.12	0.08
	GLA	-6.51±3.34	0.06
	DGLA	-0.96±0.88	0.29
	DTA	-0.18±1.23	0.89
	Eicosadienoic	-4.79±4.33	0.28
	Total n-6[c]	-0.15±0.08	0.08
n-9 FA	Mead acid	-4.54±5.56	0.42
	Oleic	0.11±0.06	0.10
	Eicosenoic	-0.14±2.65	0.96
	Nervonic	-0.21±1.17	0.86
	Total n-9[d]	0.12±0.07	0.08
Desaturases	SCD n-7	-10.09±33.40	0.77
	SCD n-9	0.55±0.43	0.22
	D6D	-9.67±13.86	0.49
	D5D	-0.12±0.19	0.53
Others	Total MUFA	0.12±0.06	**0**.08
	Total PUFA	-0.09±0.05	0.11
	T/T ratio	2.79±46.64	0.95
	n-6/n-3 ratio	0.20±0.22	0.39

[a]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric. [b]Total n-3 includes alpha-linolenic, EPA, DPA n-3, and DHA. [c]Total n-6 includes linoleic, linolaidic, GLA, eicosadienoic, DGLA, arachidonic, DTA, DPA n-6. [d]Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic. SFA, saturated fatty acid; SCD n-7, stearoyl CoA desaturase n-7; SCD n-9, stearoyl CoA desaturase n-9; D6d, delta-6-desaturase; D5d, delta-5-desaturase.

Regression analysis between selected FAs and DCCS performance, (n=38) adjusting for age, malaria, BAZ, Hb is shown in Table 29. None of the FAs were positively associated with DCCS test.

Table 29: Regression results for performance on the dimensional change card sort test and selected fatty acids

Class	Fatty acid	Regression results for total pass(both testing) (n=38)	
		B ± SE	P
SFA	Myristic	-0.93 ± 0.85	0.28
	Palimitic	0.06±0.13	0.67
	Palmitelaidic	-1.87±3.75	0.62
	Stearic acid	-0.15±0.16	0.36
	Elaidic	1.31±2.07	0.53
	Total SFA[a]	-0.14±0.17	0.41
n-3 FA	ALA	2.88±2.63	0.28
	EPA	0.56±0.65	0.40
	DHA	-0.03±0.18	0.91
	DPA	-0.94±0.92	0.31
	Omega-3 Index	0.02±0.15	0.91
	Total n-3[b]	0.00±14	1.00
n-6 FA	Linoleic	0.05±0.16	0.75
	Arachidonic	-0.12±0.13	0.37
	GLA	-3.17±3.24	0.34
	DGLA	-1.41±0.73	**0.06**
	Docosatetraenoic	-0.85±0.97	0.39
	Eicosadienoic	-6.69±4.63	0.16
	Total n-6[c]	-0.06±0.09	0.47
n-9 FA	Mead acid	-3.24±4.03	0.42
	Oleic	0.06±0.06	0.32
	Eicosenoic	-2.16±1.34	0.13
	Total n-9[d]	0.06±0.07	0.35
Desaturases	SCD n-7	-10.21±20.13	0.62
	SCD n-9	0.46±0.43	0.30
	D6D	-22.52±11.52	**0.06**
	D5D	0.12±0.19	0.32
Others	Total MUFA	0.06±0.06	0.38
	Total PUFA	-0.03±0.06	0.62
	T/T ratio	-6.31±28.81	0.83
	n-6/n-3 ratio	0.03±0.24	0.89

Model: Total pass= Fatty acid of All Children + Age + BAZ + Hemoglobin+malaria
[a]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric.
[b]Total n-3 includes alpha-linolenic, EPA, DPA n-3, and DHA.
[c]Total n-6 includes linoleic, linolaidic, GLA, eicosadienoic, DGLA, arachidonic, DTA, DPA n-6.
[d]Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic. SFA, saturated fatty acid; SCD n-7, stearoyl CoA desaturase n-7; SCD n-9, stearoyl CoA desaturase n-9; D6d, delta-6-desaturase; D5d, delta-5-desaturase.

Education and performance in the DCCS test

In the second testing some of the children were picked from private schools, public schools and others were picked from home. The information on the educational

component is presented in table 30. Of those who were picked from public schools 100% passed the initial condition while 66.7 % passed 2 or more post-switch tests. Of all those were picked from public schools, 80% of them passed the initial test while 60% passed 2 or more post-switch test. Of all those who were picked at home only 25% of them were able to pass the initial test, while none of them were able to pass 3 or more of the post tests.

Table 30: Educational component

	Children picked from private schools (n=3)	Children picked from public schools, n=5	Children picked at home, n=12
Passing the initial test	100%	80%	25%
Passes 2 or more post-tests	66.70%	60%	33.30%
Passing 3 or more post tests	66.70%	40%	0

Table S4 to S6 presents Spearman correlation and Pearson correlation coefficient and p values for the subjects, n=24, and n=38. None of the FAs were associated with a total passes except for DGLA that showed trending significance for Pearson correlation, n=24.

Discussion

The objective of this study was to characterize the whole blood FA levels of Southern Ghanaian children and determine FA associations with cognition. Based on the Mead acid levels and the T/T ratio, EFAD was found to be low in this population, 10.5%. From the first testing for the cognitive function assessment, 25 out of the 209 children (12%) were able to pass the initial condition. This indicates that majority of the children (88%) were unable to proceed to the next phases of the test based to the conditions of the test. This was lower than expected. A number of reasons were speculated, and the most probable one was attributed to inconsistencies in administering the cognitive function test.

A second testing was scheduled for 20 subjects and this time, with a supervisor who could give further instructions when the test was being administered. The second testing showed that, out of the 18 children who were unable to do the test during the first testing, 7 (38.8%) of them were able to complete the initial phase, while 11 (61.2%) were unable to complete the initial phase. These results show that, the way the test was administered could result in the low pass rate in the first testing. A lack of proper supervision of the interview team could have caused this.

The initial phase of the DCCS test requires much sensitivity to get a child settled for the entire procedure. Due to environmental factors, the children may be jittery and not pay much attention to the testing, especially during the initial phase. This may require a tactful investigator to get the accurate scores for the child. An investigator's inability to maintain calmness in a child can lead to poor performance. Further, when an investigator is burdened during questionnaire administration it may prevent him/her from actively calming down a child to perform the test. The study sample was a sub-sample from a bigger study[154], and the investigators were tasked to take lots of information from the parents of the subject. Additional information could have increased investigator burden, hence the results obtained. This can be prevented in other studies in a number of ways: 1) Supervising the investigators to patiently assess the child can be helpful. 2) Also if possible merging a number of studies can be prevented so that the investigators are not burdened.

The data were analyzed regardless to these pitfalls. Our data support the hypothesis that children with higher whole blood levels of EFAs (LA and ALA), as well as DHA and EPA, are more likely to pass the DCCS test, however, in individual regression analysis, LA was

positively associated with DCCS performance, which is consistent with results from a previous study in Tanzania [64]. The results also showed that, the children who passed the initial condition of the DCCS test were found to be older and taller, and this is consistent with data obtained from a similar study in Tanzanian children[64].

High levels of saturated fats have been linked with poor cognition because saturated FAs can lead to saturated fats-induced oxidative stress and reduced brain-derived neurotrophic factor (BDNF), resulting in compromised synaptic plasticity. Further, saturated fats increases insulin resistance in the brain and diminish the integrity of the blood brain barrier [169]. These indicate that lower levels of saturated fats may improve cognition and our study showed that children with lower levels of total saturated fatty acids and myristic acid passed the initial phase of the DCCS testing, consistent with studies by Khan et al., 2015[169].

The role of education/schooling of DCCS were assessed. In the second testing, of the 3 children who were chosen from private schools, all of them passed the initial condition while those chosen from public schools had fewer of them passing the DCCS. Children who have not had any education, thus, those chosen from home, performed poorly in all phases of the cognitive function test. This could indicate that being in school can have an effect on attention, task switching and overall executive function of the children.

From the onset, this study was presented with a number of pitfalls, among them include the irregularities in the administering of the test. This pitfall contributed to a lower sample size for this study. However, the second testing provided a new perspective to the entire study: the educational component. Although the study was not well powered, the results

of this study add to a body of knowledge that high whole blood saturated fats are associated with lower cognitive performance.

Future studies should include supervisors when administering the DCCS test so that investigators would be guided to do the right thing. Further, considering the educational level or whether the child has been exposed to any education at all can be assessed in future studies. Finally, measures should be taken to reduce investigator burden so as to get very accurate results and scores.

CHAPTER 8: QUANTIFICATION OF FATTY ACID AND MINERAL LEVELS OF SELECTED SEEDS, NUTS, AND OILS IN GHANA.

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Abstract

Background: Fatty acids (FA) and minerals play crucial roles in growth and development. However, Ghanaian diets consist mainly of starchy roots and cereals, with intake of fats and many minerals below recommended levels. The purpose of this study was to quantify FA and mineral levels of seeds, nuts, and oils in Ghana that are available but not usually incorporated in the diets. **Method:** Seven seeds and five oils collected in Ghana were analyzed for FA and mineral composition by gas chromatography mass spectrometry (GC/MS) and inductively coupled plasma (ICP) emission spectroscopy, respectively. **Results:** Soybean was found to contain high levels of alpha-linolenic acid (ALA) (3.77 mg/g). Linoleic acid (LA) was higher in peanuts (65.8 mg/g), agushie seeds (102 mg/g) and agushie flour (122 mg/g). Agushie seeds (88.4 mg/g), agushie flour (111 mg/g) and soybean (78.4 mg/g) had appreciable levels of iron. In addition, both agushie seeds and flour contained high amounts of zinc. Taken together, these data indicate that several Ghanaian seeds, nuts, and oils are high in FA, including essential fatty acids, and minerals. Future studies should investigate increased incorporation of palm oil, soybean, peanuts, cashew nut, tigernuts, agushie seeds and/or flour into Ghanaian diets in areas where nutrient deficiencies are prevalent.

Introduction

Essential fatty acids (EFA) and minerals play crucial roles in human growth and development due to their role in nutrient metabolism and cell differentiation [8, 170]. FA are precursors of structural and metabolic substances in the body as well as a major contributor of energy in diets. Although fats have critical roles in human diets, their intakes in African diets are low [33]. Ghanaian diets consist mainly of starchy roots (yam and cassava), fruits (plantain, orange) and cereals (rice, maize), which together make up about 75% of diets, while protein and fats make up 25% [134]. This indicates how it is generally accepted that there is low dietary diversity in Ghana often below adequate levels of dietary fats and proteins. In addition, only 36.6% of Ghanaian children from 6 to 24 months old have fats added to their complementary food [171]. The low content of fats in Ghanaian diets contributes to lower intakes of EFA, a probable likelihood of EFA deficiency (EFAD) in the population. Mineral deficiencies have also been reported in Ghana [134], contributing to the occurrence of iron deficiency anemia. EFAD is related to stunting and cognitive impairment, hence there is a need to include adequate amounts of EFA and minerals in diets.

The inclusion of EFA in the diet is of utmost importance because EFA cannot be synthesized in the body and must be provided through the diet. They are abundant in foods such as soybean oil, canola oil, flaxseed oil, nuts, and animal products. They include linoleic acid (LA), an n-6 FA, and alpha-linolenic acid (ALA), an n-3 FA. Downstream products of EFA, such as arachidonic acid (AA), are metabolized from LA, while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are metabolized from ALA. EPA and DHA are predominantly found in seafood. The EFA and their very

long chain polyunsaturated fatty acid (VLCPUFA) derivatives are vital for growth, cognition, and immune functions [33, 130]. Fish are a good source but they are expensive, and they are not available in sufficient amounts in the population. This is evidenced by the fact that the amount of fish required to meet the nutritional needs of Ghanaians exceeds fish production (marine, inland and aquaculture) [131]. There is a high probability of low intakes of EFA in the Ghanaian population; therefore, the exploration of alternative and affordable sources of EFA is important.

The prevalence of mineral deficiencies in different age groups has been described [34, 129, 133]. The prevalence of anemia in Ghanaian children 6 to 59 months old is 78% and iron deficiency is a major contributor [19]. A study of a community in Ghana showed that zinc deficiency among children 2 to 10 years was 35.6% [133]. Mineral deficiencies are also known to be associated with growth stunting and cognitive impairment [135, 136]. This is because some minerals such as iron and zinc are cofactor nutrients and are beneficial in EFA metabolism. For example, zinc deficiency can impair the conversion of LA to AA and ALA to EPA and DHA [137]. Iron deficiency also contributes to the occurrence of iron deficiency anemia. Studies have been conducted to evaluate the mineral composition of seeds and nuts, but these data can vary due to environmental factors such as soil type, agronomic practices and climate [138, 139]. Differences between geographical regions can directly affect the concentration of minerals in crop plants and affect dietary mineral content, impairing EFA metabolism and consequently leading to growth stunting and cognitive impairment in the population. However, the mineral content of foods in Ghana has been poorly described.

The West African food composition table published in 2012 gives an estimation of nutrients such as proteins, carbohydrates, total fat, vitamins, and minerals, with no information on the EFA concentrations of individual foods and no information on the provenance of the foods analyzed. These reports state that the data represent average values derived from compositional data from 8 countries including Ghana. Additionally, it has been documented that most of the mineral and vitamin data in the table were based on information obtained from several non-African countries. Cultivation of crops in different geographical areas is also known to affect composition of food [149]. Given these drawbacks, the nutrient content listed in the table does not represent the foods in Ghana. Therefore, analyses that quantifies individual FA, especially EFA, and mineral content of local foods in Ghana is needed. In this study, we quantified the amount of EFA, VLCPUFAs and minerals in local foods available in markets in Southern Ghana.

Materials and Methods

Preparation of selected seeds, oils, and nuts in Ghana.

Seeds, nuts, and oils (Table 31) were purchased from the Kumasi Central Market in Kumasi, Southern Ghana. To prevent the oils from oxidation they were packed in plastic containers. The seeds and nuts were crushed and freeze-dried and also stored in containers. The freeze-dried samples were stored no longer than 14 days upon arrival in the US. All samples were shipped to a Michigan State University Food Science and Human Nutrition laboratory. The samples were purged with high purity nitrogen and stored at -20°C until analysis. Freeze-drying of all solid products was chosen to maintain integrity of the foods during shipment due to shipping and power challenges in this region.

Table 31: List of seeds, nuts, and oils analyzed

Food	Abbreviation	Genera	Description
Soybean	SYBN	*Glycine sp.*	A legume, bean seeds, freeze-dried
Cashew nut	CNUT	*Anarcardium sp.*	A tropical tree with nuts. Nuts were freeze-dried
Agushie flour	AGSF	*Cucumeropsis sp.*	Climbing vine, flattened seeds. Seeds milled.
Peanut	PNUT	*Arachis sp.*	A root of a tropical legume. Seeds were freeze-dried
Tigernut	TNUT	*Cyperus sp.*	A root nut of a tropical 'grass'. Nuts were freeze-dried
Coconut	CCN	*Cocos sp.*	A palm tree fruit, flakes were freeze dried and used in analyses
Agushie seeds	AGS	*Cucumeropsis sp.*	Climbing vine, flattened seeds. Seeds were freeze dried
Palm kernel oil	PKO	*Elaeis sp.*	Oil derived from the kernel of the oil palm
Margarine	MG	NA	A commercial butter substitute made from hydrogenated vegetable oils
Vegetable oil	VGT	*Elaeis sp.*	Commercially refined oil made from crude palm oil
Coconut oil	CCNO	*Cocos sp.*	Crude oil extracted from the flesh of mature coconut
Palm oil	PALM	*Elaeis sp.*	Crude oil processed locally, red-orange, high in beta carotene
Sheabutter	SBUT	*Vitellaria sp.*	An ivory-coloured fat extracted from African shea nut.

These freeze-drying methods have been shown to give comparable extraction yields when compared to air-drying methods [141]. Also, LA and ALA from freeze-dried samples are reported to be more abundant than the air-drying methods [142].

Crude Seed Oil Extraction

Glassware for the analysis were acid washed to deactivate glassware and remove mineral residues, followed by high performance liquid chromatography (HPLC)-grade organic solvents to remove any FA contaminants. Lipids were extracted from seed material as previously described [143], but modified as specified [172]. In brief, a total of 400 mg freeze-dried seed material was incubated at RT in 10 mL of 2:1 (v/v) mixture of HPLC grade chloroform (Avantor Performance Materials, Inc., Center Valley, PA) and HPLC-grade methanol containing 100 µg butylated hydroxytoluene (BHT)/mL (Sigma – Aldrich, St. Louis, MO). Using lipid-free filters (FGE Healthcare UK Limited, Buckinghamshire, UK), the seed-solvent mixtures were gravity-filtered into glass tubes, containing 2.5 mL of 0.88% m/v aqueous KCl to remove water soluble pigments and phytochemicals (J.T. Baker, Phillipsburg, NJ). After drying under high-purity nitrogen, the total crude seed oil was weighed and calculated.

Methylation of Oils to FAMEs, Neutralization, and FAME Isolation

A total of 80 mg of the crude seed oil were weighed into individual 16x100 mm glass tubes. The samples were resuspended in chloroform/methanol (2:1 v/v, 100 µg BHT/mL) to obtain a final total lipid concentration (20 mg/mL). The resuspended oils were prepared for methylation as described by Cequier-Sanchez et al. [143]. In brief, 100 µL lipid extract

solution was transferred to a clean 16×100 mm Teflon-lined screw-capped glass tubes. To each sample, an internal standard, nonadecanoic acid (150 µg; Sigma – Aldrich, St. Louis, MO) in HPLC-chloroform was added. The resultant mixture was dried under high-purity nitrogen at RT. The samples were methylated with 2% acidified methanol as described methods by Agren et.al. [145], and as modified by Pickens et al. [70]. FAMEs were neutralized and isolated as previously described [71].

FAME Identification, Analysis, and Data Processing

Re-suspended FAMEs were transferred to GC vials with glass inserts for analysis. Prior to analysis, the sample injection order was randomized. FAMEs were identified and quantified using a dual stage quadrupole (DSQ)II quadrupole GC/MS (Thermo Scientific, Waltham, MA) equipped with a DB-23, 30-m column (0.25 mm id; Agilent Technologies, Santa Clara, CA) using helium as a carrier gas. The GC temperature profile was as follows: Initial, hold 40°C 1 min; Ramp 1, 100°C/min to 160°C; Ramp 2, 2.8°C/min to 192°C; Ramp 3, 0.5°C/min to 201°C; Ramp 4, 50°C/min to 250°C and hold for 1 min. Selective ion monitoring (SIM) was employed for enhanced sensitivity. Identification and quantification of individual FAMEs was done with standard FAME mixture (Part# CRM47885; Lot# LC06601V; Supelco, Bellefonte, PA). The certified range of each FAME measured was as follows: 600 µg/mL for palmitic acid; 400 µg/mL for myristic, stearic, (cis-9) oleic, arachidic, behenic, and lignoceric acid; 200 µg/mL for (cis-7) palmitoleic, (cis-11) eicosenoic, (cis-9,12) LA, (cis-6,9,12) gamma-linolenic acid (GLA), (cis-11,14) eicosadienoic, (cis-5,8,11,14) AA, (cis-13,16) docosadienoic, (cis-15) nervonic, (cis-11,14,17) ALA, (cis-5,8,11,14,17) EPA, and (cis-4,7,10,13,16,19) DHA. Standard curves

were created from the certified ranges given using 5-fold serial dilutions to produce a 5-point standard curve. Detected FAME concentrations below the lower limit of quantification (LLOQ) are defined for each FA in Tables 2-5. DHA, EPA, and linoelaidic acid were below the LLOQ in all samples analyzed and were excluded from the tables. Resuspended FAME samples that contained FAME concentrations above the highest standard curve were diluted 1:10 and reanalyzed on the same standard curve as undiluted samples. FAME peak integration and quantification was performed using TargetLynx V4.1 (Waters, Milford, MA) based on the FAME standard's retention time and SIM ions and ratios. The concentrations of resuspended FAMEs were normalized to the amount of food-grade oil (i.e. palm oil) or crude seed oil and total seed material for the seed samples (i.e. agushie seeds). FA concentrations are representative of a pooled food-grade oil (n=1) or pooled seed (n=1) sample, obtained from a local market in Kumasi, Southern Ghana. Figures were made in GraphPad Prism version 4 (GraphPad Software, Inc., La Jolla, CA).

Mineral analysis

For the mineral analysis, freeze-dried samples of PNUT, AGS, AGSF, CNUT, CCN, TNUT and SYBN were shipped on dry ice to a third-party contractor. The minerals that were analyzed include zinc, iron, potassium, phosphorus, sodium, magnesium, manganese and calcium. Identification and quantification of minerals was performed using ICP emission spectroscopy (ICP_S: 28) AOAC International no. 984.27, 985.01, and 2011.14. [173, 174] For each sample, concentrations of minerals are expressed as mg/kg.

Results

The composition of FA in the seeds, nuts, and food-grade oils are reported as the concentration of each FA as mg/g (Table 33–36). Samples were found to contain varying levels of several FA, as shown in the following data.

Saturated Fats

All samples analyzed were found to contain saturated FA. CCNO, PALM and VGT had high amounts of total saturated FA (112 mg/g, 184 mg/g, and 165 mg/g, respectively). In particular, PKO and CCNO had high amounts of myristic acid, while PALM and VGT contained high amounts of palmitic acid. SBUT contained high amounts of stearic acid (Table 32).

Monounsaturated Fatty Acids

Food-grade oils were more abundant in saturated fats and MUFA compared to the seeds. The MUFA that were quantified include oleic (18:1), eicosenoic (20:1), and docosenoic (22:1). PALM (202 mg/g) and SBUT (309 mg/g) had high amounts of oleic acid. Levels of eicosenoic and docosenoic acid were low in all samples (Table 33).

Table 32: Saturated fatty acids (mg FA/g crude oil or mg FA/g seed)[1]

Sample ID	Myristic C14:0	Palmitic C16:0	Stearic C18:0	Arachidic C20:0	Lignoceric C24:0	Total Saturated
Soybean	0.06	5.55	1.44	0.25	0.04	7.34
Cashew nut	0.10	7.44	5.40	1.00	0.08	14.0
Agushie flour	0.14	18.2	6.60	0.84	0.09	25.9
Peanut	0.06	9.55	1.89	2.79	0.45	14.7
Tigernut	0.06	5.80	1.26	0.47	0.05	7.64
Coconut	21.8	12.8	2.03	0.14	0.03	36.8
Agushie seeds	<LLOQ	13.3	13.0	0.55	0.04	26.9
Palm kernel oil	73.0	27.8	5.48	0.50	0.10	107
Margarine	1.13	54.6	4.03	0.85	0.08	60.7
Vegetable oil	2.60	150	10.2	2.05	0.15	165
Coconut oil	84.8	22.7	4.48	0.30	0.08	112
Palm oil	2.25	169	10.8	2.13	0.10	184
Sheabutter	0.28	16.5	263	16.1	0.28	296

[1]FA concentrations are representative of a pooled food-grade oil (n=1) or pooled seed (n=1) sample, obtained from local markets in Kumasi, Southern Ghana.

Table 33:Total monounsaturated fatty acids (mg FA/g crude oil or mg FA/g seed)[1]

Sample ID	Oleic C18:1	Eicosenoic C20:1	Docosenoic C22:1	Total MUFA
Soybean	13.8	0.11	0.03	13.9
Cashew nut	118.8	0.35	<LLOQ	119
Agushie flour	28.6	0.28	0.13	29.0
Peanut	62.6	1.36	0.15	64.1
Tigernut	56.1	0.15	<LLOQ	56.3
Coconut	12.0	0.07	<LLOQ	12.1
Agushie seeds	20.2	0.12	<LLOQ	20.3
Palm kernel oil	69.0	0.38	<LLOQ	69.4
Margarine	72.0	0.25	0.28	72.5
Vegetable oil	232	0.75	<LLOQ	233
Coconut oil	34.5	0.15	<LLOQ	34.7
Palm oil	202	0.63	0.33	203
Sheabutter	309	1.90	0.33	311

[1]FA concentrations are representative of a pooled food-grade oil (n=1) or pooled seed (n=1) sample, obtained from local markets in Kumasi, Southern Ghana.
MUFA, monounsaturated fatty acid

n-3 Fatty Acids

All samples analyzed had low amounts of n-3 FA. The n-3 FA considered in this study include ALA (18:3n3), EPA (20:5n3), and DHA (22:6n3). However, as mentioned previously, detected concentrations of EPA and DHA were below the LLOQ in all samples analyzed. SYBN (3.77 mg/g), VGT (0.98 mg/g), and PALM (1.15) were relatively abundant in ALA (Table 34).

n-6 Fatty Acids

In general, samples were more abundant in n-6 FA than n-3 FA. The n-6 FA that were measured in the samples include LA (18:2n6), GLA (18:3n6), eicosadienoic (20:2n6), linoelaidic (20:2n6), and docosadienoic acid (22:2n6). As mentioned, all detected concentrations of linoelaidic were below the LLOQ and so this FA was removed from the table. LA levels were found to be high in AGSF (122 mg/g), AGS (102 mg/g), and PNUT (65.8 mg/g). CCNO (7.63 mg/g) and CCN (2.21 mg/g) contained lower amounts of LA (Table 35). CCNO had higher amounts of all the FA analyzed than CCN.

Table 34: Total n-3 fatty acids (mg FA/g crude oil or mg FA/g seed)[1]

Sample Name	α-Linolenic (ALA) C18:3n3	Total n-3
Soybean	3.77	3.77
Cashew nut	0.25	0.25
Agushie flour	0.30	0.30
Peanut	0.15	0.15
Tigernut	0.16	0.16
Coconut	<LLOQ	<LLOQ
Agushie seeds	0.04	0.04
Palm kernel oil	0.40	0.40
Margarine	0.50	0.50
Vegetable oil	0.98	0.98
Coconut oil	<LLOQ	<LLOQ
Palm oil	1.15	1.15
Sheabutter	0.95	0.95

[1]FA concentrations are representative of a pooled food-grade oil (n=1) or pooled seed (n=1) sample, obtained from local markets in Kumasi, Southern Ghana.;

Table 35:Total n-6 FA (mg/g crude oil of mg FA/g seed)[1]

Sample ID	Linoleic C18:2n6	γ-Linolenic (GLA) C18:3n6	Eicosadienoic C20:2n6	Docosadienoic C22:2n6	Total n-6
Soybean	33.2	0.36	0.09	<LLOQ	33.7
Cashew nut	32.8	0.83	0.20	0.15	34.0
Agushie flour	122	1.27	0.26	<LLOQ	124
Peanut	65.8	0.76	0.19	0.14	66.9
Tigernut	11.9	0.47	0.10	<LLOQ	12.5
Coconut	2.21	<LLOQ	0.12	<LLOQ	2.33
Agushie seeds	102	<LLOQ	0.22	<LLOQ	102
Palm kernel oil	8.75	2.38	0.48	<LLOQ	11.6
Margarine	17.5	2.58	0.45	0.38	20.9
Vegetable oil	61.5	2.53	0.55	<LLOQ	64.6
Coconut oil	7.63	2.58	0.35	<LLOQ	10.6
Palm oil	57.8	2.75	0.58	<LLOQ	61.1
Sheabutter	39.3	3.43	0.68	<LLOQ	43.4

[1]FA concentrations are representative of a pooled food-grade oil (n=1) or pooled seed (n=1) sample, obtained from local markets in Kumasi, Southern Ghana.

Minerals

Minerals were abundant in seeds and nuts. SYBN, AGS and AGSF contained relatively high amounts of iron (78.5 mg/kg, 88.4 mg/kg, 111 mg/kg, respectively), zinc (41.6 mg/kg, 59.9 mg/kg, 65.0 mg/kg respectively) and manganese (34.7 mg/kg, 40.1 mg/kg, 25.4 mg/kg respectively) (Figure 4A-C). A high amount of magnesium (2420 mg/kg) and phosphorus (5360 mg/kg) was found in CNUT (Figure 3B and C), as well as zinc (54.3 mg/kg) (Figure 4B). High amounts of potassium and sodium were recorded for all seeds, but SYBN contained the highest amount of potassium (Figures 3A and 5B). With the exception of manganese, AGSF was relatively more abundant in minerals than AGS. TNUT had lower levels of most elements except sodium, which was present in the highest amount (200 mg/kg).

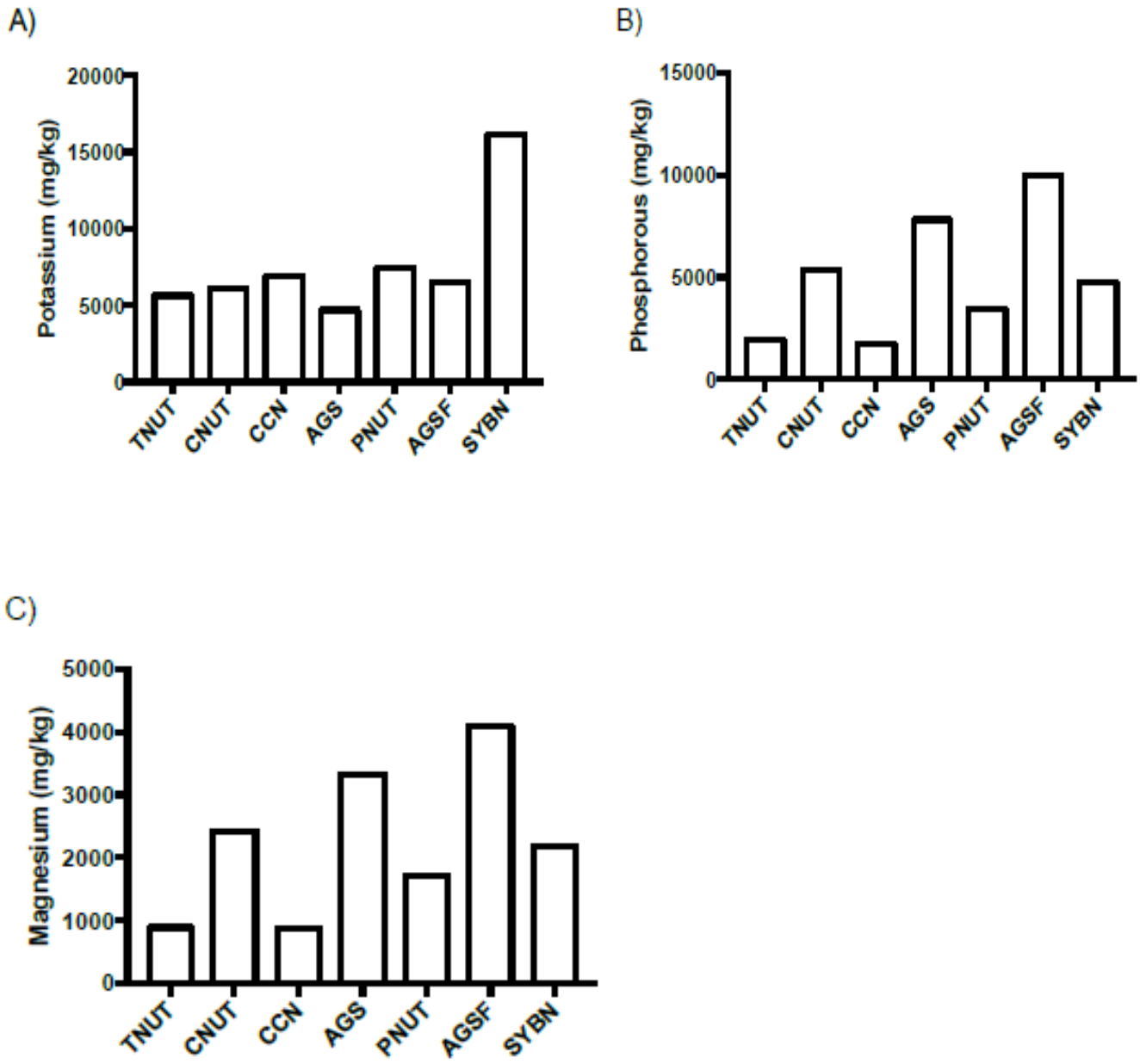

Figure 4: Concentration of potassium (A), phosphorus (B) and magnesium (D) expressed as mg/kg

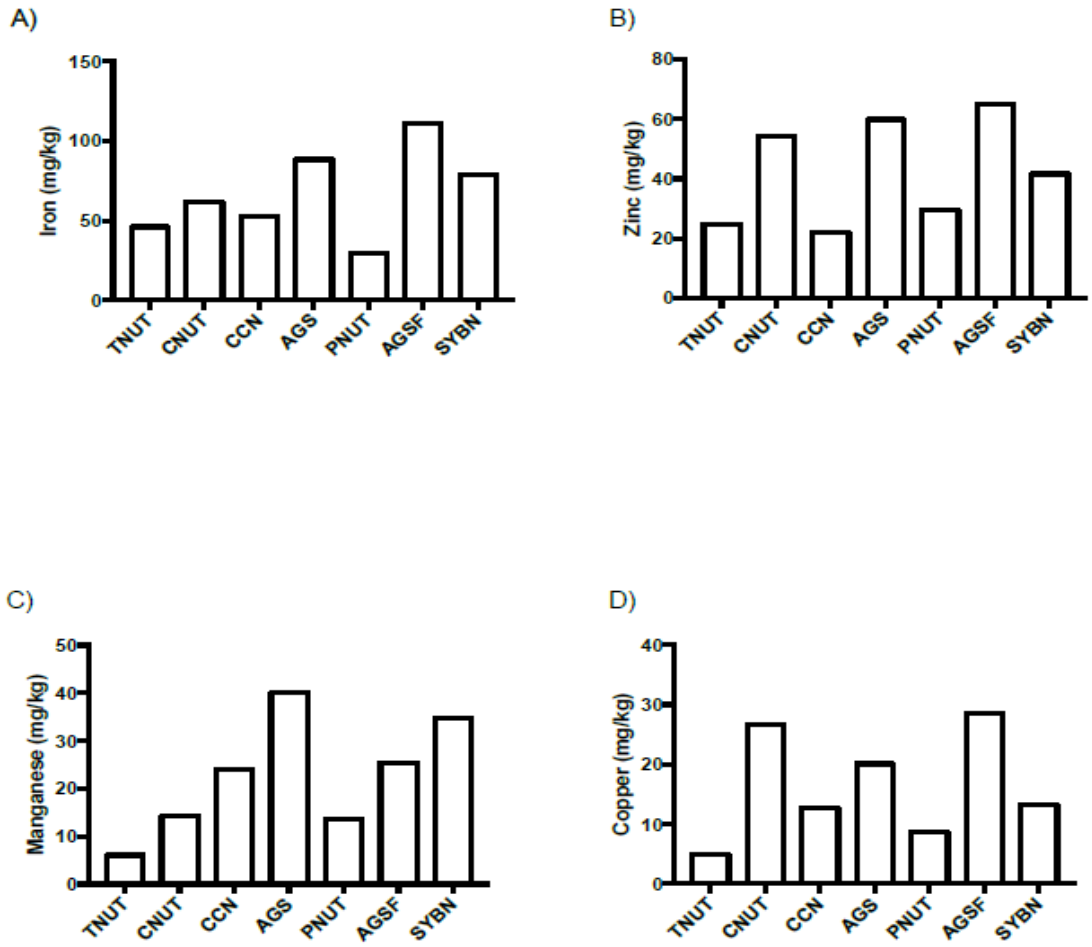

Figure 5: Concentration of iron (A), zinc (B), manganese (C) and copper (D) expressed as mg/kg.

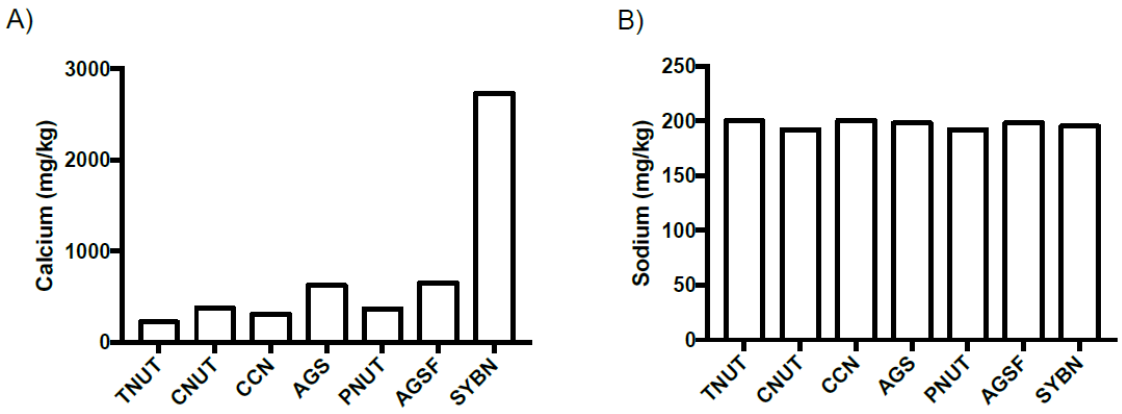

Figure 6: Concentration of Calcium (A),Sodium (B), expressed as mg/g

Discussion

In this study we show that SYBN, AGSF and AGS contained higher amounts of EFA (ALA and LA) and lower amounts of MUFA and saturated fats. MG and VGT also had high amounts of MUFA and saturated fats, but VGT contained higher amounts of n-3 FA, and this is consistent with information given by Unilever Ghana Limited manufacturers. Margarine and VGT are commercial products that are normally consumed in the Ghanaian population. They are expensive and may not be available in rural communities. However, SYBN, AGSF and AGS are relatively cheaper and widely available, but are not widely consumed, likely due to cultural differences in acceptability of these foods across the population.

As previously noted, the West African food composition table does not provide the composition of individual EFA, but rather gives the total content of protein, carbohydrates, fats, vitamins and minerals [149]. In this study, we report the individual FA content of the foods. Additionally, it has been reported that EFAD in diets is associated with growth stunting [135]. The data in this study support that SYBN, AGS and AGSF are sources of EFA and could be incorporated in diets to potentially help alleviate EFAD and growth stunting in the population. MG and VGT could also be adopted and promoted and made available to the majority of the population. PNUT is also reported to have low amounts of total saturated fats and MUFA and relatively higher amounts of total n-6 FA. However, compared to SYBN, AGSF and AGS, PNUT is widely acceptable and consumed in the population having been utilized in supplements to address undernutrition [175-177]. SYBN, AGSF, and AGS could also be promoted and used for food supplementation programs.

Furthermore, the study showed that SBUT, PALM, VGT, and PKO had substantial amounts of MUFA and total saturated fats. Oleic acid was the most abundant MUFA. PALM and VGT showed high amounts of total n-3. With the exception of SYBN, all other samples contained low amounts of ALA. CCNO had higher amounts of MUFA and total saturated fats compared to CCN, which could be due to the commercial extraction process and its effects on the recovery of oils. This is suspected because CCNO was extracted using crude field methods, while the oil from CCN was extracted in the laboratory. PALM, which is widely consumed in Ghana, was found to be high in total saturated fats and MUFA, but contained EFA. VGT and MG are currently not listed in the West African food composition table; thus, our study contributes to the existing food composition data.

In Ghanaian markets, there are two types of melon seed products: AGS (the seeds) and AGSF (the flour). The flour is made from milled seeds exposed to environmental conditions, such as oxygen and sunlight until they are purchased, but the seeds remain intact until they are purchased. It is possible that the flour would contain lower amounts of PUFA compared to the seeds because unsaturated FA are susceptible to oxidation compared to saturated FA, but the results showed otherwise. The reason for this could be that only one sample was analyzed and it may not be representative of all AGS and AGSF samples. Additionally, the process of converting the seeds to the flour involves milling with metal grinders; therefore, it is possible that the high mineral content of the flour could be due to contamination through the milling process or fortification.

As stated earlier, most minerals are needed for FA metabolism because they serve as cofactors. Although the West African food composition table has listed the amounts of

minerals in certain foods in West Africa [149], the effect of geographic location and the environment on the mineral content of seeds and nuts cannot be overemphasized [138, 139]. In addition, the data in this database are less accurate for Ghana as they were obtained from sources other than direct analysis of the foods in country. Our study showed that SYBN, AGS, and AGSF had high amounts of iron, calcium, zinc, magnesium, and phosphorus. CNUT, which is widely grown and available in Southern Ghana, contains high amounts of zinc, magnesium, and phosphorus. Most of these minerals are known to be cofactors of FA and protein metabolism. In summary, melon seeds (AGSF and AGS), SYBN, and CNUT have appreciable amounts of EFA and minerals that are necessary for normal human growth and development.

The results indicate that melon seeds and SYBN contain higher amounts of EFA and minerals. Most rural folks in Southern Ghana are small-scale farmers and traders; therefore, these foods are readily available to them. CNUT is cultivated in Ghana as a cash crop. Though it can be a great source of minerals for the people of Ghana, the bulk of it is exported. If it were available for domestic consumption, CNUT could be a valuable addition to the Ghanaian diet. MG and VGT are commercial products that are available to few people in the population. SYBN is a legume that is not as widely and culturally accepted as compared to others such as cowpea. Its use could be promoted through nutrition education. Food supplements that have been developed from seeds cultivated in Ghana include PNUT and SYBN, but melon seeds, MG, and VGT could also be considered for use in supplementation programs and promoted as valuable ingredients of children's diets.

When comparing our data to the USDA Food Composition Databases, our data are almost identical for some values, but we acknowledge there is variability in others [150]. In our opinion, the variability could be a result of how the USDA food composition databases derived individual FA values based on others' results of total fat content. Our data were directly calculated, and were not normalized to mean fats based on other researchers' findings. The USDA also does not report any statistics of variability. For our study, it was only possible to obtain samples from one location due to budgetary constraints. Future studies should investigate sources from different markets to account for variability among

In this study, local seeds, nuts, and oils available to the population in Southern Ghana were analyzed to identify dietary sources of EFA and minerals. We recognize the generalizability of these observations is limited; thus, future studies should investigate FA and mineral profiles from several. In addition, we did not directly collect or assess the foods consumed by households in this region. Another limitation of our study is that we do not have the details of how the seeds, nuts, and oils were stored prior to purchase from the market in Ghana markets in Southern Ghana and from various regions of the country.

We also do not have details regarding the processing methods of food-grade oils in Ghana. Furthermore, some of the foods analyzed are consumed without cooking while others are cooked before eating. Future studies should investigate the impact of local cooking methods on EFA degradation and measure antioxidant vitamins that may protect EFA during cooking and processing. However, to our knowledge, this is the first study to extensively characterize the EFA content of foods in Southern Ghana. Our research suggests that melon seeds and soybean in Southern Ghana can be used to increase the dietary EFA sources in the population. Future studies should investigate incorporating melon seeds, cashew nut, and soybean in diets of children in Southern Ghana, since in Ghana, 75% of children below age five suffer from iron deficiency anemia [134] and 19% are stunted [19].

Conclusion

We conclude that SYBN, melon seeds (AGS and AGSF), PNUT, MG, and VGT are good sources of EFA available in markets in Southern Ghana. SYBN and melon seeds have high amounts of EFA and minerals. It is widely known that EFAD and mineral deficiencies are associated with stunting and cognitive impairment. PNUT, MG, VGT, melon seeds, and SYBN could be incorporated into diets and dietary supplements to potentially help prevent or offset these health issues in Southern Ghana.

CHAPTER 9: SUMMARY

Main findings

A number of experimental and observational studies, have confirmed that, essential fatty acids are crucial for growth and cognition in children. Essential fatty acid deficiency leads to impaired growth and delayed brain development and cognitive abilities. Primarily, this research set out to assess associations between essential fatty acid status and growth and cognition in Ghanaian children. In Ghana, there are huge disparities in stunting levels between the North and the South[20]. The reason for this remains unknown, however, the accessibility of foods in both sectors can account for this. Numerous studies have shown that, essential fatty acids especially long-chain UFA are important for growth and cognitive development. These studies were conducted mainly in developed countries. These studies also focused on dietary and supplementation options with subjects' predominantly pregnant and lactating women, and preterm and term infants. This area is less studied in developing countries because of the logistics and technicalities involved in fatty acid research though there is high prevalence of deficiencies that are contributing to delays in growth and cognitive development. There have been few research using fat based supplements in Ghana however, the research doesn't measure the whole blood FA levels as well as its association with growth and cognition. The objective of this thesis was to assess whole blood FA content and establish an association between growth and cognition with FA status in two different populations in Ghana. The research was designed to know the prevalence of EFAD, to identify local sources of fatty acids and to determine the FA content and cognitive impairment varied in populations in Northern and Southern Ghana. In Northern Ghana, the prevalence of EFAD was 8% while stunting was

29.7%. An inverse relationship was established between n-6 FA and stunting levels. Children who had insufficient levels of n-6 fatty acids were more stunted than those with sufficient levels of n-6 fatty acids. Further, there was significant positive associations between children with omega-3 fatty acids and executive function, specifically, children with n-3 blood levels perform better on executive function tests. In Southern Ghana, the prevalence of stunting was 22.0% while EFAD was 10.64%. None of the FAs were associated with stunting, but total saturated FA and linoleic acid were associated with stunting, but was trending towards statistical significance.

Seeds, nuts and oils were purchased from markets from both populations. Melon seeds, cashew nuts, peanut, tigernuts, palm oil, palm kernel oil, coconut oil, commercial vegetable oil and margarine were identified for Southern Ghana. Dawadawa, sesame seeds, neri, soybean, peanut, palm kernel, sheabutter and palm oil were identified for Southern Ghana. The research demonstrates that, melon seeds and peanuts have high amounts of n-6 FAs specifically linoleic acid, while palm oil, and soybean contain high amounts of n-3FAs specifically alpha linolenic acid. Melon seeds and soybean contained high amounts of iron and zinc. Dawadawa and soybean contain high amounts of calcium. Some of the identified seeds, nuts and oils were rich in saturated fats, n-6 FAs and minerals. Therefore incorporation of dawadawa, soybean, palm oil, melon seeds, and peanuts in diets of children in Ghana can increase EFA intake and this may support growth and cognitive development in children in both populations.

Although FA research has received little inputs in developing countries, FAs are crucial in growth and cognition development in Ghanaian children. This research further

demonstrates that, some of the local foods are high in FAs and minerals and these can be incorporated into diets of children.

Innovation

As stated earlier, FA research have been neglected in development due to the technicalities (FA oxidation) and logistics (storage/shipment/analysis) involved in measuring whole blood fatty acids. This study is novel because we utilized a unique system to obtain blood samples for the FA analysis.[65]. Using this method ensures sample integrity at room temperature for up to two weeks during storage and shipment. The samples were shipped to US for detailed FA analysis, an analysis would have been challenging and impossible to run in Ghana. The use of whole blood FA levels is a more reliable way to establish chronic and recent FA intakes rather that depending on other methods of food intakes such as 24 hour dietary recall This study provides a primary knowledge for future intervention and the use of locally available and culturally accepted foods to address essential FA deficiency in Ghana. Another innovation of this study is because it gives a relationship between EFA status and cognitive abilities of the children. This knowledge enlightens health and nutrition care personnel on the importance of EFA on growth and cognition.

Limitations

This study has numerous limitation despite the positive findings**.** The study was a cross sectional study, hence all the association that has been reported here are correlative and not causative. The study was conducted in one village each in Northern and Southern

Ghana hence the findings cannot be generalized to the entire Ghanaian population. In this study the adequacy of other nutrients were not assessed. These nutrients such as zinc, iron or protein may be low in the diets of children. Further, the deficiencies of certain nutrients may interfere with FA metabolism and this would affect whole blood FA levels. Further, the deficiencies of some of these nutrients may contribute to poor linear growth and poor cognition. The children were not required to fast and the blood samples were collected throughout the day. This could increase the variability in the whole blood FA levels. This variability could be reduced in this setting because children in each of the populations consume relatively similar meals which are usually low in fat compared to children in other settings.

The seeds, nuts and oils that were identified to be potential sources of EFAs were in their raw and uncooked state, thus the effect of the cooking on the FAs were not assessed. The effect of heat on oxidation of FAs cannot be overemphasized, hence the FA levels that were presented in this study may not truly represent the FAs in cooked food in this population. Further, we also did not account for potential effects of short term or long-term effects of storage on the FA content of these foods. This could be challenging because during storage, there could be FA oxidation that may alter FA composition.

Cognitive abilities of these children was assess throughout day. This could be a limitation to the work because, the performance of these children may be affected by the time of the day the test was administered.

Though there were limitations this study, this study is novel in a number of ways: the application of anti-oxidant treated blood spot cards, usage of the DCCS test to assess

cognitive development as well as the identification of seeds, nuts and oils that are sources of EFAs are giant steps towards addressing malnutrition in Ghana.

Future Directions

This research contributes to the body of literature supporting the importance of fatty acids in growth and development as well as providing information about the FA status in developing countries especially Ghana. This study is also the first to assess executive function as a measure of cognitive abilities of the malnourished and well-nourished children in rural Ghana. The results of this study can be utilized to develop intervention studies and determine the ability of increased intake of n-6 FAs and n-3FAs to improve growth and executive function in young children.

There have been few studies linking FA levels in blood to dietary intake of FAs in children 2-6 year olds. An exploration of this area could be helpful. Additionally, future studies may consider FA supplementation programs and assess whole blood lipid levels connecting them with anthropometric measures over a period of time. This study could establish a link between dietary FA intakes and changes in growth and cognitive function measures. The planning and implementation of a similar project could be explored at different stages in the life cycle so that we could know the opportune time to give certain amounts of FA rich foods that can enhance growth and cognition in children

The FA contents of the seeds, nuts and oils in this study can provide direction in how these food components could be utilized in the population to ensure that children are receiving sufficient amounts of EFAs. This research also supports the addition of specific FA content of local foods to the West African Food Composition table other than their

representation as whole FAs. The foods that have been identified in this study can be utilized in ongoing and future strategies in formulating high-energy foods to eliminate malnutrition, improving growth and cognition. These foods can also be incorporated into household diets.

This study supports previous studies that states that high consumption of fish and fish oil intake can enhance linear growth in children. There is also the need for a randomized study to be conducted in other populations in Ghana in areas with both high and low fish consumption.

Finally, this study enlist food source of EFA and minerals. These foods can be formulated into a snack product and fed to children in Ghana. This research also supports the addition of specific FA content to the West African Food Composition table.

APPENDICES

APPENDIX 1: Supplementary material

Table 36, S1: Median (Q1, Q3) of fatty acid proportions in whole blood

Class	Fatty acid	Overall	Stunted	Non-stunted	P-value[b]
SFA	Myristic	0.17 (0.12, 0.27)	0.19 (0.13, 0.29)	0.16 (0.11, 0.26)	0.25
	Palmitic	21.3 (20.3, 22.3)	21.4 (20.8, 22.4)	21.2 (20.2, 22.2)	0.21
	Stearic	13.5 (12.6, 14.3)	13.4 (12.4, 14.2)	13.6 (12.8, 14.3)	0.08
	Arachidic	0.35 (0.31, 0.39)	0.35 (0.31, 0.40)	0.35 (0.31, 0.39)	0.35
	Behenic	0.86 (0.72, 0.98)	0.84 (0.69, 0.99)	0.87 (0.73, 0.98)	0.33
	Lignoceric	1.24 (1.04, 1.43)	1.21 (0.98, 1.42)	1.25 (1.06, 1.45)	0.40
	Total SFA[c]	37.5 (36.8, 38.5)	37.4 (36.8, 38.4)	37.5 (36.8, 38.5)	0.45
n-9	Oleic	20.8 (19.5, 22.6)	21.1 (20.1, 23.7)	20.6 (19.5, 22.2)	**≤0.05**
	Elaidic	0.17 (0.14, 0.23)	0.17 (0.14, 0.25)	0.17 (0.14, 0.23)	0.64
	Eicosenoic	0.31 (0.27, 0.39)	0.31 (0.27, 0.36)	0.31 (0.27, 0.40)	0.61
	Mead	0.13 (0.10, 0.16)	0.12 (0.10, 0.15)	0.13 (0.11, 0.17)	0.12
	Nervonic	0.72 (0.59, 0.91)	0.71 (0.57, 0.92)	0.73 (0.60, 0.90)	0.32
	Palmitoleic	0.32 (0.22, 0.45)	0.35 (0.25, 0.47)	0.31 (0.21, 0.42)	0.06
	Total n-9[d]	21.9 (20.7, 23.6)	22.2 (21.3, 24.5)	21.7 (20.5, 23.4)	**≤0.05**
n-7	Palmitelaidic	0.02 (0.01, 0.04)	0.03 (0.02, 0.04)	0.02 (0.01, 0.04)	0.14
n-3	ALA	0.16 (0.11, 0.21)	0.16 (0.11, 0.24)	0.15 (0.11, 0.21)	0.75
	EPA	0.18 (0.13, 0.24)	0.18 (0.13, 0.24)	0.18 (0.13, 0.24)	0.89
	DPA n-3	0.55 (0.47, 0.67)	0.54 (0.46, 0.67)	0.56 (0.47, 0.67)	0.72
	DHA	2.53 (2.18, 2.96)	2.42 (2.09, 2.76)	2.60 (2.24, 3.03)	**≤0.01**
	Total n-3[e]	3.47 (3.08, 3.95)	3.30 (2.98, 3.78)	3.51 (3.12, 4.00)	**≤0.05**
	O3I	2.70 (2.36, 3.17)	2.58 (2.29, 3.03)	2.74 (2.41, 3.20)	**≤0.01**
n-6	LA	20.7 (19.4, 21.7)	20.8 (19.7, 22.1)	20.6 (19.2, 21.5)	0.14
	Linoelaidic	0.23 (0.20, 0.28)	0.23 (0.20, 0.27)	0.23 (0.20, 0.28)	0.95
	GLA	0.15 (0.12, 0.20)	0.16 (0.12, 0.19)	0.15 (0.11, 0.20)	0.74
	EDA	0.29 (0.24, 0.33)	0.28 (0.24, 0.34)	0.29 (0.25, 0.33)	0.41
	DGLA	1.36 (1.18, 1.52)	1.35 (1.18, 1.49)	1.37 (1.19, 1.54)	0.32
	AA	11.0 (9.94, 11.9)	10.8 (9.60, 11.4)	11.2 (10.0, 11.9)	**≤0.01**
	DTA	1.68 (1.44, 1.92)	1.62 (1.36, 1.80)	1.72 (1.48, 1.95)	**≤0.01**
	DPA n-6	0.58 (0.47, 0.69)	0.54 (0.46, 0.68)	0.59 (0.48, 0.70)	0.06
	Total n-6[f]	36.0 (34.4, 37.4)	35.8 (34.5, 36.7)	36.1 (34.3, 37.6)	0.10
Ratios	GLA/LA	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	0.93
	EDA/LA	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.15
	DGLA/LA	0.07 (0.06, 0.08)	0.07 (0.06, 0.07)	0.07 (0.06, 0.08)	0.11
	AA/DGLA	8.05 (7.21, 8.99)	7.88 (7.04, 8.79)	8.11 (7.31, 9.08)	0.19
	T/T	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.01 (0.01, 0.02)	0.81

Table 36,S1: (cont'd)

[a]Values represent blood fatty acid (FA) % composition. Stunted defined by height-for-age z-score (HAZ)≤-2. SFA, saturated FA; n-9, omega-9; n-7, omega-7; n-3, omega-3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; O3I, omega-3 index; n-6, omega-6; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; T/T, triene to tetraene. [b]P-value from Wilcoxon-Mann-Whitney test comparing stunted and non-stunted children. [c]Total SFA includes myristic, palmitic, arachidic, behenic, and lignoceric. [d]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic. [e]Total n-3 includes ALA, EPA, DPA n-3, and DHA. [f]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Table 37, S2: Regression results between HAZ, WAZ, and fatty acids

		HAZ		WAZ	
Class	**Fatty acid**	**Beta ± SE**	**p-value**	**Beta ± SE**	**p-value**
SFA	Myristic	-0.47 ± 0.56	0.41	-0.16 ± 0.43	0.71
	Palmitic	-0.01 ± 0.04	0.72	-0.05 ± 0.03	0.08
	Stearic	0.05 ± 0.04	0.25	0.07 ± 0.03	**≤0.05**
	Arachidic	-0.86 ± 0.96	0.37	0.48 ± 0.74	0.52
	Behenic	0.01 ± 0.35	0.98	0.22 ± 0.27	0.40
	Lignoceric	-0.06 ± 0.20	0.76	0.02 ± 0.15	0.89
	Total SFA[b]	0.03 ± 0.05	0.54	0.01 ± 0.04	0.84
n-9	Oleic	-0.04 ± 0.02	0.10	-0.03 ± 0.02	0.14
	Elaidic	-0.02 ± 0.33	0.94	-0.04 ± 0.25	0.87
	Eicosenoic	-0.35 ± 0.60	0.56	-0.01 ± 0.46	0.98
	Mead	2.48 ± 1.21	**≤0.05**	2.08 ± 0.93	**≤0.05**
	Nervonic	0.13 ± 0.29	0.65	0.14 ± 0.23	0.53
	Palmitoleic	-0.37 ± 0.32	0.25	-0.40 ± 0.24	0.10
	Total n-9[c]	-0.04 ± 0.02	0.10	-0.03 ± 0.02	0.15
n-7	Palmitelaidic	3.13 ± 2.47	0.21	2.15 ± 1.90	0.26
n-3	ALA	-0.67 ± 0.56	0.23	-0.32 ± 0.43	0.45
	EPA	0.07 ± 0.25	0.77	0.17 ± 0.19	0.36
	DPA n-3	0.03 ± 0.37	0.93	0.20 ± 0.28	0.48
	DHA	0.08 ± 0.10	0.43	0.09 ± 0.08	0.26
	Total n-3[d]	0.04 ± 0.07	0.62	0.06 ± 0.06	0.27
	O3I	0.06 ± 0.08	0.47	0.08 ± 0.06	0.23
n-6	LA	-0.06 ± 0.03	0.07	-0.04 ± 0.03	0.11
	Linoelaidic	1.44 ± 0.99	0.15	0.16 ± 0.76	0.83
	GLA	-0.19 ± 0.95	0.84	-0.21± 0.73	0.78
	EDA	-1.48 ± 0.94	0.12	-0.80 ± 0.72	0.27
	DGLA	0.51 ± 0.25	**≤0.05**	0.39 ± 0.19	**≤0.05**
	AA	0.12 ± 0.04	**≤0.01**	0.08 ± 0.03	**≤0.01**
	DTA	0.50 ± 0.17	**≤0.01**	0.39 ± 0.13	**≤0.01**
	DPA n-6	0.56 ± 0.38	0.14	0.18 ± 0.29	0.54
	Total n-6[e]	0.03 ± 0.03	0.18	0.02 ± 0.02	0.26
Ratios	GLA/LA	4.19 ± 17.7	0.81	0.54 ± 13.6	0.97
	EDA/LA	-13.8 ± 18.3	0.45	-6.52 ± 14.0	0.64
	DGLA/LA	11.7 ± 4.39	**≤0.01**	8.34 ± 3.38	**≤0.01**
	AA/DGLA	0.02 ± 0.04	0.68	0.00 ± 0.03	0.92
	T/T	11.3 ± 12.1	0.35	9.75 ± 9.28	0.29

Model: HAZ= fatty acid + hemoglobin; WAZ= fatty acid + hemoglobin; HAZ= ratio + hemoglobin; WAZ= ratio + hemoglobin

[a]Model is not adjusted for sex as there were few significantly different FAs between sexes and regression values were essentially unaffected when evaluated with sex adjustment.

HAZ, height-for-age z-score; WAZ, weight-for-age z-score; SFA, saturated fatty acid; n-9, omega-9; n-7, omega-7; n-3, omega-3; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA n-3, omega-3 docosapentaenoic acid; DHA, docosahexaenoic acid; O3I, omega-3 index; n-6, omega-6; LA, linoleic acid; GLA, gamma-linolenic acid; EDA, eicosadienoic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; DPA n-6, omega-6 docosapentaenoic acid; T/T, triene to tetraene ratio. [b]Total SFA includes myristic, palmitic, arachidic, behenic, and lignoceric. [c]Total n-9 includes oleic, elaidic, eicosenoic, Mead, and Nervonic. [d]Total n-3 includes ALA, EPA, DPA n-3, and DHA. [e]Total n-6 includes LA, linoelaidic, GLA, EDA, DGLA, AA, DTA, and DPA n-6.

Table 38,S3: Spearhman correlation (n=23)

	height	weight	Total passes	WHZ	HAZ	WAZ	BAZ	Oleic	LA	ALA	Mead	DGLA	AA	EPA	DHA	Tot n-3	n-6/n-3 ratio	Omega-3 Index	Tot. PUFA	d6d	d5d	TT
Age	.609**	0.394	0.157	-0.169	0.237	0.062	-0.207	0.096	0.119	0.244	0.049	-0.027	-0.212	-0.277	-0.181	-0.246	0.320	-0.196	-0.080	-0.068	-0.384	0.086
	0.002	0.057	0.475	0.430	0.264	0.774	0.333	0.656	0.579	0.250	0.822	0.900	0.320	0.191	0.397	0.247	0.127	0.358	0.710	0.753	0.064	0.689
height	1.000	.685**	0.093	-0.144	.877**	.528**	-0.210	-0.097	0.195	0.102	0.305	-0.020	0.154	-0.274	0.167	0.019	0.124	0.049	0.271	-0.101	-0.044	0.280
		0.000	0.672	0.503	0.000	0.008	0.325	0.652	0.361	0.635	0.147	0.924	0.474	0.195	0.437	0.931	0.563	0.820	0.200	0.639	0.837	0.184
weight		1.000	-0.254	.471*	.683**	.903**	.414*	-0.064	0.047	.422*	-0.033	0.062	-0.020	-0.149	-0.065	-0.073	0.086	-0.092	0.057	0.066	-0.179	0.042
			0.242	0.020	0.000	0.000	0.044	0.767	0.829	0.040	0.880	0.773	0.924	0.488	0.763	0.736	0.689	0.670	0.790	0.760	0.403	0.845
Totalpasses			1.000	-.446*	-0.019	-0.297	-0.411	0.088	0.105	-0.020	0.004	-0.108	-0.107	-0.091	-0.127	-0.171	0.221	-0.099	0.074	-0.242	-0.012	0.088
				0.033	0.932	0.168	0.052	0.691	0.633	0.928	0.985	0.625	0.627	0.680	0.564	0.434	0.310	0.653	0.736	0.266	0.958	0.691
WHZ				1.000	-0.099	.573**	.991**	-0.064	-0.170	.496*	-0.270	0.212	0.001	0.117	-0.065	0.010	-0.092	-0.016	-0.100	0.270	-0.077	-0.214
					0.645	0.003	0.000	0.765	0.428	0.014	0.201	0.320	0.995	0.586	0.764	0.961	0.670	0.941	0.640	0.203	0.719	0.314
HAZ					1.000	.690**	-0.156	-0.180	0.030	0.019	0.288	-0.037	0.308	-0.198	0.237	0.116	0.011	0.109	0.337	-0.074	0.164	0.277
						0.000	0.468	0.400	0.888	0.929	0.173	0.862	0.143	0.353	0.266	0.590	0.958	0.613	0.108	0.731	0.443	0.191
WAZ						1.000	.523**	-0.123	-0.103	.426*	-0.121	0.129	0.152	-0.112	0.003	0.009	0.007	-0.042	0.127	0.138	-0.012	-0.044
							0.009	0.568	0.630	0.038	0.574	0.549	0.478	0.602	0.990	0.968	0.974	0.846	0.554	0.519	0.955	0.837
BAZ							1.000	-0.072	-0.171	.483*	-0.268	0.196	-0.017	0.118	-0.108	-0.019	-0.063	-0.045	-0.115	0.250	-0.070	-0.203
								0.738	0.424	0.017	0.206	0.360	0.936	0.582	0.616	0.929	0.771	0.834	0.593	0.238	0.747	0.340
Oleic								1.000	-0.271	0.105	0.109	-.479*	-.819**	-.515*	-.648**	-.715**	.546**	-.697**	-.902**	-0.341	-0.342	0.388
									0.200	0.625	0.613	0.018	0.000	0.010	0.001	0.000	0.006	0.000	0.000	0.103	0.102	0.061
LA									1.000	-0.132	-0.179	-0.122	-0.005	0.163	0.083	0.093	0.013	0.129	0.375	-.437*	0.274	-0.369
										0.538	0.402	0.571	0.981	0.448	0.698	0.665	0.952	0.549	0.071	0.033	0.195	0.076
ALA										1.000	-0.330	0.364	-0.146	-0.137	-0.298	-0.192	0.258	-0.255	-0.113	0.324	-.536**	-0.183
											0.116	0.080	0.496	0.525	0.157	0.368	0.223	0.230	0.599	0.122	0.007	0.393
Mead											1.000	0.057	0.080	-.489*	0.118	-0.133	0.211	-0.082	-0.040	0.152	-0.151	.900**
												0.790	0.710	0.015	0.582	0.535	0.322	0.704	0.853	0.478	0.480	0.000
DGLA												1.000	.576**	-0.004	0.374	0.368	-0.227	0.321	.443*	.913**	-0.357	-0.148
														0.003	0.984	0.072	0.077	0.286	0.126	0.030	0.000	0.087
AA													1.000	0.323	.758**	.719**	-.523**	.702**	.837**	.482*	.407*	-0.217
														0.124	0.000	0.000	0.009	0.000	0.000	0.017	0.048	0.310
EPA														1.000	.526**	.735**	-.821**	.732**	.480*	0.003	.470*	-.603**
															0.008	0.000	0.000	0.000	0.018	0.987	0.021	0.002
DHA															1.000	.923**	-.843**	.946**	.763**	0.317	0.390	-0.135
																0.000	0.000	0.000	0.000	0.131	0.060	0.530
Tot n-3																1.000	-.935**	.988**	.779**	0.320	0.363	-0.357
																		0.000	0.000	0.000	0.127	0.082
n-6/n-3 ratio																	1.000	-.930**	-.573**	-0.265	-0.365	0.366
																			0.000	0.003	0.210	0.079
Omega-3 Index																		1.000	.776**	0.264	0.397	-0.310
																				0.000	0.212	0.054
Tot. PUFA																			1.000	0.248	.407*	-0.337
																				0.243	0.048	0.108
d6d																				1.000	-.430*	0.011
																					0.036	0.958
d5d																					1.000	-0.323
																						0.123
TT																						1.000

Hb, Hemoglobin; WAZ, weight-for-age z-scores; BAZ, BMI-for-age z-scores; HAZ, height-for-age z-score; AA, arachidonic acid; SCD n-7, Stearoyl CoA Desaturase n-7; SCD n-9, Stearoyl CoA Desaturase n-9; D6D, delta-9-desaturase; D5D, delta-5-desaturase; DGLA, di-homo-gamma-linolenic. [a]Top number is the Spearman correlation coefficient. Bottom number is the p-value. All significant associations (p<0.05) are in bold. [b]Total PUFA includes total n-3 and total n-6. [c]Total n-3 includes alpha linolenic, EPA, DPA n-3, and DHA.

Table 39, S4: Pearson correlation, n=23

	height	weight	Total passes	WHZ	HAZ	WAZ	BAZ	Oleic	LA	ALA	Mead	DGLA	AA	EPA	DHA	Tot n-3	n-6/n-3 ratio	Omega-3 Index	Tot. PUFA	d6d	d5d	TT
Age	.609**	0.394	0.157	-0.169	0.237	0.062	-0.207	0.096	0.119	0.244	0.049	-0.027	-0.212	-0.277	-0.181	-0.246	0.320	-0.196	-0.080	-0.068	-0.384	0.086
	0.002	0.057	0.475	0.430	0.264	0.774	0.333	0.656	0.579	0.250	0.822	0.900	0.320	0.191	0.397	0.247	0.127	0.358	0.710	0.753	0.064	0.689
height	1.000	.685**	0.093	-0.144	.877**	.528**	-0.210	-0.097	0.195	0.102	0.305	-0.020	0.154	-0.274	0.167	0.019	0.124	0.049	0.271	-0.101	-0.044	0.280
		0.000	0.672	0.503	0.000	0.008	0.325	0.652	0.361	0.635	0.147	0.924	0.474	0.195	0.437	0.931	0.563	0.820	0.200	0.639	0.837	0.184
weight		1.000	-0.254	.471*	.683**	.903**	.414*	-0.064	0.047	.422*	-0.033	0.062	-0.020	-0.149	-0.065	-0.073	0.086	-0.092	0.057	0.066	-0.179	0.042
			0.242	0.020	0.000	0.000	0.044	0.767	0.829	0.040	0.880	0.773	0.924	0.488	0.763	0.736	0.689	0.670	0.790	0.760	0.403	0.845
Total passes			1.000	-.446*	-0.019	-0.297	-0.411	0.088	0.105	-0.020	0.004	-0.108	-0.107	-0.091	-0.127	-0.171	0.221	-0.099	0.074	-0.242	-0.012	0.088
				0.033	0.932	0.168	0.052	0.691	0.633	0.928	0.985	0.625	0.627	0.680	0.564	0.434	0.310	0.653	0.736	0.266	0.958	0.691
WHZ				1.000	-0.099	.573**	.991**	-0.064	-0.170	.496*	-0.270	0.212	0.001	0.117	-0.065	0.010	-0.092	-0.016	-0.100	0.270	-0.077	-0.214
					0.645	0.003	0.000	0.765	0.428	0.014	0.201	0.320	0.995	0.586	0.764	0.961	0.670	0.941	0.640	0.203	0.719	0.314
HAZ					1.000	.690**	-0.156	-0.180	0.030	0.019	0.288	-0.037	0.308	-0.198	0.237	0.116	0.011	0.109	0.337	-0.074	0.164	0.277
						0.000	0.468	0.400	0.888	0.929	0.173	0.862	0.143	0.353	0.266	0.590	0.958	0.613	0.108	0.731	0.443	0.191
WAZ						1.000	.523**	-0.123	-0.103	.426*	-0.121	0.129	0.152	-0.112	0.003	0.009	0.007	-0.042	0.127	0.138	-0.012	-0.044
							0.009	0.568	0.630	0.038	0.574	0.549	0.478	0.602	0.990	0.968	0.974	0.846	0.554	0.519	0.955	0.837
BAZ							1.000	-0.072	-0.171	.483*	-0.268	0.196	-0.017	0.118	-0.108	-0.019	-0.063	-0.045	-0.115	0.250	-0.070	-0.203
								0.738	0.424	0.017	0.206	0.360	0.936	0.582	0.616	0.929	0.771	0.834	0.593	0.238	0.747	0.340
Oleic								1.000	-0.271	0.105	0.109	-.479*	-.819**	-.515*	-.648**	-.715**	.546**	-.697**	-.902**	-0.341	-0.342	0.388
									0.200	0.625	0.613	0.018	0.000	0.010	0.001	0.000	0.006	0.000	0.000	0.103	0.102	0.061
LA									1.000	-0.132	-0.179	-0.122	-0.005	0.163	0.083	0.093	0.013	0.129	0.375	-.437*	0.274	-0.369
										0.538	0.402	0.571	0.981	0.448	0.698	0.665	0.952	0.549	0.071	0.033	0.195	0.076
ALA										1.000	-0.330	0.364	-0.146	-0.137	-0.298	-0.192	0.258	-0.255	-0.113	0.324	-.536**	-0.183
											0.116	0.080	0.496	0.525	0.157	0.368	0.223	0.230	0.599	0.122	0.007	0.393
Mead											1.000	0.057	0.080	-.489*	0.118	-0.133	0.211	-0.082	-0.040	0.152	-0.151	.900**
												0.790	0.710	0.015	0.582	0.535	0.322	0.704	0.853	0.478	0.480	0.000
DGLA												1.000	.576**	-0.004	0.374	0.368	-0.227	0.321	.443*	.913**	-0.357	-0.148
														0.003	0.984	0.072	0.077	0.286	0.126	0.030	0.000	0.087
AA													1.000	0.323	.758**	.719**	-.523**	.702**	.837**	.482*	.407*	-0.217
														0.124	0.000	0.000	0.009	0.000	0.000	0.017	0.048	0.310
EPA														1.000	.526**	.735**	-.821**	.732**	.480*	0.003	.470*	-.603**
															0.008	0.000	0.000	0.000	0.018	0.987	0.021	0.002
DHA															1.000	.923**	-.843**	.946**	.763**	0.317	0.390	-0.135
																0.000	0.000	0.000	0.000	0.131	0.060	0.530
Tot n-3																1.000	-.935**	.988**	.779**	0.320	0.363	-0.357
																		0.000	0.000	0.000	0.127	0.082
n-6/n-3 ratio																	1.000	-.930**	-.573**	-0.265	-0.365	0.366
																			0.000	0.003	0.210	0.079
Omega-3 Index																		1.000	.776**	0.264	0.397	-0.310
																				0.000	0.212	0.054
Tot. PUFA																			1.000	0.248	.407*	-0.337
																				0.243	0.048	0.108
d6d																				1.000	-.430*	0.011
																					0.036	0.958
d5d																					1.000	-0.323
																						0.123
TT																						1.000

Hb, Hemoglobin; WAZ, weight-for-age z-scores; BAZ, BMI-for-age z-scores; HAZ, height-for-age z-score; AA, arachidonic acid; SCD n-7, Stearoyl CoA Desaturase n-7; SCD n-9, Stearoyl CoA Desaturase n-9; D6D, delta-9-desaturase; D5D, delta-5-desaturase; DGLA, di-homo-gamma-linolenic. aTop number is the Pearson correlation coefficient. Bottom number is the p-value. All significant associations (p<0.05) are in bold. bTotal PUFA includes total n-3 and total n-6. cTotal n-3 includes alpha linolenic, EPA, DPA n-3, and DHA.

Table 40, S5: Pearson correlation, n=38

	height	weight	Total passes	WHZ	HAZ	WAZ	BAZ	Oleic	LA	ALA	Mead	DGLA	AA	EPA	DHA	Tot n-3	n-6/n-3	Omega3Index	Tot PUFA	d6d	d5d	TT
Age	.499**	0.193	0.253	-0.154	0.041	-0.099	-0.180	0.091	0.301	0.096	0.039	0.034	-0.033	-0.288	-0.151	-0.215	.366*	-0.200	0.001	-0.088	-0.104	0.059
	0.001	0.245	0.125	0.355	0.805	0.553	0.279	0.585	0.066	0.568	0.815	0.842	0.843	0.080	0.365	0.195	0.024	0.229	0.997	0.598	0.533	0.723
height	1	.711**	0.211	0.125	.881**	.576**	0.023	0.116	0.082	0.258	0.216	0.052	-0.016	-0.294	-0.060	-0.125	0.221	-0.126	-0.033	0.014	-0.152	0.195
		0.000	0.203	0.455	0.000	0.000	0.893	0.488	0.623	0.118	0.192	0.756	0.926	0.073	0.722	0.454	0.182	0.453	0.843	0.933	0.363	0.242
weight		1	-0.004	.778**	.716**	.947**	.712**	0.107	-0.170	.444**	0.064	0.058	-0.100	-0.172	-0.082	-0.082	0.051	-0.113	-0.153	0.121	-0.202	0.088
			0.983	0.000	0.000	0.000	0.000	0.522	0.309	0.005	0.704	0.727	0.550	0.301	0.624	0.625	0.761	0.500	0.361	0.468	0.223	0.601
Totalpasses			1	-0.151	0.137	-0.040	-0.182	0.171	0.162	0.138	-0.052	-0.284	-0.133	0.033	-0.061	-0.068	0.139	-0.043	-0.056	-.334*	0.137	0.034
				0.366	0.413	0.811	0.275	0.305	0.330	0.408	0.758	0.084	0.425	0.844	0.714	0.686	0.404	0.799	0.740	0.041	0.412	0.840
WHZ				1	0.240	.839**	.993**	0.033	-0.279	.398*	-0.122	0.024	-0.096	0.042	-0.038	0.022	-0.135	-0.021	-0.150	0.137	-0.116	-0.071
					0.147	0.000	0.000	0.843	0.090	0.013	0.467	0.884	0.566	0.803	0.822	0.895	0.420	0.902	0.370	0.411	0.488	0.670
HAZ					1	.728**	0.132	0.084	-0.069	0.266	0.213	0.037	0.014	-0.172	0.034	-0.008	0.044	-0.016	-0.023	0.062	-0.110	0.176
						0.000	0.430	0.618	0.682	0.106	0.200	0.823	0.932	0.302	0.841	0.961	0.794	0.922	0.893	0.711	0.510	0.291
WAZ						1	.775**	0.076	-0.246	.425**	0.040	0.040	-0.065	-0.067	-0.017	0.003	-0.068	-0.032	-0.129	0.137	-0.147	0.055
							0.000	0.652	0.137	0.008	0.813	0.810	0.698	0.689	0.919	0.984	0.687	0.851	0.442	0.413	0.377	0.743
BAZ							1	0.026	-0.285	.372*	-0.147	0.023	-0.108	0.055	-0.051	0.015	-0.137	-0.028	-0.160	0.136	-0.112	-0.089
								0.876	0.083	0.022	0.379	0.892	0.520	0.741	0.762	0.928	0.413	0.868	0.336	0.415	0.503	0.595
Oleic								1	-0.247	0.223	0.290	-.468**	-.872**	-.528**	-.870**	-.862**	.751**	-.861**	-.912**	-.361*	-.325*	.584**
									0.135	0.179	0.078	0.003	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.026	0.046	0.000
LA									1	-0.080	-0.296	0.070	0.162	0.020	0.087	0.061	0.144	0.077	.487**	-0.295	0.016	-.325*
										0.632	0.071	0.678	0.332	0.905	0.604	0.715	0.387	0.644	0.002	0.072	0.924	0.047
ALA										1	-0.191	0.047	-0.300	0.022	-0.162	-0.078	0.033	-0.130	-0.209	0.067	-0.308	-0.045
											0.250	0.779	0.067	0.898	0.330	0.641	0.844	0.438	0.208	0.688	0.060	0.788
Mead											1	0.117	-0.124	-.352*	-0.211	-0.296	0.213	-0.267	-0.288	0.213	-0.241	.923**
												0.483	0.457	0.030	0.203	0.071	0.199	0.105	0.080	0.199	0.145	0.000
DGLA												1	.549**	-0.109	.325*	0.245	-0.167	0.243	.478**	.929**	-.580**	-0.086
														0.000	0.516	0.046	0.139	0.317	0.142	0.002	0.000	0.000
AA													1	.372*	.802**	.750**	-.596**	.763**	.899**	.473**	0.303	-.462**
														0.022	0.000	0.000	0.000	0.000	0.000	0.003	0.064	0.004
EPA														1	.558**	.744**	-.732**	.723**	.467**	-0.109	.551**	-.454**
															0.000	0.000	0.000	0.000	0.003	0.513	0.000	0.004
DHA															1	.963**	-.882**	.977**	.849**	0.287	.384*	-.495**
																0.000	0.000	0.000	0.000	0.081	0.017	0.002
Tot n-3																1	-.936**	.994**	.818**	0.218	.446**	-.547**
																		0.000	0.000	0.000	0.188	0.005
n-6/n-3																	1	-.923**	-.635**	-0.224	-.421**	.436**
																			0.000	0.000	0.177	0.008
Omega-3 Index																		1	.827**	0.210	.462**	-.529**
																				0.000	0.205	0.003
Tot PUFA																			1	0.288	0.280	-.582**
																				0.080	0.089	0.000
d6d																				1	-.560**	0.026
																					0.000	0.877
d5d																					1	-.338*
																						0.038
TT																						1

Hb, Hemoglobin; WAZ, weight-for-age z-scores; BAZ, BMI-for-age z-scores; HAZ, height-for-age z-score; AA, arachidonic acid; SCD n-7, Stearoyl CoA Desaturase n-7; SCD n-9, Stearoyl CoA Desaturase n-9; D6D, delta-9-desaturase; D5D, delta-5-desaturase; DGLA, di-homo-gamma-linolenic. aTop number is the Pearson correlation coefficient. Bottom number is the p-value. All significant associations (p<0.05) are in bold. bTotal PUFA includes total n-3 and total n-6. cTotal n-3 includes alpha linolenic, EPA, DPA n-3, and DHA.

Table 41, S6: Spearman correlation, n=38

	height	weight	Totalpasses	WHZ	HAZ	WAZ	BAZ	Oleic	LA	ALA	Mead	DGLA	AA	EPA	DHA	Tot n-3	n-6/n-3	Omega3Index	Tot.PUFA	d6d	d5d	TT
Age	.544**	0.221	0.272	-0.159	0.049	-0.056	-0.159	-0.088	0.229	0.127	-0.023	-0.029	0.075	-0.313	-0.102	-0.156	0.272	-0.143	0.134	-0.062	-0.060	-0.048
	0.000	0.183	0.098	0.341	0.772	0.737	0.341	0.599	0.167	0.446	0.890	0.864	0.655	0.056	0.542	0.351	0.099	0.391	0.423	0.712	0.720	0.776
height	1.000	.678**	0.257	0.053	.829**	.541**	-0.003	-0.090	0.133	0.173	0.233	-0.034	0.133	-0.289	0.094	-0.005	0.094	-0.003	0.161	-0.076	-0.018	0.248
		0.000	0.120	0.752	0.000	0.000	0.984	0.591	0.427	0.300	0.160	0.841	0.427	0.079	0.573	0.977	0.576	0.984	0.336	0.651	0.914	0.134
weight		1.000	-0.002	.707**	.727**	.944**	.670**	-0.020	-0.124	.387*	0.065	0.077	-0.051	-0.148	-0.032	-0.039	0.013	-0.070	-0.080	0.121	-0.192	0.158
			0.990	0.000	0.000	0.000	0.000	0.903	0.459	0.016	0.700	0.644	0.761	0.375	0.848	0.817	0.940	0.678	0.631	0.469	0.248	0.345
Total passes			1.000	-0.216	0.140	-0.058	-0.207	0.100	0.153	0.182	-0.122	-0.181	-0.100	0.025	0.019	-0.004	0.029	0.038	0.034	-0.287	0.194	-0.090
				0.192	0.402	0.728	0.212	0.550	0.359	0.275	0.465	0.276	0.550	0.882	0.911	0.982	0.862	0.822	0.839	0.081	0.243	0.590
WHZ				1.000	0.212	.770**	.994**	0.001	-0.265	.344*	-0.152	0.097	-0.052	0.155	0.000	0.064	-0.166	0.042	-0.168	0.182	-0.071	-0.080
					0.201	0.000	0.000	0.993	0.108	0.035	0.361	0.562	0.755	0.354	0.998	0.702	0.320	0.802	0.312	0.274	0.673	0.632
HAZ					1.000	.752**	0.149	-0.040	-0.066	0.192	0.272	0.035	0.119	-0.188	0.126	0.053	-0.012	0.034	0.093	0.031	-0.046	.331*
						0.000	0.372	0.811	0.696	0.248	0.098	0.833	0.476	0.259	0.450	0.753	0.942	0.841	0.578	0.856	0.785	0.042
WAZ						1.000	.730**	0.012	-0.219	.379*	0.026	0.066	-0.029	-0.073	-0.002	0.011	-0.057	-0.028	-0.100	0.126	-0.118	0.137
							0.000	0.941	0.188	0.019	0.875	0.692	0.862	0.661	0.991	0.945	0.735	0.867	0.552	0.450	0.479	0.413
BAZ							1.000	-0.004	-0.261	.335*	-0.173	0.092	-0.073	0.167	-0.028	0.047	-0.153	0.025	-0.184	0.177	-0.066	-0.103
								0.982	0.113	0.040	0.298	0.584	0.663	0.316	0.866	0.778	0.359	0.879	0.269	0.287	0.693	0.539
Oleic								1.000	-0.156	0.205	0.183	-.386*	-.816**	-.451**	-.684**	-.711**	.559**	-.687**	-.818**	-0.287	-.361*	.431**
									0.349	0.217	0.271	0.017	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.080	0.026	0.007
LA									1.000	-0.082	-0.276	-0.040	0.093	0.024	0.008	-0.021	0.199	0.011	.457**	-.376*	0.178	-.389*
										0.623	0.093	0.810	0.580	0.889	0.961	0.901	0.230	0.946	0.004	0.020	0.286	0.016
ALA										1.000	-0.300	0.072	-0.292	-0.002	-0.152	-0.077	0.054	-0.123	-0.189	0.074	-.332*	-0.154
											0.067	0.667	0.075	0.991	0.362	0.646	0.746	0.463	0.256	0.658	0.042	0.355
Mead											1.000	0.156	-0.046	-.379*	-0.074	-0.226	0.181	-0.203	-0.234	0.309	-.338*	.918**
												0.349	0.784	0.019	0.659	0.172	0.277	0.222	0.157	0.059	0.038	0.000
DGLA												1.000	.502**	-0.073	0.214	0.159	-0.087	0.137	.383*	.907**	-.472**	-0.057
														0.001	0.665	0.196	0.341	0.603	0.411	0.018	0.000	0.003
AA													1.000	0.284	.688**	.655**	-.452**	.648**	.857**	.398*	.389*	-0.313
														0.084	0.000	0.000	0.004	0.000	0.000	0.013	0.016	0.055
EPA														1.000	.546**	.770**	-.822**	.757**	.369*	-0.084	.483**	-.464**
															0.000	0.000	0.000	0.000	0.023	0.615	0.002	0.003
DHA															1.000	.922**	-.848**	.941**	.721**	0.168	.488**	-0.261
																0.000	0.000	0.000	0.000	0.314	0.002	0.114
Tot n-3																1.000	-.940**	.989**	.704**	0.127	.520**	-.396*
																		0.000	0.000	0.000	0.446	0.001
n-6/n-3																	1.000	-.930**	-.458**	-0.135	-.441**	0.302
																			0.000	0.004	0.420	0.006
Omega-3 Index																		1.000	.707**	0.089	.545**	-.378*
																				0.000	0.594	0.000
Tot PUFA																			1.000	0.166	.420**	-.478**
																				0.319	0.009	0.002
d6d																				1.000	-.524**	0.145
																					0.001	0.386
d5d																					1.000	-.426**
																						0.008
TT																						1.000

Hb, Hemoglobin; WAZ, weight-for-age z-scores; BAZ, BMI-for-age z-scores; HAZ, height-for-age z-score; AA, arachidonic acid; SCD n-7, Stearoyl CoA Desaturase n-7; SCD n-9, Stearoyl CoA Desaturase n-9; D6D, delta-9-desaturase; D5D, delta-5-desaturase; DGLA, di-homo-gamma-linolenic. aTop number is the Pearson correlation coefficient. Bottom number is the p-value. All significant associations ($p<0.05$) are in bold. bTotal PUFA includes total n-3 and total n-6. cTotal n-3 includes alpha linolenic, EPA, DPA n-3, and DHA.

APPENDIX 2: Script to the consent form

Title: Association between essential fatty acid in growth and cognitive function in Ghanaian children 2 to 6 years of age

Background

During infancy and childhood, there is an increased need for nutrients because of the rapid growth and development occurring during this stage. This increased need renders infants and children susceptible to nutrient deficiencies. UNICEF's Global Damage Assessment Report states many are living lives below their physical, mental and intellectual capabilities due to nutritional deficiencies and among these is cognitive impairment.

Apart from proteins and carbohydrates, fatty acids are one such nutritional component essential for growth and development and associated with cognitive abilities. Essential fatty acids are fatty acids that cannot be synthesized by the body and are required from the diet. They are needed for brain and retinal tissues development especially in the regulation of membrane fluidity and signal transduction. They are also responsible for myelination of neurons and maturation of synapses. Despite these crucial roles in development, dietary intake of fatty acids in most developing countries is low.

In Ghana, only 36.6% of children from 6 to 24 months have fats added to their complementary food. In Ghana, 19% of children below 5 years of age are stunted, 5% are wasted and 11% are underweight. Though there has been a reduction in stunting, the situation still persists in Northern Ghana compared to southern Ghana, where 33.1% of children are stunted. Though the reasons for these regional differences in Ghana are not

entirely clear, it is likely that location or local culture may govern limited access to foods rich in essential fatty acids.

In this study, the researcher hope to learn whether essential fatty acids is associated in growth and cognitive function of Ghanaian children from 2 to 6 years of age.

Purpose of research:

You are being asked to participate in a research to know the relationship between essential fatty acid status in growth and cognitive function in children. Your child has been selected as a possible participant because he/she is aged from 2 to 6 years. Your participation in this study will take about 30 minutes in a single session. Your child cannot partake in this study except the parent agrees for him/her to be part In the entire study, 500 people are being asked to participate (250 children from each sector, north and south).

Procedure of the research, what shall be required of each participant and approximate total number of participants that would be involved in the research:

Dr. Jenifer Fenton and her associates will be randomly select children 2 to 6 years of age to participate in this study. Parents will be asked to give consent for their children in order for their children to participate. Anthropometric measurements (weight, height, head circumference) will be measured. Drops of blood will be taken from finger prick and 40ul will be placed onto an antioxidant treated card and a dried blood spot (DBS) card. Whole

blood fatty acid and the presence of inflammatory markers will be assessed. Haemoglobin levels and malaria status will also be assessed. A questionnaire will be used to assess the dietary intake as well as other relevant information pertaining to the study.

Risk(s):

The potential risks of participating in this study include enduring pains of finger prick. They may also suffer from bleeding and swelling of the finger. Apart from this, there is no unforeseeable risk to the subject.

Benefit(s):

The potential benefits to your child for taking part in this study is that his/her hemoglobin level at the time of the study will be known. If the child is anaemic, they will be treated however beneficial effects of the treatments cannot be guaranteed. By the end of the study, we seek to establish an evidence-based approach to know the physiological roles of essential fatty acid in growth and cognitive function in Ghanaian children. If we identify that the population is deficient in essential fatty acids, studies will be done to include culturally acceptable foods that could be incorporated into complementary weaning foods to improve fatty acid status.

Confidentiality:

All information obtained from the volunteer will be coded and this ensure confidentiality of any information obtained from them. Values obtained from the analysis will be documented and the samples disposed off. The coded samples will be sent to a laboratory

in South Dakota for FA analysis to take place. The data can be accessed by investigators and IRB staff.

Voluntariness:

Participation is voluntary. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled. You may discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. You have the right to say no. You may change your mind at any time and withdraw. You may choose not to answer specific questions or to stop participating at any time.

Alternatives to participation:

A child's participation in this research may not affect his access to community benefits or any other benefits.

Withdrawal from the research:

Participants can choose to be part of the study and withdraw from the study at any time of the study. Participants can be withdrawn from the study when they are found to be sick.

Consequence of Withdrawal: When participants withdraw from the study it will not affect them in any way. However the participant should note that some of the information that may have been obtained from him/her without identifiers, before withdrawal, may have been modified or used in analysis reports and publications

Costs/Compensation: Your participation in this research will not involve any additional cost to you. For your time/convenience we will compensate you with non-monetary token such as household utensil or soap or rice to show our appreciation for your participation.

Contacts: If you have any questions concerning this study contact:

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Michigan State University

East Lansing (madjepong2020@gmail.com/adjepong@msu.edu)

(0246410959)

Dr. Reginald Annan

Department of Biochemistry

KNUST (reggie@imft.org)

PMB (0201237169)

Dr. Jenifer Fenton

Michigan State University

East Lansing (imigjeni@msu.edu)

(5173533342)

Further, if you have any concern about the conduct of this study, your welfare or your rights as a research participant, you may contact:

The Office of the Chairman

Committee on Human Research and Publication Ethics

Kumasi

Tel: 03220 63248 or 020 5453785

CONSENT FORM

(Copy will be given to participant)

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information about the study, including that on procedures, risks and benefits, to enable the prospective participant make an informed decision to or not to participate.

DATE: _____ NAME: _____

Statement of person giving consent:

I have read the information on this study or have had it translated into a language I understand. I have also talked it over with the interviewer to my satisfaction.

I understand that my child's participation is voluntary (not compulsory).

I know enough about the purpose, methods, risks and benefits of the research study to decide for my child to take part in it.

I understand that my child may freely stop being part of this study at any time without having to explain.

NAME:_____

DATE: _____ SIGNATURE/THUMB PRINT: _____

Statement of person witnessing consent (Process for Non-Literate Participants):

I _____ (Name of Witness) certify that information given to

_____(Name of Participant), in the local language, is a true reflection of what I have read from the study Participant Information Leaflet, attached.

WITNESS' SIGNATURE (maintain if participant is non-literate): _____

GUARDIAN'S SIGNATURE (maintain if participant is under 18 years): _____

GUARDIAN'S NAME: _____

APPENDIX 3: Participant information collection sheet

A Participants characteristics

Child's code number

Survey Date (dd/mm/yyyy) …../…../…...

Village

Name of Child

Sex (0=Male, 1 Female)

Date of Birth (dd/mm/yyyy)

Name of mother/guardian

B Anthropometric measurements

Height of child in cm

Weight of child in kg

Head circumference in cm

C Biochemical measurements

Hemoglobin (g/dl)

Malaria test (RDT-1= Positive /2= negative)

Dried Blood spots taken (1=Yes, 2=No)

D **Other Information (To be answered by mother/caregiver)**

Did child ever breast feed? 1=Yes, 2=No

Is child still breastfeeding? 1=Yes, 2=No

When did child stop breastfeeding (Age in months)

Number of siblings

Has child been sick in the past two weeks (0=no 1=yes)

Common type of complementary food child is eating

Dietary sources of fats

1= agushie seeds, 2= agushie seed flour, 3=peanut, 4=coconut, 5= tiger nut, 6= cashew nut, 7= soybean, 8=palm kernel oil, 9= blue band margarine, 10= other brand of margarine 11=frytol, 12= other brand of vegetable oil 13=coconut oil, 14=palm oil 15=sheabutter 16=baobab seeds 17=neri/were were 18=dawadawa 19=others

APPENDIX 4: Policy component

IFPRI Discussion Paper…..

September 2017

Drivers of Micronutrient Policy Change in Ghana: An Application of the Kaleidoscope Model

Mary Adjepong, Jenifer Fenton
Suresh Chandra Babu

Director General Office

The International Food Policy Research Institute (IFPRI), established in 1975, provides evidence-based policy solutions to sustainably end hunger and malnutrition and reduce poverty. The Institute conducts research, communicates results, optimizes partnerships, and builds capacity to ensure sustainable food production, promote healthy food systems, improve markets and trade, transform agriculture, build resilience, and strengthen institutions and governance. Gender is considered in all of the Institute's work. IFPRI collaborates with partners around the world, including development implementers, public institutions, the private sector, and farmers' organizations, to ensure that local, national, regional, and global food policies are based on evidence.

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ABSTRACT

This paper reviews the policy processes that takes place in the micronutrients policies in Ghana. All the studies utilizes the kaleidoscope model of policy change in order to explore what influences micronutrient policy change in Ghana.

The paper starts with outlining the micronutrient policy process in Ghana with a major focus on iodine, iron and Vitamin A. Although, there have been a decline of Vitamin A deficiency and Iron deficiency in the population there are a number of people who still suffer adversities due to these deficiencies. In an effort to find effective tools for combating these deficiencies, there have been changes in the policies overtime. This paper analyzes the causes of policy change and the progression of micronutrient policies in Ghana.

These data assessed the 16 Kaleidoscope hypotheses about the dynamics driving policy change at each of the five crucial stages of the policy process; agenda setting, design, decision making, implementation and monitoring and reform.

Agenda setting: A strong empirical evidence of the need of a particular micronutrient in the population triggered the inception of most policies in Ghana. Among these include international conferences passing on the mandate to countries in need. Like other developing countries, Ghana's policy environment is crowded hence there was a need of strong and effective advocates who pushed the great mandates unto the policy agenda in Ghana. These empirical evidences weren't treated in isolation, it was usually backed by baseline survey in the population.

Design: Like other countries, the design for Ghana's micronutrient policies was based on what was being done by other countries. There were few policy modifications however, most of the designs were chosen because they were cost effective. Some of the designs that were chosen were also dependent on the severity of the condition. Either short term or long-term options were considered.

Decision making: Usually decision on policies are made by the relative powers on proponents and opposers as well as veto players. In this paper, there were few veto players but donors and some international agencies were major proponents of the policies. This is because of their support in funding the policy programs. In some cases, the MOH played a role.

Implementation: The main public agency responsible for implementing micronutrient policy in Ghana is the GHS. The fortification mandate was taken up by the food industries and various actors in the private sector.

Monitoring and evaluation and reform: In Ghana monitoring of these policies are minimal however there have been strong evaluation to know that these policies have huge and positive implications on the population. There have been changes in the policies due to new scientific evidence.

From the interviews conducted, Ghana's micronutrient policy is donor driven with minimal opposition from public agencies. The policies were usually designed from international design spill overs from other nations. International donor agencies faced little opposition

with the policy because of the funds they had to support the policies. The data suggests that, any nutrition policy needs the help of foreign advocates in order for it to appear on Ghana's policy agenda

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LIST OF ACRONYMS

ADRA	Adventist Development Relief Agency
CEPS	Customs Excise and Preventive Service
CRS	Catholic Relief Services
DCE	District Chief Executive
DANIDA	Danish International Development Agency
FDA	Food and Drugs Agency
FDB	Food and Drug Board
GES	Ghana Education Service
GHS	Ghana Health Service
GSS	Ghana Statistical Service
DHS	Demographic Health Survey
GOG	Government of Ghana
IDA	Iron Deficiency Anemia
IDD	Iodine Deficiency Disorders
IDRC	International Development Research Chairs
MDG	Millennium Development Goal
MICS	Multiple Indicator Cluster Survey
MOFA	Ministry of Food and Agriculture
MOH	Ministry of Health
MOWAC	Ministry of Women and Children's Affairs
NGO	Non-governmental Organization
RCC	Regional Coordinating Council

UIS	Universal iodization of salt
UNICEF	United Nations Children's Fund
USAID	United States Agency for International Development
WFP	World Food Program
WHO	World Health Organization
WIADD	Women in Agriculture Development Directorate
HIV	Human Immuno-deficiency virus

INTRODUCTION

There is a variation of micronutrient policies across countries and over time and there are a number of drivers of policy change in any given setting.

The aim of this study is to provide insights on national policymaking processes and identify key drivers of micronutrient policy change. This paper focuses on Ghana's micronutrient policies and explores how and why the policies have changed over time. The case study also aims to address the following two objectives:

1. *Map nutrition policy institutions and policy processes*. This aim include identifying key stakeholders and institutions that drive nutrition policy decisions, how do nutrition policy institutions and stakeholders interact, how has the institutional architecture for nutrition policy changed over time? How has that institutional framework affected policy change over time? How has that institutional framework affected policy outcomes?
2. *Assess key drivers of change for specific micronutrient policies.* There is not a distinct micronutrient policy in Ghana, however, there are various programs for key micronutrients in Ghana. Some of these key programs include Vitamin A supplementation and fortification, iron supplementation and fortification and iodine fortification. The aims of this paper include finding how each of these intervention get into the policy agenda, who championed the policies? Who opposed them? Who financed? How they have implemented, monitored and modified over time?

The framework for understanding policy processes was through the development of a model of policy change. The kaleidoscope model offers testable hypothesis covering the

five key stages of the policy cycle; agenda setting, design, adoption, implementation and evaluation and reform [178]

Section 2 describes Ghana's micronutrient policy institutions and processes as well as major changes over time. The methods for the Kaleidoscope model are outlined and described in section 3. The paper proceeds to test key hypothesis about drivers of micronutrient policies: iodine, iron and vitamin A. Section 5 sums up the major conclusions emerging from Ghana's micronutrient policy/program review.

OVERVIEW OF MICRONUTRIENT POLICIES AND POLICY PROCESSES IN GHANA

2.1 Major micronutrient deficiencies

There are three major micronutrients that are of public health interest worldwide. Iron deficiency affects over two billion people worldwide. Iron deficiency has diverse effects on different sections of the population. For preschoolers and school aged children, it affects their cognitive performance, behavior and physical development. During pregnancy, iron deficiency anemia increases infant mortality and perinatal risks for mothers and neonates. For all ages, iron affects the immune status of individuals [127].Similarly, iodine deficiency affects nearly 2 billion of the world's population leading to fetal birth defects, goiter as well as serious cognitive dysfunction including cretinism [3] . Furthermore, Vitamin A deficiency affects an estimated million, leads to night blindness and increase maternal mortality and increased risk from severe infections.

There have been numerous efforts to address micronutrient deficiencies by both national and international advocates. Many countries adopted the iodine fortification of salt mandate in 1940s while most countries in the developing world adopted it in the 1990s with the goal of eliminating iodine deficiencies by 2000 [179].

The international nutrition and public health communities have made a number of effort to address micronutrient deficiency and they first focused on iodine. The efficacy and affordability of iodized salt in 1920s caused a number of countries to adopt fortified iodized salt. There was huge acceleration of iodine fortification in developing countries in 1990 [180] and this was as a result of the International Summit on children ,and this produced

a global agreement on universal salt iodization with the goal of eliminating iodine deficiency by 2000 [179].

The role of vitamin A in immunity led to the promotion of bi-annual supplements of vitamin A mega doses, which the liver can store. Comorbidities that are common in children decreases with vitamin A supplementation hence children were supplemented with mega doses of vitamin A. This is important in child survival [181].

The human body cannot store iron easily hence bi-annual mega doses are not feasible hence effective prevention of iron deficiency requires improved diet or regular supplementation. They are costly and can be complex, hence preventing iron deficiency in populations have been difficult. [182].

In Ghana, these micronutrients have been of public health interest as well. Multiple Indicator Cluster Survey (MICS) indicates that 35% of household salt in Ghana are adequately iodized while 22% of the salts did not have iodine at all. Further, the annual mortality rate per 100,000 people from iodine deficiency in Ghana has increased by 64% since 1990, an average increase of 2.8% a year [183]. Iron deficiency in under-five year old is 65.7% in 2014. In women of child bearing age, there have been little improvement in IDA. Thus 45% in 2003 to 42% in 2014 [20] (Table 1).

Table 1. Trends in major micronutrient deficiencies in Ghana

	Children					Women				
	1993	1997	2003	2008	2014	1997	1998	2003	2008	2014
Vitamin A (<0.7umol/l		75.8%					18.1%			
Iron			76%	82.8	78.6%			45%	58.7%	42.4%
Iodine					65.7%	23.8%				

Sources: GSS, GHS, and ICF Macro 2009; GSS, GHS, and ICF International 2015

2.2 Micronutrient policies

Since independence, micronutrient policy and interventions have focused mainly on three nutrients; iron, iodine and vitamin A. Some other programs have also focused on micronutrient and complementary feeding. Over the years, stunting, which is an indicator of chronic malnutrition has decreased but there are still disparities in Northern Ghana[20]. Ghana has a Nutrition Policy that was launched in 2016, however, there is no separate document for micronutrient policy but the National nutrition policy has sections that address micronutrients [184]. Policy measures in Ghana focuses on iron/folic acid supplementation as well as food based approaches-such as fortification, promotion of micronutrient and infant feeding. There is however a number of micronutrient programs that are run in the country. The target groups for the nutrition policy in Ghana include children, pregnant and lactating women and infants under 5 years of age[184]. Table 2 summarizes current policy and programs while table 3 describes the chronology of micronutrient policies in Ghana

Table 2: Snapshot of micronutrient policies/programs in Ghana, 2017 (Current and proposed)

Micronutrients	Targets	Which delivery mechanism		
		Supplements	Fortification	Bio-fortifcation
Iodine	General Public		Salt fortification mandate since 1987	
Iron/folate	Children, pregnant women and lactating mothers	Provided through ante-natal care(pregnant women)		
Vitamin A	Children 6-59 month	Bi-annual doses of the supplement (1996) Expanded Programme of Immunization (EPI) and National Child Health Days		
	Post-partum women			
Zinc	Children with diarrhoea			

Source: National Nutrition Policy,

Table 3. Summary Chronology of key Micronutrient Policies and programs in Ghana

	International Events	Ghana Policy environment (Nutrition policies and programs)	Iodine	Vitamin A	Iron
1950s		Food Demonstration and Education			
1960s		Identification and Attitudes and behavior change needs Food demonstration and education			
1970s		Weaning foods Identification of attitudes & behavior change needs Food demonstration and education			
1980s		1987-1990:Addressing micronutrient requirements	Salt fortification mandate 1987		
1990s	1990 UN World Summit for Children 1992 International Conference on Nutrition 1994 UNICEF-WHO endorse universal salt iodization (USI)	Micronutrients, iodine, Vitamin A, iron and exclusive micronutrient		1992 VAST study 1993 VAS trial 1997 MOH survey	1996 UNICEF survey
2000s	2000 OAU Abuja summit Rolling Back Malaria 2002 UN General Assembly on Children set foal of IDD elimination by 2005 2006 Pemba iron study documents danger of iron supplementation in high malaria zones (Sazawal et al., 2006)	2000-2008 Consolidation of strategies to address micronutrient deficiencies			
2010s		The National Nutrition Policy was launched			

Source: Field interviews, Hagblade et al., 2015, Ghartey et al., 2010

2.3 Overview of micronutrient policies and processes in Ghana

There are three groups of actors which interact to design implement micronutrient policies/programs in Ghana. The individuals and institutions in these groups have distinct roles. They have distinct, differing roles, responsibilities and resources. They also differ in their priorities leading to complexities of interactions among them. These interactions lead to policy processes in Ghana.

Government actors

The government actors include the Nutrition Department of Ministry of health which is responsible for implementing health and some nutrition policies and enforce regulations. Children's Department of the Ministry of Women and Children's Affair (MOWAC) is responsible for child right promotion, child protection, and early childhood care development (child health including nutrition through; education sensitization, awareness creation and advocacy; child welfare, registration etc.). Also, the Women in Agriculture Development Directorate (WIADD) Ministry of Food and Agriculture (MOFA) responsible for promoting food utilization aspects of MOFA through education, sensitization, awareness creation and advocacy. The Ghana Education Service (GES) of the Ministry of Education is responsible for school feeding and health education among others. The Department of Community Development of the Ministry of Local Government, Rural Development and Environment is responsible for the promotion of rural development issues, including welfare and development of vulnerable groups in the rural and urban areas with focus on women and children. The Department of Social Welfare of the Ministry

of Manpower Development, Youth and Employment charged with the promotion of the welfare of children the youth and other vulnerable groups. [185]

Although Ghana doesn't have a separate micronutrient policy, it has a nutrition policy which was launched in 2013. All policies including the Nutrition policy are passed in this wise:

In Ghana, the policy direction is through the ministries and departments which is usually headed by a Minister that is nominated by the president and approved by parliament. With the help of decentralized departments and agencies, there is coordination and monitoring roles at the district and regional levels in the implementation of policies. This is headed by the Regional Coordination Councils (RCCs) with each regional level headed by regional minister. At the district levels, the district coordinating directors and the District Chief executive coordinates the implementation of the policies through decentralized departments. The DCEs are nominated by the president and approved by the Metropolitan, Municipal and District Assemblies under the GOG:Local Government Act 462. [185]

In the formulation of policies, the government is guided by international agreements as well as allegiance to international goals such as the Millennium Development goals (MDGs). Some policies are also formulated based on the country's needs in response to its development agenda.

Donors

Like other African countries, in Ghana, donors play a vibrant role in priority setting, financing and implementing micronutrient policies and programs [182]. A detailed account of the role of donors in micronutrient policies in Ghana is outlined in table 3.

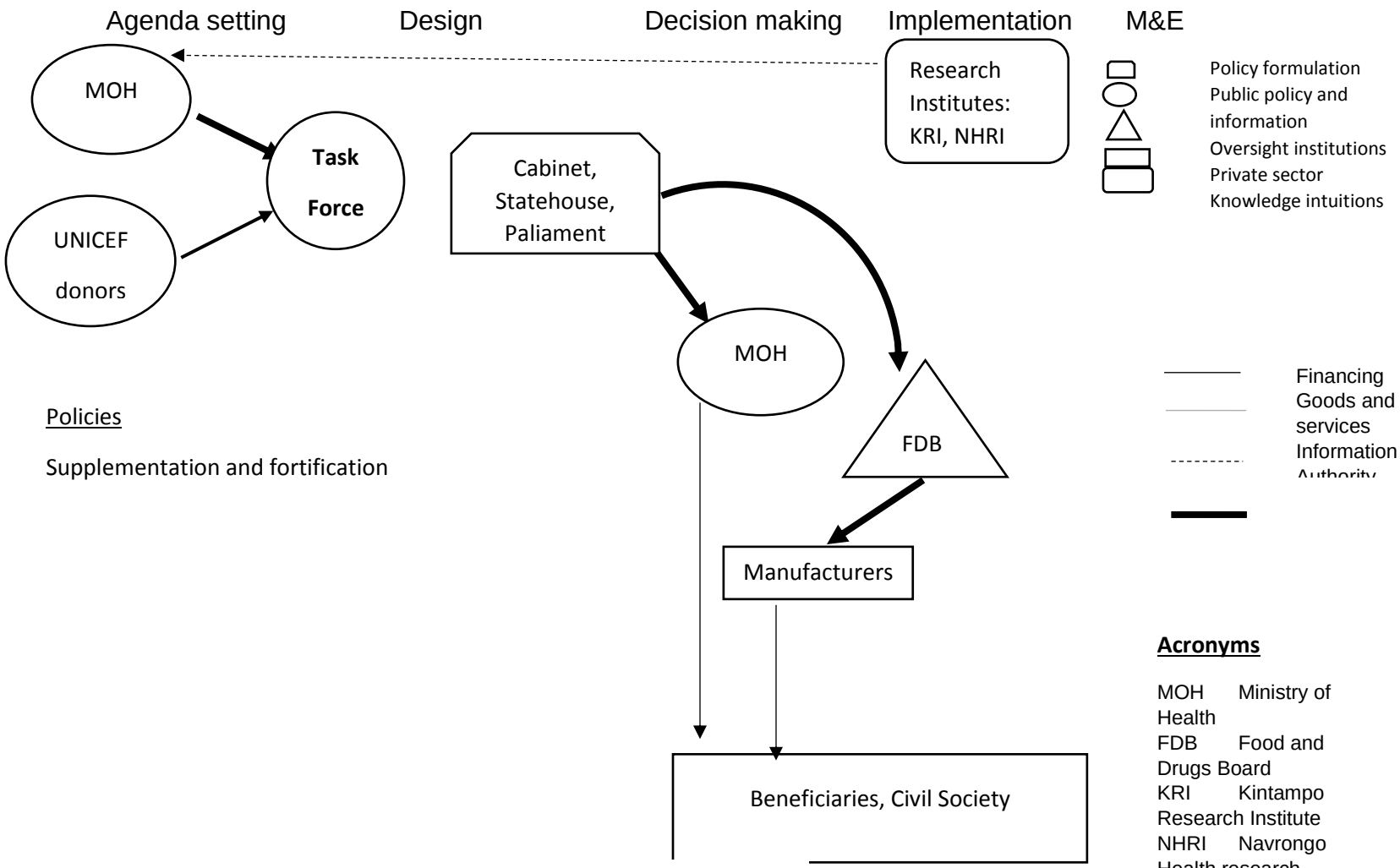
The leader amongst the donors in Ghana was UNICEF through the UN Summit for children in 1990. The UN general assembly that is devoted to children welfare and nutrition have been involved in a series of global conferences on nutrition and micronutrients and this has led to an increase in donor funding for a series of micronutrient. Systematically, the first micronutrient that gained attention was iodine [182].

The Nutrition Department, WIADD and Department of Community Development also receives support for World Food Program (WFP) and United States Agency for International Development (USAID). The resources from these donors are for implementation of policies and programs. There is the Multi Donor Budget Support (MDBS) that comprises of donors such as EU, USAID, JICA, DANIDA, Netherlands, World Bank and the UN called the Health Sector Group. The group hold meetings with MOH, universities and research institutions to review implementations of health intervention in the sector as well as discussing strategies and interventions to address challenges in the Health sectors[185].

Private sector

The private sector plays a crucial role in micronutrient policy design and in Ghana these include the non-governmental organizations private cooperation and industries. Although NGOs play a significant role in implementing nutrition interventions no mapping has been

done on NGO actors in nutrition. They include Christian Relief Service (CRS), World Vision and Adventist Development and Relief Agency (ADRA). A number of food industries are involved in micronutrient policies due to fortification strategies. Among them include Uniliever, Flour milling industries as well as other vegetable oil and condiment producing industries. Salt producing industries have been targeted by the policy makers because of the universal iodization of salt program [185].



Source: Field interviews

Figure 1: Ghana's Micronutrient policy/Programs processes

Table 4: Some Institutional roles and responsibilities for Nutrition Programs in Ghana*

Institution	Specific sectors	Roles and Responsibilities
Ministry of Health	Ghana Health Service Nutrition Division	Provide health services Implement health and some nutrition policies Enforce regulations
Ministry of Agriculture	Women in Agriculture Development Directorate	Involved in the implementation of some of the nutrition programs such as universal salt iodation and complementary food
Ministry of Local Government and Rural development	Department of Community Development	Promotion of rural development issues, including the welfare and development of vulnerable groups (women and children)
Education/Research Institution	Crop Research Institute and Food Research Institute	Development of improved variety of crops
Food and Drugs Board		Tests food and drugs for compliance with standards
Department of Social Welfare		Promotion of welfare of children, the youth and vulnerable groups
Ministry of Education	Ghana Education Service	School feeding and health education programmes in schools
Ministry of Women and Children Affairs	Children's department	Child right promotion, child protection and early childhood care and development

*Ghana has does not have a separate micronutrient policy documents but these institutions have roles in Nutrition policy in Ghana

METHODS

3.1 The Kaleidoscope Model

There have been numerous studies to explore what influences nutrition and agricultural policy [178]. There is a huge gap that exist in the literature on policy processes in the developing country context especially when nutrition policies are considered.

Kaleidoscope model identifies key hypotheses about factors driving policy change. The model aims at answering the causes of policy change based on the influences on and the action of national policy makers. The model also aims to identify key variables that define the necessary and sufficient conditions in each of the 5 stages in the policy process. Kaleidoscope model has 6 key hypothesis and they are presented in the table below. The model derives key variables that help in policy change combining insights from the policy process and political economy literature. Qualitative comparative analysis is also utilized with enables the identification of sufficient conditions for causal patterns across case studies. As part of the process variables are defined this helps in have too many variables and too few cases. This definition also help to identify the ‘key determinants of policy change’.[178]

The framework presented in figure 2 focuses on five key elements of the policy cycle: agenda setting, design, adoption, implementation, and evaluation and reform. Like the kaleidoscope, focusing on a particular stage of the policy process reveal different constellation of key variables. Also some factor tend to have large roles in the driving of policy change[182]

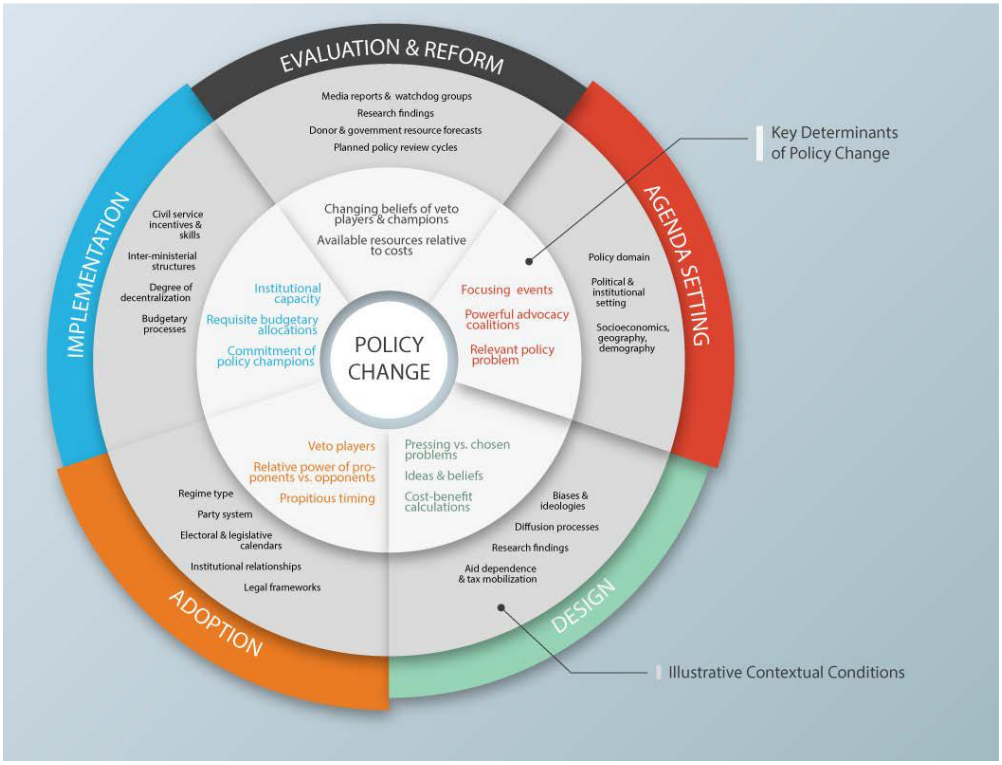


Figure 2: The Kaleidoscope Model of Food Security Change

Table 5. Kaleidoscope Model hypothesis: Key Variables Affecting Policy Change

Policy stages
Key variables affecting policy change
1. Agenda setting
1.1 Powerful advocates
1.2 Focusing event
1.3 Recognized, relevant problem
2. Design
2.1 Pressing vrs chosen problem
2.2 Ideas and beliefs
2.3 Cost-benefit calculations
2.4 International design spillovers
3. Adoption
3.1 Propitious timing
3.2 Veto players
3.3 Relative power:proponents vs opponents
4. Implementation
4.1 Institutional capacity
4.2 Requisite budgetry allocations
4.3 Commitment of policy champoins
5. Evaluation, Reform
5.1 Changing conditions
5.2 Changing information or beliefs
5.3 Resource availability relative to cost

Source: Resnick et al., (2014)

3.2 Data

Information from semi-structured interviews with key stakeholders and data from published documentation were used to test the Kaleidoscope hypothesis. The collection of data involved the use of questionnaires with a set of pre-determined open questions, questions that prompted discussion giving an opportunity for the exploration of further questions or ideas. Additional data and interviews were initiated due to the outcomes of initial interviews. The interview was conducted by student from Michigan State University. The background of this document includes policy documents, published data, gray literature and published research. Key stakeholders were interviewed using semi-structured interviews, where each of the participants provided critical insights into the policy process and interaction among various stakeholders. There was stakeholder mapping which provided a list of key informants. The interview guide is shown in appendix

1. This document was used to guide the interview process. Most of the interviews included specific questions about micronutrients of interest.

Semi-structured interviews were conducted on a number of policy stakeholders in Ghana in July and August 2016. Appendix 2 provides a list of the persons interviewed

3.3 Tools for testing of the model's hypothesis

a) Policy Chronology

b) Stakeholder Mapping

- Stakeholder inventory
- Policy system schematic
- Circle of influence

c) Hypothesis testing template

The detailed sequence of policy decisions and the results in its implementations and actions are outlined in the policy Chronology.

Key interest groups involved in any specific policy formulation or implementation were identified. This report also summarizes the role, resources, and the position of stakeholder inventory. It also shows how various stakeholders interact to produce policy outcomes.

Hypothesis testing focuses on the tabular representation of the 16 specific Kaleidoscope hypothesis about factors that drives policy change. Using the sum total of available documentary and oral evidence reviewed, the research team assigns an initial qualitative score in the hypothesis table under each of the 16 hypothesis. A "+" indicates a significant, positive impact of that particular variable, while a "-"indicates a significant negative

impact. A blank cell indicates no impact of that particular variable on the policy outcome. [178]

3.4 Validation and counterfactuals

The Kaleidoscope research protocol calls for verification of each policy hypothesis from multiple respondents. This is because qualitative interview data recorded by the research team is composed of biases of individual respondents because the different respondents which of whom may have different information, perspectives, objectives and stakes in the policy outcomes. Written documents in both gray and published literature provided additional testimony about the factors that affect policy change.

Counterfactuals are rare in social science research especially in interactive processes involving multiple stakeholders. The Kaleidoscope Model addresses this problem in two ways. Firstly, multiple restatement of similar, individual policy events come close to providing repeated testing within the same framework conditions.

DRIVERS OF POLICY CHANGE A FORMAL TEST OF THE KALEIDOSCOPE HYPOTHESIS

4.1 Iodine

4.1.1 Policy Chronology

Ghana has an iodine policy which is poorly implemented due to ineffective regulation. After the Otawa and the Cameroon conference, discussion about measures to rid of

iodine deficiency in Ghana began in earnest. This was followed by an international development research chairs (IDRC) survey to know the prevalence of IDD in Northern Ghana. The study included education of universal salt iodization as well as the distribution of iodine capsules to individuals who are severely affected. Iodine policy began its implementation in 1996 and continues till date. This involved the fortification of salt and the target was the entire population[185].

The programme focuses on the promotion of iodized salt consumption to eliminate iodine deficiency disorders (IDD) which is prevalent in Ghana. This is achieved through the enforcement of the Public Health Amendment law 2012 Act 851. This states that salts for human and animal consumption must be iodized[185].

Apart from proceeds from international conferences, a National Workshop on Iodine Deficiency disorder on IDD held in 1994 was an immense contributor to the policy formulation. .Other programs that has ensured the policy's continuity include workshops that has assessed the biological impact on salt iodization program in Ghana (2007). Market and research study on the iodization of salt was conducted to document the knowledge, attitudes and practices of consumers, traders and producers in Ghana.[185].

4.1.2 Stakeholder mapping

A number of stakeholders drive iodine policies in Ghana. Ghana Health Service and Food and Drugs board designed the policy intervention. GHS, ministry of trade and industry, food and drugs board implements this policy, with ministry of local government and FDB providing institutional oversights.

Academic and research institutions such as University of Ghana and Navrongo Research Institute make up the major group of stakeholders. The government formulates and enforces the iodine fortification mandate, The Food and drugs authority designs the policy.

The funding sources for this policy include the health ministry, United Nations Children's fund (UNICEF) and the World food programme (WFP). Other private sectors involved include National Salt producers Association of Ghana, a private sector contributor.

4.1.3 *Hypothesis testing*

Agenda setting: Universal iodization of salt got to the policy agenda with inputs from WHO and UNICEF. This was after the Otawa and Cameroon Conference in 1987. UNICEF was involved in the policy process from the very beginning. Apart from UNICEF, WHO and USAID who are the major international advocates some academic institutions were the domestic advocates. Due to probably illiteracy some herbal practitioners opposed it. UIS was considered a priority issue because a survey conducted by IDRC showed that IDD symptoms are prevalent. Moreover, it was move from the international community.

Design: The policy intervention was designed by the Ghana Health service and the Food and Drugs board. Fortification and supplementation options were considered but fortification was chosen because supplementation was short term, but long term approaches was needed because of the severity of the problem. Also universal salt iodization is a sustainable, long term and a globally acceptable design. The policy targeted the whole population. UNICEF played a role on capacity building as well as

communication of the policy measures to the population. The annual cost of the program is huge and it is financed by the Canadian government, GHS, WFP and UNICEF. This design was chosen because it was cost effective. This is a pressing problem and the iodine rich foods are expensive.

Adoption: The final decision was made by the MOH, GHS and the cabinet. Some clinicians opposed it, but was still favored because it was a worldwide intervention and the timing was good.

Implementation: Ghana health service, ministry of trade and industry and food and drugs board are involved in the implementation of the policy. A number of regulatory and legislative changes took place in the implementation of this policy. Other implementing bodies include the nutrition and reproductive and child health of the Ghana health service. School health and education programme of the Ghana education service was involved. The women and Children's desk of the Ministry of women and Children Affairs also play roles in its implementation. Other agencies such as ministry of trade and industry, security agency, (CEPS and Ghana Police) and the Environmental Health Department of the Local government. However, the policy did not require the setting up of new institutions and there have been no changes since its introduction, there are no separate cost for implementation. Ghana produces salt but the iodine is imported from India. Food and Drugs board is responsible for the monitoring the impact of this policy.

Monitoring: Food and drugs board monitors the implementation of this policy. The monitoring of this policy is not at its peak because of the varying levels of the iodized salt in the supply chain. The amount of iodine to be added to the salt should be monitored taking into consideration the amount of iodine which will vaporize since iodine is volatile. There are losses of iodine in the salt upon transportation and handling before they get to the market. Hence in the market, there should be random checks to confirm that the salt that is being sold to consumers have the right amount of iodine. Additionally, some salt are sold in the open market which means that they are open to the atmospheric conditions that can decrease iodine content. Monitoring can include proper packaging of the salt so as to preserve the amount of iodine

Though it has a number of flaws in its implementation, this policy has a number of advantages, among them include the fact that it is population sensitive however, it does not adequately cover the food industries. In that food industries aren't monitored on their use of iodized salt.

There have been set-backs with implementing this policy and it can be overcomed by strict monitoring of the food industries. Government should invest in the salt industry because Ghana may have enough salt to feed the whole West Africa.

UIS in other countries like Nigeria and Burkina Faso work well than in Ghana. This is because Ghanaian people do not cautiously purchase iodized salt and this could be a result of little sensitization. Additionally, Ghana produces local salt, and there are un-iodized salt in the market for purchasing and these are cheaper than the iodized salt. Most consumers who have not been informed would prefer the un-iodized salt

The FDA is responsible for the monitoring of the efficacy of the program while UNICEF and USAID monitors the impact of this policy. The implication of this policy include the improvement of some important biomarkers, which may be leading to IDD in various age groups.

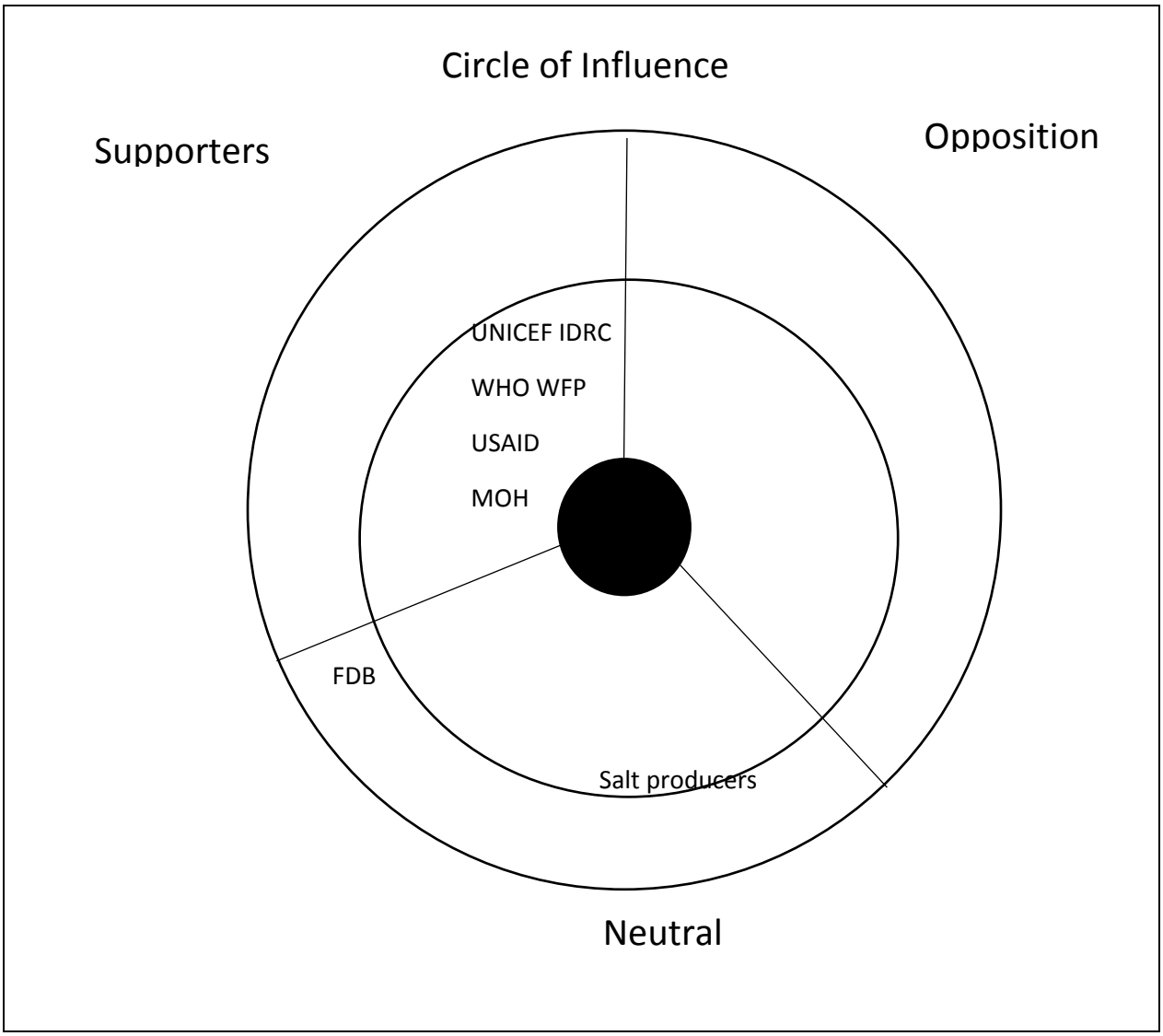
Some gaps have been identified and this have a negative effect among these include the use of other condiments that may inhibit other nutrient absorption. Food industries should be monitored. Also, surveys to know the quantities of iodine in salt should be monitored. Additionally, supply chain of this policy should be monitored to ensure that the consumer gets the right amounts of iodine.

Table 6: Iodine fortification policy hypothesis testing

Policy stages	
Kaleidoscope hypothesis	Policy action: Iodine fortification of salt
1. Agenda setting	
1.1 Powerful advocates	+
1.2 Focusing event	
1.3 Recognized, relevant problem	+
2. Design	**+**
2.1 Pressing vrs chosen problem	
2.2 Ideas and beliefs	+
2.3 Cost-benefit calculations	+
2.4 International design spillovers	+
3. Adoption	**+**
3.1 Propitious timing	
3.2 Veto players	
3.3 Relative power: proponents vs opponents	
4. Implementation	
4.1 Institutional capacity	++
4.2 Requisite budgetary allocations	+
4.3 Commitment of policy champions	
5. Evaluation, Reform	
5.1 Changing conditions	
5.2 Changing information or beliefs	
5.3 Resource availability relative to cost	+

Legend
+ Significant positive impact of this variable on policy outcomes
-Significant negative impact of this variable on policy process

Source : Field interviews



Source: Field interviews

Figure 3: Iodine Fortification Circle of Influence

4.2 Vitamin A

4.2.1 Policy Chronology

The period of 1990 -2000 began a number of great waves of micronutrient policies and among these is the Vitamin A policy/program. Although government's involvement in policy advocacy was very little through donor support, a number of international conferences were organized to raise the awareness of micronutrient deficiency in the

country. Among these conferences included World Summit for Children, 1990, the International conference on Nutrition (ICN) in 1992, the International Conference on Population development (1994), the World Summit for Social Development (1995) and the World Food Summit (1996) [182].

The global concern for Vitamin A deficiency led to the 1992 VAST study by the Navrongo Research Centre in the Kasena-Nankana District (Upper East Region). The survey indicated that, there are high levels of Vitamin A deficiency among children, pregnant women and lactating mothers. This led to the development of Vitamin A framework by the Multi-Sectoral Central Committee. The framework has four components which includes a) food supplementation b) conducting a survey in the south to know the prevalence of VAD, c) using Vitamin A for the management of measles d) food based approaches to increasing vitamin A intake. [185]

The Vitamin A supplementation project was supported by the government of Ghana, Rotary club, UNICEF and USAID. During this period, a number of surveys and studies were conducted to for assess Vitamin A deficiency. A ministry of health (MOH) survey conducted reported that, VAD affected 72% of the country's under five year olds. It is also reported that 1 out of 3 of all cases of child mortality between 6-59 months. Vitamin A supplementation trial was done in 1993 and this revealed that VAD accounted for 12% of all clinic attendance of all clinic attendance of pre-school children and 38% of all hospital admissions. Additionally, the MOH survey also revealed that, lactating mothers have low breastmilk retinol levels[185].

The effect of these conferences and these surveys led the government of Ghana and donors to support and address the problem. A policy on Vitamin A supplementation was

adopted in 1996 with the aim of administering Vitamin A to children 6-59 months old biannually. The coverage was 100% from 1996-2000 but fell to 80% in 2005.

4.2.2 Stakeholder mapping

There are numerous agencies that were involved in the formulation of Vitamin A policy. The Ghana health service designed and monitors the vitamin A policy. WHO, UNICEF and USAID have been strong advocates from the beginning.

Local organization such as Kintampo and Navrongo research institutions and NGOs such as Christian Health association of Ghana have been instrumental in the policy formulation.

Since its inception, the funding sources for this policy have been the Government of Canada. UNICEF have been active in providing of the supplements. The government of Ghana is set to take over the funding from 2017.

4.2.3 Hypothesis testing

Agenda setting: A number of domestic and international advocates were involved in the Vitamin A supplementation and fortification policies and they include WHO, UNICEF, USAID (international advocates), and Kintampo and Navrongo research stations. After the baseline survey was conducted based on WHO recommendations, the committee presented its report to the ministry of health. This led to the consideration of Vitamin A policy in Ghana. The policy agenda was opposed from some few element from medical stations but was still considered an important issue because there was evidence based research knowing the role of Vitamin A on the survival of children. The policy intervention

was designed by the Ghana Health Service, the implementing body of the ministry of health. Fortification and supplementation was considered but supplementation was considered because it is a short term approach. Apart from the above mentioned institutions, Non-governmental organizations such as Christian Health association of Ghana was also involved as partners. The vitamin A policy targeted children below 5 years, nursing mothers, however, nursing mother do not take the supplements anymore because evidence shows that, supplements for nursing mothers were not useful. Specifically, Vitamin A supplementation in postpartum women is not recommended for the prevention of maternal and infant morbidity and mortality[186]

Design: UNICEF provided the supplements with sponsorship from the Canadian government. With a huge annual cost, the Canadian government funds the vitamin A policy. The supplements are known to be cost effective. Vitamin A issues were considered a priority issue because it was a pressing problem considering the effects of Vitamin A deficiency on infants and children. Although dietary approaches were considered, supplementation was considered because vitamin A rich foods are expensive and also dietary patterns may not change in a short time.

Adoption: The final decision to implement this policy was made by the Ministry of health, UNICEF and WHO. Some scientists and researchers lobbied in favor whereas some clinicians opposed it. In this policy process there were no known veto players at work.

Implementation: The Ghana health service are the sole implementers of the Vitamin A supplementation policy, however there were no regulatory and legislative changes that took place to implement the policy decision. Due to this the only institutional oversight was from the Ghana health service. The policy did not require the setting up of new institutions. The policy has had changes since its inception. Currently, lactating mothers and mothers after delivery do not take the supplements because evidence shows that it is not as useful. The policy is monitored by UNICEF, WHO, GHS through the demographic health surveys and the Multiple Indicator survey (MICS)

This policy has a number of advantages which includes the elimination of night blindness and other clinical and subclinical deficiency symptoms from the population. The coverage in children 12-59 months is low (22%) but the coverage is higher in children below 60 months because these children are immunized till 12 months, hence they are able to attend clinics. To increase the coverage, the supplements can be delivered at community levels through the Community Health Post (CHPs). Although they have been stated as expensive and have other pitfalls, Vitamin A fortification and the introduction of Vitamin A rich foods (food based approaches) can be pursued by the government as other alternatives to Vitamin A supplementation. These approaches may have a broader coverage. Vitamin A supplementation in other countries such as Zambia works better because its distribution in child health is exceptional.

The government engages the help of UNICEF, WHO, GHS and researchers in academia to get feedback on Vitamin A policies, and the impact of the policy is monitored by the Ghana health service. There have been a number of researches on the bearing of this policy. Changing beliefs have led to change in the policy.

Implications

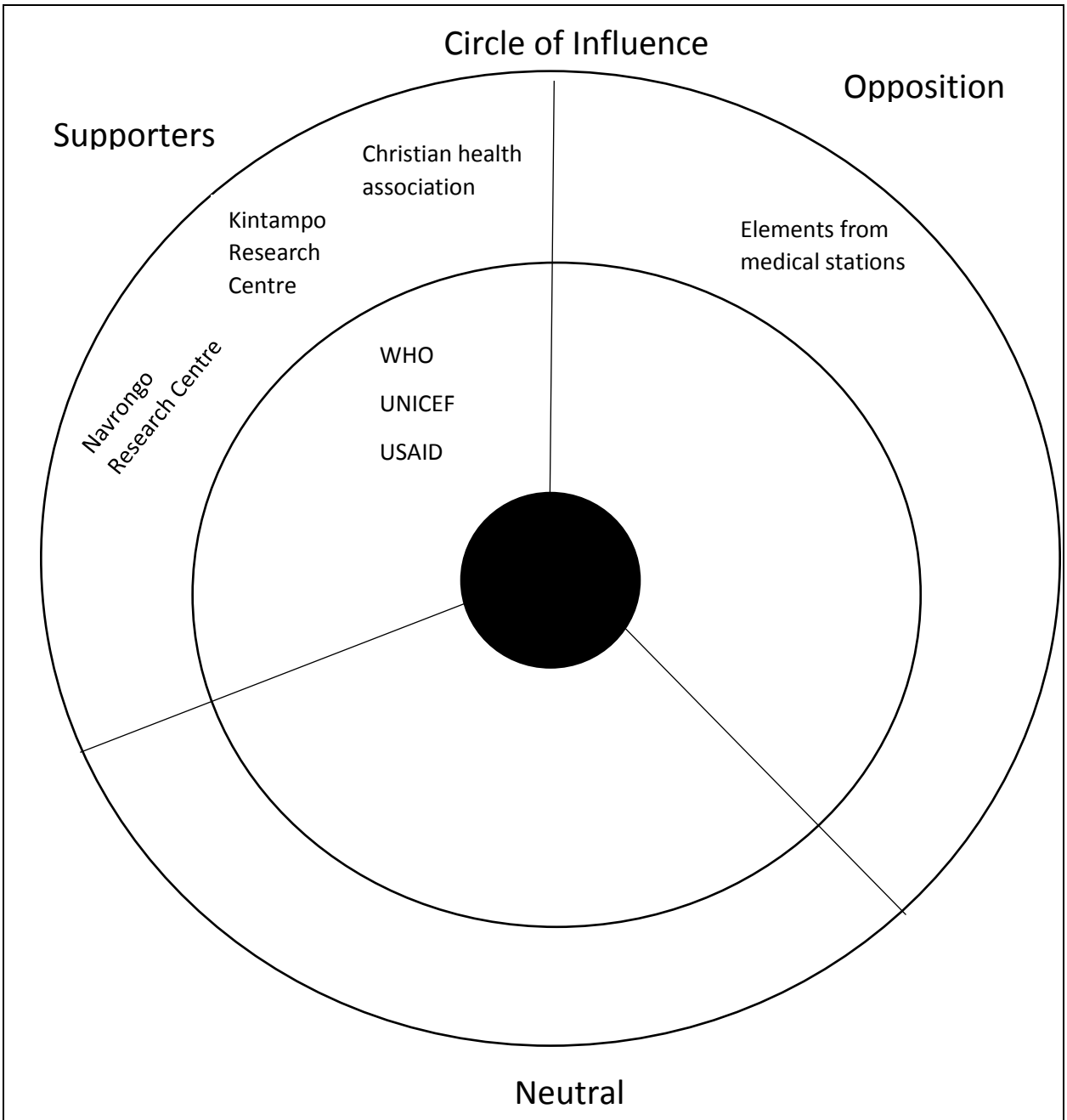
This policy has been able to eliminate deficiency from the policy, however, the main gap is that data in the Vitamin A policy is old hence there needs to be improved monitoring and research. The program should be assessed again for current information.

Table 7: Vitamin A Supplementation policy hypothesis testing

Policy stages Kaleidoscope hypothesis	Policy action: Vitamin A Supplementation
1. Agenda setting	
1.1 Powerful advocates	+
1.2 Focusing event	
1.3 Recognized, relevant problem	+
2. Design	**+**
2.1 Pressing vrs chosen problem	
2.2 Ideas and beliefs	+
2.3 Cost-benefit calculations	+
2.4 International design spillovers	+
3. Adoption	
3.1 Propitious timing	
3.2 Veto players	
3.3 Relative power: proponents vs opponents	+
4. Implementation	
4.1 Institutional capacity	++
4.2 Requisite budgetary allocations	+
4.3 Commitment of policy champions	
5. Evaluation, Reform	
5.1 Changing conditions	
5.2 Changing information or beliefs	+
5.3 Resource availability relative to cost	+

Legend
+ Significant positive impact of this variable on policy outcomes
-Significant negative impact of this variable on policy process

Source: Field interviews



Source: Field interviews

Figure 4: Vitamin A Supplementation Circle of Influence

4.3 Iron

4.3.1 Policy Chronology

A survey conducted by UNICEF to address anemia revealed a high prevalence of iron deficiency anemia among children and pregnant women in Ghana. The results of the survey showed that, preschool children had anemia levels of 84%, school aged children had 71% and pregnant women reported 69%. Through UNICEF, the USAID funded Micronutrient Operational Strategies and Technology (MOST) project was initiated to support the anemia control. The target group was children, pregnant women and lactating mothers. The iron supplements were given to school aged children in addition to de-worming treatments however, the iron supplements which were introduced in conjunction with a new malaria drug has been halted because of the negative side effect of the malaria drugs.[185]

4.3.2 Stakeholder mapping

There have been numerous stakeholders for iron deficiency control program in Ghana. They include the USAID funded Micronutrient Operational Strategies and Technology (MOST) initiated the program. Ministry of health another important stakeholder designed the policy intervention and delivers supplements to all the target groups. UNICEF and USAID were responsible for baseline survey to ascertain the levels of IDA in the population. Summing up, USAID, UNICEF and GHS are the strongest advocates for this policy.

Fortification of food and consumables with iron is mainly by private sector whose main responsibility is to implement and finance the fortification programs. The government of

Ghana is responsible for monitoring and evaluation of the firms as well as educate consumers and enforce the fortification mandate.

The government of Ghana, UNICEF and USAID funds the policy intervention.

4.3.3 Hypothesis testing

Agenda setting: The world health organization policy states that, when a population has a prevalence of anemia greater than 40%, there is a need for iron supplementation and fortification programs. WHO, UNICEF, GHS were the strongest advocates for the iron supplementation policy/program. Discussions of the iron supplementation policy began in 1996 and the GHS were involved in the discussion from the very beginning from 1996. The domestic advocates who supported this policy intervention is GHS, whereas USAID, WHO and UNICEF were the international advocates. There were no known opposers to this policy/program. Iron supplementation policy/program was considered important because of its roles in child survival and maternal mortality.

Design: The policy was designed by ministry of health, Ghana health Service. A number of options were considered and this include, dietary intervention dietary diversification and the fortification of flour with iron, folic acid, vitamins B_2, B_3, B_1, B_6 and zinc. Supplementation was chosen because the prevalence of anemia is high and supplementation was short term. Short term approaches are considered in high prevalence conditions because the change in d dietary pattern cannot be achieved in a short time. Pregnant women and women of child bearing age and children. GHS is the lead institution and implementing body which monitors, evaluate and conduct baseline

reports. The annual cost is unknown but it is financed by the Government of Ghana. The methods were chosen because it is a pressing problem. Supplementation was chosen based on scientific evidence of the prevalence of anemia and the operational feasibility of the supplements

Adoption: The final decision of this policy was made by the ministry of health who also lobbied in favor. There were no opposers and no veto players existed. The timing was good because it was on the global front and most developing countries were considering this policy.

Implementation: Ghana health service, the implementing body of ministry of health implements the iron supplementation policy/program. The institution overhead is MOH and GHS. However, the policy did not require the setting up of new institutions. The policy has seen some changes in the fact that, children are no longer given the supplements because there is evidence of increased parasitemia in children supplemented with iron. The cost of implementing is huge and the supplements are imported.

Monitoring and Evaluation: The impact of this policy was monitored by the GHS. The policy/program has helped in the improvement of anemia and maternal health hence decreasing maternal mortality. Compliance problems and coverage issues can be dealt with and this will improve the current program. Fortification and dietary diversification are other alternatives to iron supplementation. The government engage institutions such as MICS, GHS. GSS to get feedback on its iron policy. GHS monitors the efficacy of the supplements while the DHS and MICS monitors the impact of the iron supplements.

Research about the supplementing children with iron that was conducted has bearings on this policy because it led to changes in the distribution in the supplements in children. The policy has led to the improvement of maternal health and maternal mortality has also decreased. The coverage of this policy is low. Also strengthening the monitoring tool and coverage of this policy will be beneficial.

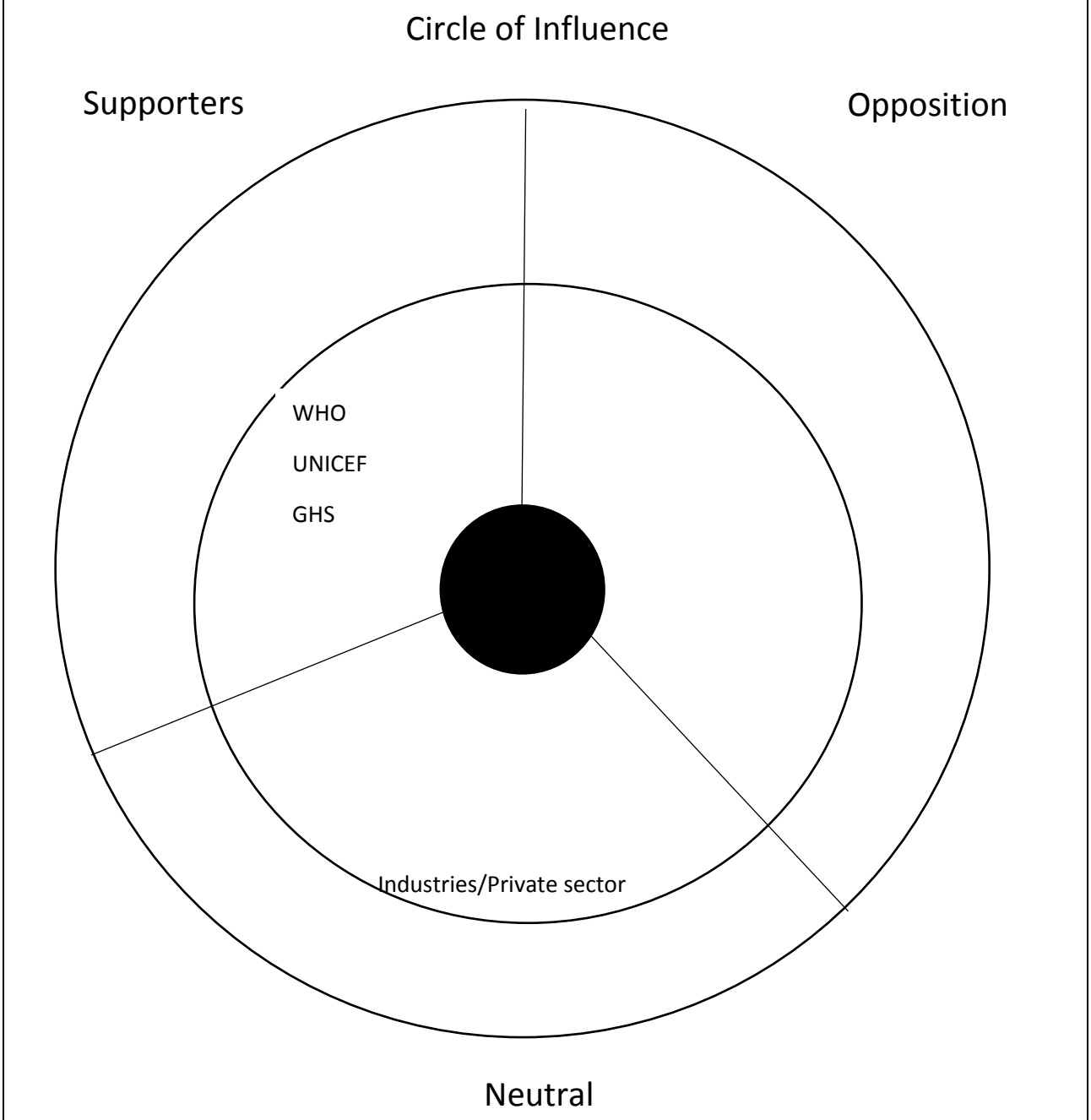
Table 8: Iron Supplementation policy hypothesis testing

Policy stages Kaleidoscope hypothesis	Policy action: Iron supplementation
1. Agenda setting	
1.1 Powerful advocates	+
1.2 Focusing event	
1.3 Recognized, relevant problem	+
2. Design	
2.1 Pressing vrs chosen problem	**+**
2.2 Ideas and beliefs	+
2.3 Cost-benefit calculations	+
2.4 International design spillovers	+
3. Adoption	
3.1 Propitious timing	
3.2 Veto players	
3.3 Relative power: proponents vs opponents	+
4. Implementation	
4.1 Institutional capacity	++
4.2 Requisite budgetary allocations	+
4.3 Commitment of policy champions	
5. Evaluation, Reform	
5.1 Changing conditions	
5.2 Changing information or beliefs	+
5.3 Resource availability relative to cost	+

Legend
+ Significant positive impact of this variable on policy outcomes
-Significant negative impact of this variable on policy process

Source: Field interviews

Figure 5: Iron supplementation Circle of Influence



Source: Field interviews

CONCLUSION

5.1 Summing up key hypotheses about what drives micronutrient policy change

The micronutrient policies/programs that have been reviewed in this paper have been put on Ghana's policy. The table below outlines the number of times each of the Kaleidoscope Model's key hypothesized variables emerged as a significant cause of policy change.

Agenda setting: Majority of the time, and for all the micronutrient policies reviewed, the advocates that drove Ghana's micronutrient policy were international organizations and donors like UNICEF, WHO and USAID. Discussions of these policies began in earnest after meetings by international bodies to create awareness about a nutritional deficiency in populations.

Design: Similar to other developing countries, international design spillovers contributed to majority of the design options. Specifically, GHS designed the overall policies in Ghana. In some cases, with the help of the FDB. The chosen designs were mostly sustainable, long term and were globally accepted. In some few instances, short term approaches are considered when there were high prevalence of the nutritional deficiency.

Adoption: In adopting a micronutrient policy in Ghana, there were no veto players. Rather, all the policies were adopted based on the relative powers of the proponents and the opponents. In some cases the proponents were able to overcome the opponents because they were donors and had money to support the policies.

The timing of the inception of the policies played a great role. Mostly the timing of these policies were favorable because at that time other nations were also in line doing the same thing. Most of these policies were seen as global interventions to a problem, hence its adoption.

Implementation: The main implementing body for the policies reviewed is the GHS and in some cases the FDB. There was a need to set up regulatory and legislative changes in the implementation of policies in some few cases. Overall the GHS was the institutional oversight in the implementation.

Evaluation and Reform: Information on new scientific findings led to some changes in some of the policies. Scientific evidence that shows that children with increased iron intake have increased malaria parasitemia hence the supplement was discontinued for children. Also, lactating mothers and mothers after delivery do not take the vitamin A supplements anymore because evidence shows that it is not as useful.

Table 9: What drives micronutrient policy change?

Policy stages Kaleidoscope hypothesis	Percent significant cases	Significant cases	Total cases
1. Agenda setting			
1.1 Powerful advocates	80%	4	5
1.2 Focusing event	20%	1	5
1.3 Recognized, relevant problem	80%	4	5
2. Design			
2.1 Pressing vrs chosen problem	80%	4	5
2.2 Ideas and beliefs	80%	4	5
2.3 Cost-benefit calculations	40%	2	5
2.4 International design spillovers	80%	4	5
3. Adoption			
3.1 Propitious timing	20%	1	5
3.2 Veto players	0	0	5
3.3 Relative power: proponents vs opponents	20%	1	5
4. Implementation			
4.1 Institutional capacity	80%	4	5
4.2 Requisite budgetary allocations	60%	3	5
4.3 Commitment of policy champions	60%	3	3
5. Evaluation, Reform			
5.1 Changing conditions			
5.2 Changing information or beliefs	60%	3	5
5.3 Resource availability relative to cost	60%	3	5

Legend
+ Significant positive impact of this variable on policy outcomes
-Significant negative impact of this variable on policy process

Source: Field interviews

Common factors influencing the effectiveness of micronutrient advocates

In Ghana, majority of the advocates in micronutrient policies have been international agencies. They are useful in shaping agenda, evaluation of the design, lobbying for decisions to be made as well as monitoring the policy implementation and evaluation. The power and influence of micronutrient advocates at each state in the policy process depends on three major factors- information, resources and nature of opposition

Information: Credible empirical information has been crucial in providing information for micronutrient policy advocates in Ghana. A number of research have provided numerous evidence to attest to the fact that micronutrient deficiencies leads to loss of health and human resources. IDRC sponsored by the government of Canada, conducted a survey to know the prevalence of IDD. UNICEF also conducted a survey that revealed a high prevalence of iron deficiency anemia. The results of these surveys served as ammunitions to persuade Ghana's cabinet and other stakeholders on the importance and potential benefits of micronutrient supplementation and fortification.

Resources: For micronutrient policies reviewed in this paper, there is an indication that, donors frequently drive the agenda by choosing micronutrients they are willing to fund. This has become necessary because governments face resource constraints. Donors have therefore been involved modeling micronutrient policy agenda, designs, implementation and monitoring. Additionally in all the micronutrient policies reviewed, there was evidence of the monetary influence of donors on policy inception.

Nature of opposition: In summary, there were few and minimal opposition to the inception of micronutrient policies in Ghana. For instance the main opposers to the vitamin A and iodine polices were herbal practitioners and some elements in the medical sectors as well as some clinicians. Interestingly, the power of the proponents (advocates, donors) overcame the influence of the opposers.

To conclude, Ghana's policy is donor driven with minimal opposition from public agencies. Ghana's policy process is similar to other developing countries. The salt fortification

mandate is similar to Zambia, Malawi and South Africa. One challenge faced with Ghana's policy processes is the lack of coordination among institutions in the management of policies. There is therefore the need for policy makers in Ghana to understand the roles in this coordination and implement it in policy institutions in Ghana. Also government budgets should include micronutrient policies in their budget. This can be beneficial in all the phases of the micronutrient policy change process.

6. APPENDICES

APPENDIX A: KEY INFORMANT INTERVIEW GUIDE

Name:

Institution:

Position:

1. Policy process:

a) Does Ghana have a Nutrition policy?

b) When was it launched?

c) Which sectors are involved in the implementation of the policy?

d) What are the strategic areas in the policy and what are the target groups?

e) Does Ghana have a separate strategy for micronutrient/nutritional intervention implementation?

f) If yes, when was it launched?

g) Which governmental and non-governmental sectors are involved?

h) What is the role of your institution in the policy formulation and review in Ghana?

i) Do you know whose initiative it was to involve your institution in micronutrient policy discussions?

a. Agenda-setting

a) How did Micronutrient get onto the nutrition policy agenda?

b) Who were the strongest advocates? When did the Micronutrient discussions begin in earnest?

c) When in the process did your institution become involved in micronutrient discussions:

d) Who championed this cause?

- Domestic advocates:
- International advocates:

e) Who opposed it?

f) Why was this considered a priority issue?

b. Design

a) Who designed the policy intervention?

b) What design options were considered?

c) What implementation options were considered (fortification, bio-fortification etc?

d) Why did designers choose:

a. supplementation;

b. fortification

c. biofortification?

d. Which institutions/partners are involved in micronutrient supplementation?

e. What are the target groups and target population?

f. What was your institution's role in the implementation of this?

g. What is the annual cost?

h. Who finances the cost? Government of Ghana,

- How cost-effective are the various alternatives?
- Was this a pressing or a chosen problem?
- What ideas and beliefs underlie the chosen design?

c. Decision making

a) Who made the final decision?

b) Who lobbied in favor?

c) Who opposed it?

d) What factors led to a favorable decision? (propitious timing?)

- What veto players exist?
- Evaluate the relative power of the proponents and opponents.

d. Implementation

a) Who implements Micronutrient supplementation in Ghana?

b) What regulatory and legislative changes took place to implement the policy decision?

c) Did this policy require setting up new institutions?

d) Any policy changes since introduction? When? Why?

- institutional capacity of implementing institution
- commitment of policy makers
- Budget resources: what cost? Who pays? Are the resources sustainable?

f) What is the cost of implementing?

g) Are the supplements imported or are they are made in Ghana?

e) Who monitors the impact of this policy: Not sure

i. What are the main advantages and disadvantages of the policy from your perspective?
ii. Do you have any ideas regarding how to improve the current program?
iii. Are there alternatives to the Micronutrient supplementation that the Government should be pursuing?
iv. Do you think that Micronutrient supplementation programs in other countries work better?
v. Who does the government engage with to get feedback on its policies?
vi. Who monitors the efficacy of the supplements?
vii. Who monitors the impact of this policy
viii. Any other relevant research bearing on this policy?
    - Did changing conditions lead to policy change?
    - Changing beliefs? Did understanding or awareness change?
    - Did resource constraints trigger reform?

3. Implications

a) What are the policy and program implications of this for Ghana?
b) What implications for policy process and inclusiveness of various actor and players?

4. Gaps

a) What are the gaps in the implementation and monitoring of these policies?
b) Can you give some suggested solutions to the gaps?

APPENDIX B: LIST OF PERSONS INTERVIEWED

Prof. Matilda Steiner

University of Ghana, Department of Nutrition.

Felix Asante

ISSER, University of Ghana

Esi Amoafo, Nutrition Department, Ghana Health Service

Lilian Selenje, UNICEF

Victoria Tekpo, WIADD

INTERNATIONAL FOOD POLICY

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APPENDIX 5: IRB approval letters

MICHIGAN STATE
UNIVERSITY

Initial IRB Application Determination
Exempt

March 28, 2016

To: Jenifer Fenton
208B G.M. Trout Bldg

Re: **IRB# x16-461e** Category: Exempt 2
Approval Date: March 28, 2016

Title: Review of Micronutrient Policy in Ghana

The Institutional Review Board has completed their review of your project. I am pleased to advise you that **your project has been deemed as exempt** in accordance with federal regulations.

The IRB has found that your research project meets the criteria for exempt status and the criteria for the protection of human subjects in exempt research. **Under our exempt policy the Principal Investigator assumes the responsibilities for the protection of human subjects** in this project as outlined in the assurance letter and exempt educational material. The IRB office has received your signed assurance for exempt research. A copy of this signed agreement is appended for your information and records.

Renewals: Exempt protocols do not need to be renewed. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: Exempt protocols do not require revisions. However, if changes are made to a protocol that may no longer meet the exempt criteria, a new initial application will be required.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects and change the category of review, notify the IRB office promptly. Any complaints from participants regarding the risk and benefits of the project must be reported to the IRB.

Follow-up: If your exempt project is not completed and closed after three years, the IRB office will contact you regarding the status of the project and to verify that no changes have occurred that may affect exempt status.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with the IRB office.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at IRB@msu.edu. Thank you for your cooperation.

Sincerely,

Harry McGee, MPH
SIRB Chair

c: Mary Adjepong

Office of Regulatory Affairs
Human Research Protection Programs

Biomedical & Health Institutional Review Board (BIRB)

Community Research Institutional Review Board (CRIRB)

Social Science Behavioral/Education Institutional Review Board (SIRB)

Olds Hall
408 West Circle Drive, #207
East Lansing, MI 48824
(517) 355-2180
Fax: (517) 432-4503
Email: irb@msu.edu
www.hrpp.msu.edu

MICHIGAN STATE
UNIVERSITY

Initial IRB Application Approval

May 13, 2016

To: Jenifer Fenton
469 Wilson Rd
Rm 206B

Re: **IRB#** 16-557 Category: EXPEDITED 2(b), 4, 7
Approval Date: May 13, 2016
Expiration Date: May 12, 2017

Title: Association of whole blood fatty acid levels with growth and cognition in Ghanian children 2-5 (OGA#147309, RC102095)

The Institutional Review Board has completed their review of your project. I am pleased to advise you that **your project has been approved.**

Please send approval letters from the Committee on Human Research Publication and Ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, and the Noguchi Memorial Institute for Medical Research, Institutional Review Board, and approved consent forms to the MSU IRB upon receipt. Note if the consent forms approved by the MSU IRB are revised by the local ethics committees, the revised forms must be submitted to the MSU IRB via a revision before implementation.

The committee has found that your research project is appropriate in design, protects the rights and welfare of human subjects, and meets the requirements of MSU's Federal Wide Assurance and the Federal Guidelines (45 CFR 46 and 21 CFR Part 50). The protection of human subjects in research is a partnership between the IRB and the investigators. We look forward to working with you as we both fulfill our responsibilities.

Renewals: IRB approval is valid until the expiration date listed above. If you are continuing your project, you must submit an *Application for Renewal* application at least one month before expiration. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: The IRB must review any changes in the project, prior to initiation of the change. Please submit an *Application for Revision* to have your changes reviewed. If changes are made at the time of renewal, please include an *Application for Revision* with the renewal application.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects, notify the IRB office promptly. Forms are available to report these issues.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with the IRB office.

Good luck in your research. If we can be of further assistance, please contact us at 517-355-2180 or via email at IRB@msu.edu. Thank you for your cooperation.

Sincerely,

Ashir Kumar, M.D.
BIRB Chair

c: Mary Adjepong, Matthew Pontifex

Office of Regulatory Affairs
Human Research Protection Programs

Biomedical & Health Institutional Review Board (BIRB)

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MICHIGAN STATE
UNIVERSITY

Revision Application Approval

February 2, 2017

To: Jenifer Fenton
469 Wilson Rd
Rm 208B

Re: **IRB# 16-557 Category: EXPEDITED 2(b), 4, 7**
Revision Approval Date: February 1, 2017
Project Expiration Date: May 12, 2017

Title: Association of whole blood fatty acid levels with growth and cognition in Ghanian children 2-5 (CGA#147309, RC102095)

The Institutional Review Board has completed their review of your project. I am pleased to advise you that **the revision has been approved.**

This letter notes approval for the revised McGill/University of Ghana consent form; the MSU IRB acknowledges that the NMIMR IRB approved consent form will be used. This letter also notes approval for the revised consent process and data collection procedures, and for the removal of compensation.

The review by the committee has found that your revision is consistent with the continued protection of the rights and welfare of human subjects, and meets the requirements of MSU's Federal Wide Assurance and the Federal Guidelines (45 CFR 46 and 21 CFR Part 50). The protection of human subjects in research is a partnership between the IRB and the investigators. We look forward to working with you as we both fulfill our responsibilities.

Renewals: IRB approval is valid until the expiration date listed above. If you are continuing your project, you must submit an *Application for Renewal* application at least one month before expiration. If the project is completed, please submit an *Application for Permanent Closure*.

Revisions: The IRB must review any changes in the project, prior to initiation of the change. Please submit an *Application for Revision* to have your changes reviewed. If changes are made at the time of renewal, please include an *Application for Revision* with the renewal application.

Problems: If issues should arise during the conduct of the research, such as unanticipated problems, adverse events, or any problem that may increase the risk to the human subjects, notify the IRB office promptly. Forms are available to report these issues.

Please use the IRB number listed above on any forms submitted which relate to this project, or on any correspondence with the IRB office.

If we can be of further assistance, please contact us at 517-355-2180 or via email at IRB@msu.edu. Thank you for your cooperation.

c: Mary Adjepong, Matthew Pontifex

Office of Regulatory Affairs
Human Research Protection Programs

Biomedical & Health Institutional Review Board (BIRB)

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NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
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NMIMR-IRB
P. O. Box LG 581
Legon, Accra

My Reference: DF 22

May 4, 2018

Grace S. Marquis PhD

Dietetics and Human Nutriton

McGill University 21,111 Lakeshore Road,

QC H9X 3V(

RE: Our Study # 027/13-14 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Grace S. Marquis PhD:

Meeting Date: 11/2/2016 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Building capacity of sustainable livelihoods and health through public-private linkages in agriculture and health systems

(Cohort surveys to improve health and well-being of infants, adolescents and their families in Upper Manya Krobo District, Ghana)

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation

provided below.

Internal #: 1490

Expiration Date: 9/6/2017

On Agenda For: Procedure

Reason 1: Amendment **Reason 2:**

Description: The PI is requesting to collect additional information on whole bloody fatty acids, malaria and cognitive function test of study children.

IRB ACTION: Approved

Condition 1:

Action

Explanation: The amendments to the protocol are approved.

Yours Sincerely,

NMIMR-IRB

IRB Administrator

cc: Anna Lartey PhD , Nutrition and Food Science Department, University of Ghana, Anna Lartey, PhD , Department of Nutrition and Food Science, University of Ghana, Esi Colecraft, PhD , University of Ghana, Department of Nutrition and Food Science, Frances Aboud, PhD. , Richmond Aryeetey, PhD, MPH, University of Ghana, Dept. of Population, Family and Reproductive Health, Theresa Gyorkos, PhD.

McGill University
ETHICS REVIEW
RENEWAL REQUEST/STUDY CLOSURE FORM

Continuing review of research involving humans requires, at a minimum, the submission of an annual status report to the REB. This form must be completed to request renewal of ethics approval. If a renewal is not received before the expiry date, the project is not considered to be approved and no further research activity may be conducted. When a project has been completed, this form can also be used to officially close the study. To avoid expired approvals and, in the case of funded projects, the freezing of funds, this form should be returned 2-3 weeks before the current approval expires.

REB File #: 180-1011 3
Project Title: Cohort surveys to improve health and well-being of infants, adolescents and their families in Upper Krobo District, Ghana.
Principal Investigator: Prof. G. Marquis / School of Dietetics and Human Nutrition
Email: : grace.marquis@mcgill.ca **Faculty Supervisor (if PI is a student):**

1. Were there any significant changes made to this research project that have any ethical implications that have not already been reported to the REB? ____YES __X__NO
   If yes, complete an amendment form indicating these changes and attach to this form.

2. Are there any ethical concerns that arose during the course of this research? ___ YES _X__ NO
   If yes, please describe.
3. Have any participants experienced any unanticipated issues or adverse events in connection with this research project? ___ YES _X__ NO
   If yes, please describe. However there have been deaths unrelated to the project (this is a cross-sectional survey that includes no intervention) that have been discovered during subsequent annual surveys. All of these have been reported to the REB.

4. Is this a currently funded study? _X__ YES ___ NO
   If yes, list the agency name and project title and the Principal Investigator of the award if not yourself. This information is necessary to ensure compliance with agency requirements and that there is no interruption in funds.
   Global Affairs Canada (previously, Foreign Affairs, Trade and Development Canada.)
   Title: Building Capacity for Sustainable Livelihoods and Health Through Public-Private Linkages in Agriculture and Health Systems.

5. Does this project require REB approval from another Institution/Board? _X__YES ___ NO
   If yes, and the project is continuing, attach a copy of the current approval.

Attached.

Principal Investigator Signature: Grace S Marquis [redacted] Date: __7 July 2017_____

Faculty Supervisor Signature: _____ Date:_____
(if PI is a student)

____ Check here if the study is to be closed and continuing ethics approval is no longer required. A study can be closed when all data collection has been completed and there will be no further contact with participants.
_X__ Check here if this is a request for renewal of ethics approval.

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
Established 1979 A Constituent of the College of Health Sciences
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NMIMR-IRB
P. O. Box LG 581
Legon, Accra
Ghana

My Reference: DF 22

November 10, 2016
Grace S. Marquis PhD
Dietetics and Human Nutriton
McGill University 21,111 Lakeshore Road,
QC H9X 3V(

RE: Our Study # 027/13-14 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Grace S. Marquis PhD:

Meeting Date: 9/7/2016 **At:** NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Building capacity of sustainable livelihoods and health through public-private linkages in agriculture and health systems

(Cohort surveys to improve health and well-being of infants, adolescents and their families in Upper Manya Krobo District, Ghana)

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation provided below.

Internal #: 1408
Expiration Date: 9/6/2017
On Agenda For: Renewal
Reason 1: Progress Report **Reason 2:**
Description:
IRB ACTION: Renewed
Condition 1:
Action Explanation:

Yours Sincerely,

NMIMR-IRB
IRB Administrator
cc: Anna Lartey PhD , Nutrition and Food Science Department, University of Ghana, Anna Lartey, PhD , Department of Nutrition and Food Science, University of Ghana, Esi Colecraft, PhD , University of Ghana, Department of Nutrition and Food Science, Frances Aboud, PhD. , Richmond Aryeetey, PhD, MPH, University of Ghana, Dept. of Population, Family and Reproductive Health, Theresa Gyorkos, PhD.

For Administrative Use	REB: ___REB-I ___REB-II X REB-III
X Delegated Review ___ Full Review	
Signature of REB Chair or designate: _____	Date: July 7, 2017
Approval Renewal Period: July 7, 2017 to July 6, 2018	

Submit by email to lynda.mercil@mcgill.ca; REB Office: James Administration Building, 845 Sherbrooke Street West suite 429, Mtl., QC H3A0G4; tel: 514-398-6831/6193; fax: 514-398-4644;www.mcgill.ca/research/researchers/compliance/human

McGill University

ETHICS REVIEW
AMENDMENT REQUEST FORM

This form can be used to submit any changes/updates to be made to a currently approved research project. Changes must be reviewed and approved by the REB before they can be implemented.

Significant or numerous changes to study methods, participant populations, location of research or the research question or where the amendment will change the overall purpose or objective of the originally approved study will require the submission of a complete new application.

REB File #: 180-1013
Project Title: Cohort surveys to improve health and well-being of infants, adolescents and their families in Upper Krobo District, Ghana.
Principal Investigator: Prof. G. Marquis / School of Dietetics and Human Nutrition
Email: grace.marquis@mcgill.ca
Faculty Supervisor (for student PI): --

1) Explain what these changes are, why they are needed, and if the risks or benefits to participants will change.

The project takes one drop of blood to measure hemoglobin. We are requesting to take an additional couple of drops of blood from the same finger prick to assess whole blood fatty acids and presence of malaria parasites. In addition, we will add a very brief questionnaire to assess cognitive function. The research on the association between fatty acids and cognition is being carried out in collaboration with researchers at Michigan State University, Dr. Jennifer Fenton and Mary Adjepong (doctoral student of Dr. Fenton). They have been added to the consent form. The assessment of malaria is to adjust the results for a major cause of illness if the area.

There are no additional risks to the participant as the finger prick is already part of the protocol. The questionnaire asks about a few additional foods that are rich in fatty acids and knowledge about colors and animals and is not invasive. The participants will receive additional benefits by learning about their malarial status and referred to the local health center if positive.

2) Attach relevant additional or revised documents such as questionnaires, consent forms, recruitment ads.

The revised consent form is attached with changes highlighted.
The draft of additional questions is attached.

Grace S. Marquis

Principal Investigator Signature: _____ Date: Dec 6, 2016

Faculty Supervisor Signature: _____ Date: _____
(for student PI)

Submit by email to lynda.mcneil@mcgill.ca. REB Office: James Administration Building, 845 Sherbrooke Street West suite 429, fax: 398-4644 tel: 398-6831/6193; www.mcgill.ca/research/researchers/compliance/human
(August 2014)

For Administrative Use REB: ___REB-I ___REB-II X REB-III

X Delegated Review ___ Full Review

X This amendment request has been approved.

Signature of REB Chair/ delegate: _____ Date: Jan. 10, 2017

Project Approval Expires: Aug. 14, 2017

Submit by email to lynda.mcneil@mcgill.ca. REB Office: James Administration Building, 845 Sherbrooke Street West suite 450, fax: 398-4644 tel: 398-6831/6193; www.mcgill.ca/research/researchers/compliance/human

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICAL SCIENCES / KOMFO ANOKYE TEACHING HOSPITAL

COMMITTEE ON HUMAN RESEARCH, PUBLICATION AND ETHICS

Our Ref: CHRPE/AP/236/16

3rd May, 2016

Miss Mary Adepong
Department of Food Science
and Human Nutrition
Michigan State University
USA.

Dear Madam,

LETTER OF APPROVAL

Protocol Title:	*"Association between Essential Fatty Acids in Growth and Cognitive Function in Ghanaian Children, 2 to 5 Years of Age."*
Proposed Site:	*Ofoikrom, Kumasi.*
Sponsor:	**Michigan State University and Borlaug LEAP (University of California). United States Agency for International Development (USAID), Ghana.**

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee reviewed the following documents:

- A Completed CHRPE Application Form.
- Participant Information Leaflet and Consent form.
- Research Protocol.
- Interview Guide.

The Committee has considered the ethical merit of your submission and approved the protocol. The approval is for a fixed period of one year, beginning 3rd May, 2016 to 2nd May, 2017 renewable thereafter. The Committee may however, suspend or withdraw ethical approval at any time if your study is found to contravene the approved protocol.

Data gathered for the study should be used for the approved purposes only. Permission should be sought from the Committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee should be notified of the actual start date of the project and would expect a report on your study, annually or at the close of the project, whichever one comes first. It should also be informed of any publications arising from the study.

Thank you Madam, for your application.

Yours faithfully,

Osomfuor Prof. Sir J. W. Ahenkorah MD, FWACP
Chairman

Room 7 Block J, School of Medical Sciences, KNUST, University Post Office, Kumasi, Ghana
Phone: +233 3220 63248 Mobile: +233 20 5455705 Email: chrpe.knust.kath@gmail.com / chrpe@knust.edu.gh

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Huffman, S.L., et al., *Essential fats: how do they affect growth and development of infants and young children in developing countries? A literature review.* Matern Child Nutr, 2011. **7 Suppl 3**: p. 44-65.

2. Arimond, M.Z., M. Jungjohann, S. Brown, K. H. Ashorn, P. Allen, L. H. Dewey, K. G., *Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project.* Matern Child Nutr, 2013.

3. S Adu-Afarwuah, A.L., K H Brown, S Zlotkin, A Briend and K G Dewey, *A fat-based supplement containing essential fatty acids increased plasma {alpha}-linolenic acid and linear growth of Ghanaian infants.* FASEB Journal 2007. **21**(223.7).

4. UNICEF. *A vitamin and mineral deficeincy, a global damage assessment report*. [cited 2015 21st April, 2015]; Available from: http://www.unicef.org/media/files/davos_micronutrient.pdf

5. Hoddinott, J., *The economic cost of malnutrition*, in *The road to good nutrition, a global perspective*, M. Eggersdorfer, et al., Editors. 2013, Karger Publishers. p. 60-74.

6. Reinhardt, K. and J. Fanzo, *Addressing Chronic Malnutrition through Multi-Sectoral, Sustainable Approaches: A Review of the Causes and Consequences.* Front Nutr, 2014. **1**: p. 13.

7. FAO, *Fats and fatty acids in human nutrition Report of an expert consultation*. 2010: Food and agriculture organization of the united nations.

8. Georgieff, M.K., *Nutrition and the developing brain: nutrient priorities and measurement.* Am J Clin Nutr 2007, 2007. **85**: p. 614S-20S. .

9. Kang, J.X., J.B. Wan, and C. He, *Concise review: Regulation of stem cell proliferation and differentiation by essential fatty acids and their metabolites.* Stem Cells, 2014. **32**(5): p. 1092-8.

10. Maria Makrides, M.A.N., Brett Jeffrey, Eric L Lien, and Robert A Gibson, *Are long-chain polyunsaturated fatty acids essential nutrients in infancy?* Lancet, 1995. **345**(1): p. 1463-68.

11. Deborah L. O'Connor, R.H., David Adamkin,Nancy Auestad, Marcella Castillo, William E Connor, Sonja L. Connor, ; Kathleen Fitzgerald, Sharon Groh-Wargo, E. Eugenie Hartmann, Joan Jacobs, Jeri Janowsky, Alan Lucas, Dean Margeson,Patricia Mena, Martha Neuringer, Mirjana Nesin, Lynn Singer, Terence Stephenson, Joanne Szabo, Vance Zemon,, *Growth and Development in Preterm*

Infants Fed Long-Chain Polyunsaturated Fatty Acids: A Prospective, Randomized Controlled Trial. Pediatrics, 2001. **108**(1): p. 359-374.

12. Dalton, A.W., P. Witthuhn, R. C. van Stuijvenberg, M. E. Swanevelder, S. A. Smuts, C. M., *A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source.* Prostaglandins Leukot Essent Fatty Acids, 2009. **80**(2-3): p. 143-9.

13. Muthayya, S., et al., *Effect of fortification with multiple micronutrients and n-3 fatty acids on growth and cognitive performance in Indian schoolchildren: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study.* Am J Clin Nutr, 2009. **89**(6): p. 1766-75.

14. G van Wezel-Meijler, M.v.d.K., J Huisman, EJ Jonkman, J Valk and HN Lafeber, *Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation* Acta Pñ diatr 2002. **91**(1): p. 942 - 950.

15. Nancy Auestad, R.H., Robert T. Hall, Mark Blatter,, et al., *Growth and Development in Term Infants Fed Long-Chain Polyunsaturated Fatty Acids: A Double-Masked, Randomized, Parallel,Prospective, Multivariate Study* Pediatrics 2001. **108**: p. 359-71.

16. Drover, J.H., D. R. Castaneda, Y. S. Morale, S. E. Birch, E. E., *Three randomized controlled trials of early long-chain polyunsaturated Fatty Acid supplementation on means-end problem solving in 9-month-olds.* Child Dev, 2009. **80**(5): p. 1376-84.

17. Henriksen, C., et al., *Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid.* Pediatrics, 2008. **121**(6): p. 1137-45.

18. Bernard et al., *The Dietary n6:n3 Fatty Acid Ratio during Pregnancy Is Inversely Associated with Child Neurodevelopment in the EDEN Mother-Child Cohort1-3* J. Nutr. , 2013. **143**: p. 1481 - 1488.

19. NATIONS, F.A.A.O.O.T.U. *Ghana Nutrition Profile – Nutrition and Consumer Protection Division, FAO, 2009* 2009; Available from: ftp://ftp.fao.org/ag/agn/nutrition/ncp/gha.pdf.

20. Ghana Statistical Service, G.H.S., ICF, Macro. *Ghana Demographic Health Survey, 2008*. 2008 [cited 2014 21st January, 2015]; Available from: http://dhsprogram.com/pubs/pdf/FR221/FR221%5B13Aug2012%5D.pdf.

21. Ghana Statistical Service Accra, G., Ghana Health Service Accra, Ghana. *Ghana Demographic Health Survey, Key Indicators, 2014*. 2014 [cited 2015 27th June, 2015]; Available from: http://dhsprogram.com/pubs/pdf/PR59/PR59.pdf.

22. Black, M.B., *Micronutrient Deficiencies and Cognitive Functioning.* J. Nutr. , 2003. **133**(3927S-3931S).

23. Carlson, S.J.F., E. M. Kalish, B. T. Gura, K. M. Puder, M., *The role of the omega-3 fatty acid DHA in the human life cycle.* JPEN J Parenter Enteral Nutr, 2013. **37**(1): p. 15-22.

24. Fokkema, M.R., et al., *Assessment of essential fatty acid and ω3-fatty acid status by measurement of erythrocyte 20:3ω9 (Mead acid), 22:5ω6/20:4ω6 and 22:5ω6/22:6ω3.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2002. **67**(5): p. 345-356.

25. Jennifer J. Otten, J.P.H., Linda D. and F.J. Meyers, *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements* 2006: National academy press 1324 - 1325.

26. Poulsen, R.C., P.J. Moughan, and M.C. Kruger, *Long-chain polyunsaturated fatty acids and the regulation of bone metabolism.* Exp Biol Med (Maywood), 2007. **232**(10): p. 1275-88.

27. Rzehak, P., et al., *Growth of infants fed formula rich in canola oil (low erucic acid rapeseed oil).* Clin Nutr, 2011. **30**(3): p. 339-45.

28. Tinoco, S.M.S., R. Setta, C. L. Moura, A. S. Carmo, M. G., *n-3 polyunsaturated fatty acids in milk is associate to weight gain and growth in premature infants.* Lipids Health Dis, 2009. **8**: p. 23.

29. Smit, E.N.M., F. A. Boersma, E. R., *The possible role of essential fatty acids in the pathophysiology of malnutrition: a review.* Prostaglandins Leukot Essent Fatty Acids, 2004. **71**(4): p. 241-50.

30. Fokkema, M.R.S., E. N. Martini, I. A. Woltil, H. A. Boersma, E. R. Muskiet, F. A. J., *Assessment of essential fatty acid and ω3-fatty acid status by measurement of erythrocyte 20:3ω9 (Mead acid), 22:5ω6/20:4ω6 and 22:5ω6/22:6ω3.* Prostaglandins, Leukotrienes and Essential Fatty Acids, 2002. **67**(5): p. 345-356.

31. Makrides, M., et al., *Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants.* The American Journal of Clinical Nutrition, 1994. **60**(2): p. 189-194.

32. Carlson, S.E., J.D. Carver, and S.G. House, *High Fat Diets Varying in Ratios of Polyunsaturated to Saturated Fatty Acid and Linoleic to Linolenic Acid: A Comparison of Rat Neural and Red Cell Membrane Phospholipids.* The Journal of Nutrition, 1986. **116**(5): p. 718-725.

33. Sugasini, D., et al., *Dietary docosahexaenoic acid (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves memory in adult mice.* Scientific Reports, 2017. **7**: p. 11263.

34. Agble, R., et al., *Nutrition Country Profile: Republic of Ghana.* Nutrition and Consumer Protection Division, FAO, 2009.

35. Seth Adu-Afarwuah, A.L., Harriet Okronipa, Janet Peerson, Per Ashorn and Kathryn Dewey, *Effects of Small-Quantity Lipid-Based Nutrient Supplement on Hemoglobin and Iron Status of Pregnant Ghanaian Women.* FASEB Journal, 2015. **29**(1: Supplement 39.5).

36. Moses Klevor, M.H., Anna Lartey, Seth Adu-Afarwuah, Mamane Zeilani and and K. Dewey, *Effect of Small-Quantity Lipid-Based Nutrient Supplement (SQ-LNS) on Breast Milk Vitamin A Concentration among Ghanaian Women.* The FASEB Journal, 2015. **29:1**(Supplement 898.17).

37. Ghana Statistical Service (GSS), G.H.S.G., and ICF International., *Ghana Demographic and Health Survey 2014*, G. GSS, and ICF International, Editor. 2015: Rockville, Maryland, USA

38. Kuipers, R.S., et al., *Postdelivery changes in maternal and infant erythrocyte fatty acids in 3 populations differing in fresh water fish intakes.* Prostaglandins Leukot Essent Fatty Acids, 2011. **85**(6): p. 387-97.

39. Black, R.E., et al., *Maternal and child undernutrition and overweight in low-income and middle-income countries.* Lancet, 2013. **382**(9890): p. 427-451.

40. McDonald, C.M., et al., *The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries.* Am J Clin Nutr, 2013. **97**(4): p. 896-901.

41. Adu-Afarwuah, S., et al., *A fat-based supplement containing essential fatty acids increased plasma alpha-linolenic acid and linear growth of Ghanaian infants.* Faseb Journal, 2007. **21**(5):

42. Ho, P.P., et al., *Identification of naturally occurring fatty acids of the myelin sheath that resolve neuroinflammation.* Sci Transl Med, 2012. **4**(137): p. 137-73.

43. Innis, S.M., *Dietary omega 3 fatty acids and the developing brain.* Brain Res, 2008. **1237**: p. 35-43.

44. Innis, S.M., *Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids.* J Pediatr, 2003. **143**(4 Suppl): p. S1-8.

45. Ricciotti, E. and G.A. FitzGerald, *Prostaglandins and inflammation.* Arterioscler Thromb Vasc Biol, 2011. **31**(5): p. 986-1000.

46. G. C. Burdge, H.R., J. A. Kohler, and K. A. Lillycrop,, *Effect of Fatty Acid Supplementation on Growth and Differentiation of Human IMR-32 Neuroblastoma Cells In Vitro.* Journal of Cellular Biochemistry, 2000. **80**(266-273).

47. Fulco, A.J. and J.F. Mead, *Metabolism of essential fatty acids. VIII. Origin of 5,8,11-eicosatrienoic acid in the fat-deficient rat.* J Biol Chem, 1959. **234**(6): p. 1411-6.

48. Guillou, H., et al., *The key roles of elongases and desaturases in mammalian fatty acid metabolism: Insights from transgenic mice.* Prog Lipid Res, 2010. **49**(2): p. 186-99.

49. Smit, E.N., F.A. Muskiet, and E.R. Boersma, *The possible role of essential fatty acids in the pathophysiology of malnutrition: a review.* Prostaglandins Leukot Essent Fatty Acids, 2004. **71**(4): p. 241-50.

50. Ichi, I., et al., *Identification of genes and pathways involved in the synthesis of Mead acid (20:3n-9), an indicator of essential fatty acid deficiency.* Biochim Biophys Acta, 2014. **1841**(1): p. 204-13.

51. Ling, P.R., et al., *Disturbances in essential fatty acid metabolism in patients receiving long-term home parenteral nutrition.* Dig Dis Sci, 2002. **47**(8): p. 1679-85.

52. Wene, J.D., W.E. Connor, and L. DenBesten, *The development of essential fatty acid deficiency in healthy men fed fat-free diets intravenously and orally.* J Clin Invest, 1975. **56**(1): p. 127-34.

53. Siguel, E., *Diagnosing Essential Fatty Acid Deficiency.* Circulation 1998. **97**: p. 2580 -2583.

54. Siguel, E.N., et al., *Criteria for essential fatty acid deficiency in plasma as assessed by capillary column gas-liquid chromatography.* Clin Chem, 1987. **33**(10): p. 1869-73.

55. Holman, R.T., *The Ratio of Trienoic : Tetraenoic Acids in Tissue Lipids as a Measure of Essential Fatty Acid Requirement.* Journal of Nutrition, 1960. **70**.

56. Adjepong, M., et al., *Quantification of fatty acid and mineral levels of selected seeds, nuts, and oils in Ghana.* Journal of Food Composition and Analysis, 2017. **59**: p. 43-49.

57. Seth Adu-Afarwuah, A.L., Kenneth H Brown, Stanley Zlotkin, André Briend, and Kathryn G Dewey, *Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on*

growth and motor development. American Journal of Clinical Nutrition, 2007. **86:**(412-20.)

58. Issaka, A.I., et al., *Determinants of inadequate complementary feeding practices among children aged 6-23 months in Ghana.* Public Health Nutr, 2015. **18**(4): p. 669-78.

59. Service, G.S., *Population and housing census, Savelugu.* Data analytical reporting, 2014.

60. Alhassan, L., *Nutritional Status of Status of Pre-School Children; a Comparative Study of Two Communities in Savelugu-Nanton District Northern Region*, in *Department of Community Health KNUST*. 2008, Kwame Nkrumah University of Science and Technology.

61. METSS, U., *Savelugu Nanton*, USAID, Editor. 2017.

62. Sullivan, G. and R. Feinn, *Using Effect Size—or Why the P Value Is Not Enough.* Journal of Graduate Medical Education 2012.

63. Jumbe, T., et al., *Whole Blood Levels of the n-6 Essential Fatty Acid Linoleic Acid Are Inversely Associated with Stunting in 2-to-6 Year Old Tanzanian Children: A Cross-Sectional Study.* PLoS One, 2016. **11**(5): p. e0154715.

64. Jumbe, T., et al., *Whole-blood fatty acids are associated with executive function in Tanzanian children aged 4-6 years: a cross-sectional study.* Br J Nutr, 2016. **116**(9): p. 1537-1545.

65. Polreis, W.S.H.a.J., *Measurement of the Omega-3 Index in Dried Blood Spots* Annals of Clinical and Laboratory Research, 2016. **4**(137).

66. Morrison, W.R. and L.M. Smith, *Preparation of Fatty Acid Methyl Esters and Dimethylacetals from Lipids with Boron Fluoride--Methanol.* J Lipid Res, 1964. **5**: p. 600-8.

67. Min, Y.J., et al., *Effect of storage temperature and length on fatty acid composition of fingertip blood collected on filter paper.* Prostaglandins Leukotrienes and Essential Fatty Acids, 2011. **84**(1-2): p. 13-18.

68. Araujo, P., et al., *Evaluation of a rapid method for the quantitative analysis of fatty acids in various matrices.* J Chromatogr A, 2008. **1212**(1-2): p. 106-13.

69. WHO, *WHO anthro for personal computers. Geneva: World Health Organization; Software for assessing growth and development of the world's children (0 to 60 months of age)*. 2016.

70. Pickens, C.A., et al., *Plasma phospholipids, non-esterified plasma polyunsaturated fatty acids and oxylipids are associated with BMI.* Prostaglandins Leukot Essent Fatty Acids, 2015. **95**: p. 31-40.

71. Gurzell, E.A., et al., *Is the omega-3 index a valid marker of intestinal membrane phospholipid EPA+DHA content?* Prostaglandins Leukot Essent Fatty Acids, 2014. **91**(3): p. 87-96.

72. WHO, *WHO AnthroPlus for personal computers. Software for assessing growth of the world's children and adolescents (5to19 years)*. 2009: Geneva.

73. Taiyun, W. and V. Simko. *R package "corrplot": Visualization of a Correlation Matrix (Version 0.84).* 2017; Available from: Available from https://github.com/taiyun/corrplot.

74. WHO, *Guideline: Updates on the management of severe acute malnutrition in infants and children.* 2013, World Health Organization: Geneva, Switzerland.

75. Hadley, K.B., et al., *The Essentiality of Arachidonic Acid in Infant Development.* Nutrients, 2016. **8**(4): p. 216.

76. Damsgaard, C.T., et al., *The effects of n-3 long-chain polyunsaturated fatty acids on bone formation and growth factors in adolescent boys.* Pediatr Res, 2012. **71**(6): p. 713-9.

77. Carlson, S.E., et al., *Arachidonic acid status correlates with first year growth in preterm infants.* Proc Natl Acad Sci U S A, 1993. **90**(3): p. 1073-7.

78. Ahmed, S.F. and C. Farquharson, *The effect of GH and IGF1 on linear growth and skeletal development and their modulation by SOCS proteins.* J Endocrinol, 2010. **206**(3): p. 249-59.

79. Matteo Fumagalli, I.M., Niels Grarup, Fernando Racimo et al., , *Greenlandic Inuit showgenetic signatures of diet and climate adaptation.* Science, 2015. **349**(6254).

80. Cormier, H., et al., *Effects of FADS and ELOVL polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation.* Genes Nutr, 2014. **9**(6): p. 437.

81. Park, W.J., et al., *An alternate pathway to long-chain polyunsaturates: the FADS2 gene product Delta8-desaturates 20:2n-6 and 20:3n-3.* J Lipid Res, 2009. **50**(6): p. 1195-202.

82. Feingold, K.R. and P.M. Elias, *Role of lipids in the formation and maintenance of the cutaneous permeability barrier.* Biochim Biophys Acta, 2014. **1841**(3): p. 280-94.

83. Martin-Venegas, R., et al., *Arachidonic acid cascade and epithelial barrier function during Caco-2 cell differentiation.* J Lipid Res, 2006. **47**(7): p. 1416-23.

84. Fumagalli, M., et al., *Greenlandic Inuit show genetic signatures of diet and climate adaptation.* Science, 2015. **349**(6254): p. 1343-7.

85. Carlson, S.J., et al., *The role of the omega-3 fatty acid DHA in the human life cycle.* JPEN J Parenter Enteral Nutr, 2013. **37**(1): p. 15-22.

86. Lapillonne, A. and S.E. Carlson, *Polyunsaturated fatty acids and infant growth.* Lipids, 2001. **36**(9): p. 901-911.

87. Colombo, J., et al., *Long-term effects of LCPUFA supplementation on childhood cognitive outcomes.* Am J Clin Nutr, 2013. **98**(2): p. 403-12.

88. Uauy, R. and A.D. Dangour, *Nutrition in Brain Development and Aging: Role of Essential Fatty Acids.* Nutrition Reviews, 2006. **64**(5): p. 24-33.

89. Gomez-Pinilla, F., *Brain foods: the effects of nutrients on brain function.* Nat Rev Neurosci, 2008. **9**(7): p. 568-78.

90. Briend, A., K.G. Dewey, and G.A. Reinhart, *Fatty acid status in early life in low-income countries--overview of the situation, policy and research priorities.* Matern Child Nutr, 2011. **7 Suppl 2**: p. 141-8.

91. Lenroot, R.K., et al., *Sexual dimorphism of brain developmental trajectories during childhood and adolescence.* Neuroimage, 2007. **36**(4): p. 1065-73.

92. Helland, I.B., et al., *Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age.* Pediatrics, 2003. **111**(1): p. e39-44.

93. Helland, I.B., et al., *Effect of supplementing pregnant and lactating mothers with n−3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age* Pediatrics 2008. **122**: p. 472–479.

94. Clandinin, M.T., J.E. Chappell, and J.E. van Aerde, *Requirements of newborn infants for long chain polyunsaturated fatty acids.* Acta Paediatr Scand Suppl, 1989. **351**: p. 63-71.

95. Jumbe, T.J., et al., *Evaluation of fatty acid and mineral content of Tanzanian seeds and oils.* Journal of Food Composition and Analysis, 2016. **50**: p. 108-113.

96. USAID. *Ghana Nutrition Profile – Nutrition and Consumer Protection Division, FAO* 2009; Available from: ftp://ftp.fao.org/ag/agn/nutrition/ncp/gha.pdf.

97. Birch, E.E.G., S. Castaneda, Y. Hughbanks-Wheaton, D. Uauy, R. Hoffman, D., *Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula.* Early Hum Dev, 2007. **83**(5): p. 279-84.

98. Voigt, R.G., et al., *Relationship between omega3 long-chain polyunsaturated fatty acid status during early infancy and neurodevelopmental status at 1 year of age.* J Hum Nutr Diet, 2002. **15**(2): p. 111-20.

99. Sheppard, K.W. and C.L. Cheatham, *Omega-6 to omega-3 fatty acid ratio and higher-order cognitive functions in 7- to 9-y-olds: a cross-sectional study.* Am J Clin Nutr, 2013. **98**(3): p. 659-67.

100. Zelazo, P.D., *The Dimensional Change Card Sort (DCCS): a method of assessing executive function in children.* Nat Protoc, 2006. **1**(1): p. 297-301.

101. Hughes, D. and J. Bryan, *The assessment of cognitive performance in children: considerations for detecting nutritional influences.* Nutr Rev, 2003. **61**(12): p. 413-22.

102. Doebel, S. and P.D. Zelazo, *A meta-analysis of the Dimensional Change Card Sort: Implications for developmental theories and the measurement of executive function in children.* Dev Rev, 2015. **38**: p. 241-268.

103. Mustapha, H. and M. Abubakari. *Ghana Statistical Service Population and housing census, Savelugu*. 2014.

104. WHO Anthro for personal computers, v., 2011, *Software for assessing growth and development of the world's children. Geneva: WHO, 2010.*

105. Harris, W.S., et al., *Comparative effects of an acute dose of fish oil on omega-3 fatty acid levels in red blood cells versus plasma: Implications for clinical utility.* Journal of Clinical Lipidology, 2013. **7**(5): p. 433-440.

106. Zelazo, P.D., et al., *The development of executive function in early childhood.* Monogr Soc Res Child Dev, 2003. **68**(3): p. vii-137.

107. Rennie, D.A., R. Bull, and A. Diamond, *Executive functioning in preschoolers: reducing the inhibitory demands of the dimensional change card sort task.* Dev Neuropsychol, 2004. **26**(1): p. 423-43.

108. Diamond Adele, S.C., Beck Danielle, *Preschool Children's Performance in Task Switching on the Dimensional Change Card Sort Task: Separating the Dimensions Aids the Ability to Switch.* Dev Neuropsychol 2005. **28**(2): p. 689–729.

109. Cohen, J., *Statistical Power Analysis for the Behavioral Sciences*. 1988: Routledge. -1.

110. Bhargava, A., et al., *Modeling the effects of health status and the educational infrastructure on the cognitive development of Tanzanian schoolchildren.* Am J Hum Biol, 2005. **17**(3): p. 280-92.

111. Ayoya, M.A., et al., *Prevalence and Risk Factors of Anemia among Children 6-59 Months Old in Haiti.* Anemia, 2013. **2013**: p. 502968.

112. WHO. *Guideline: Updates on the management of severe acute malnutrition in infants and children. Geneva: World Health Organization*. 2013.

113. Abubakari, A., G. Kynast-Wolf, and A. Jahn, *Maternal Determinants of Birth Weight in Northern Ghana.* PLoS One, 2015. **10**(8): p. e0135641.

114. Parletta, N., et al., *Effects of fish oil supplementation on learning and behaviour of children from Australian Indigenous remote community schools: a randomised controlled trial.* Prostaglandins Leukot Essent Fatty Acids, 2013. **89**(2-3): p. 71-9.

115. Campoy, C., et al., *Effects of prenatal fish-oil and 5-methyltetrahydrofolate supplementation on cognitive development of children at 6.5 y of age.* Am J Clin Nutr, 2011. **94**(6 Suppl): p. 1880S-1888S.

116. Sinn, N., C. Milte, and P.R. Howe, *Oiling the brain: a review of randomized controlled trials of omega-3 fatty acids in psychopathology across the lifespan.* Nutrients, 2010. **2**(2): p. 128-70.

117. Robinson, J.G., N. Ijioma, and W. Harris, *Omega-3 fatty acids and cognitive function in women.* Womens Health (Lond), 2010. **6**(1): p. 119-34.

118. Shaikh, S.R., *Biophysical and biochemical mechanisms by which dietary N-3 polyunsaturated fatty acids from fish oil disrupt membrane lipid rafts.* J Nutr Biochem, 2012. **23**(2): p. 101-5.

119. Liou, S. *HOPES Huntington's Disease Information*. 2010 [cited 2010; Available from: http://web.stanford.edu/group/hopes/cgi-bin/hopes_test/author/stephanie-liou/page/4/.

120. van der Wurff, I.S., et al., *Association between Blood Omega-3 Index and Cognition in Typically Developing Dutch Adolescents.* Nutrients, 2016. **8**(1).

121. Sheppard, K.W. and C.L. Cheatham, *Executive functions and the omega-6-to-omega-3 fatty acid ratio: a cross-sectional study.* Am J Clin Nutr, 2017. **105**(1): p. 32-41.

122. Harauma, A., et al., *The essentiality of arachidonic acid in addition to docosahexaenoic acid for brain growth and function.* Prostaglandins Leukot Essent Fatty Acids, 2017. **116**: p. 9-18.

123. Harris, W.S., et al., *Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: observations from 160,000 patients.* Prostaglandins Leukot Essent Fatty Acids, 2013. **88**(4): p. 257-63.

124. Wolters, M., et al., *Reference values of whole-blood fatty acids by age and sex from European children aged 3-8 years.* Int J Obes (Lond), 2014. **38 Suppl 2**: p. S86-98.

125. Valentini, K.J., et al., *The effect of fish oil supplementation on brain DHA and EPA content and fatty acid profile in mice.* Int J Food Sci Nutr, 2017: p. 1-13.

126. Kirkham, N.Z., L. Cruess, and A. Diamond, *Helping children apply their knowledge to their behavior on a dimension-switching task.* Developmental Science, 2003. **6**(5): p. 449-476.

127. A. Catherine Ross, B.C., Robert J. Cousins, Katherine L. Tucker, Thomas R. Ziegler, *Modern Nutrition in Health and Disease*. Eleventh edition ed. 2013: Lippincott Williams & Wilkins, a Wolters Kluwer business.

128. World Food programme, G., focus on Northern Ghana, *Comprehensive Food Security & Vulnerability Analysis.* 2012.

129. Engmann, C., et al., *Anemia and iron deficiency in pregnant Ghanaian women from urban areas.* Int J Gynaecol Obstet, 2008. **101**(1): p. 62-6.

130. FAO, *Fats and fatty acids in human nutrition Report of an expert consultation.* Food and agriculture organization of the united nations, 2010.

131. FAO, *FAO Fishery Country Profile - The Republic of Ghana*. 2004.

132. Hauck, J., *Fisheries in small reservoirs in northern ghana:.* Ghana journal of developmental studies, 2010. **7**(4).

133. Egbi, G., *Prevalence of vitamin a, zinc, iodine deficiency and anaemia among 2-10 year- old Ghanaian children.* African Journal of Food, Agriculture, Nutrition and Development, 2012. **12**(2).

134. Agble, R., et al., *Nutrition Country Profile: Republic of Ghana.* Nutrition and Consumer Protection Division, FAO, 2009.

135. Adu-Afarwuah, S., et al., *Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on*

growth and motor development. American Journal of Clinical Nutrition, 2007. **86**(2): p. 412-420.

136. Ackatia-Armah, R.S., et al., *Malian children with moderate acute malnutrition who are treated with lipid-based dietary supplements have greater weight gains and recovery rates than those treated with locally produced cereal-legume products: a community-based, cluster-randomized trial.* Am J Clin Nutr, 2015. **101**(3): p. 632-45.

137. Harris, W.S., et al., *Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids.* J Nutr, 2009. **139**(4): p. 804S-19S.

138. Glew, R.H., et al., *Amino acid, mineral and fatty acid content of pumpkin seeds (Cucurbita spp) and Cyperus esculentus nuts in the Republic of Niger.* Plant Foods Hum Nutr, 2006. **61**(2): p. 51-6.

139. Sadiq, M.M., et al., *Extraction and Characterization of Nigeria Shea Butter Oil.* JOSTMED, 2012. **8(2)**.

140. Stadlmayr B., C., U. R., Enujiugha, V. N., Bayili, R. G., Fagbohoun, E. G., Samb, B. et al., , *West African Food Composition Table*. 2012, FAO.

141. Dulf, F.V., *Fatty acids in berry lipids of six sea buckthorn (Hippophae rhamnoides L., subspecies carpatica) cultivars grown in Romania.* Chem Cent J, 2012. **6**(1): p. 106.

142. Gutierrez, L.F., C. Ratti, and K. Belkacemi, *Effects of drying method on the extraction yields and quality of oils from quebec sea buckthorn (Hippophae rhamnoides L.) seeds and pulp.* Food Chemistry, 2008. **106**(3): p. 896-904.

143. Cequier-Sanchez, E., et al., *Dichloromethane as a solvent for lipid extraction and assessment of lipid classes and fatty acids from samples of different natures.* J Agric Food Chem, 2008. **56**(12): p. 4297-303.

144. Jumbe, T., et al., *Evaluation of fatty acid and mineral content of Tanzanian seeds and oils.* Journal of Food Composition and Analysis, 2016. **50**: p. 108-113.

145. Agren, J.J., A. Julkunen, and I. Penttila, *Rapid Separation of Serum-Lipids for Fatty-Acid Analysis by a Single Aminopropyl Column.* J Lipid Res, 1992. **33**(12): p. 1871-1876.

146. Gurzell, E.A., et al., *Is the omega-3 index a valid marker of intestinal membrane phospholipid EPA+DHA content?* Prostaglandins Leukot Essent Fatty Acids, 2014.

147. Prentice, A., et al., *Calcium supplementation increases stature and bone mineral mass of 16- to 18-year-old boys.* J Clin Endocrinol Metab, 2005. **90**(6): p. 3153-61.

148. Mahesh S, P.G., 1 Parvathi MS,1 Rajashekara Reddy1and Shankar A G1, *Effect of processing on phytic acid content and nutrient availability in food grains* International Journal of Agricultural Sciences, 2015. **5**.

149. Stadlmayr, B., et al., *West African Food Composition Table.* INFOODS, FAO, 2012.

150. US Department of Agriculture, A.R.S., *National Nutrient Database for Standard Reference Release 27*. 2015, US Government: Beltsville, MD.

151. Black, R.E.A., Lindsay H. Bhutta, Zulfiqar A. Caulfield, Laura E. de Onis, Mercedes Ezzati, Majid Mathers, Colin Rivera, Juan, *Maternal and child undernutrition: global and regional exposures and health consequences.* The Lancet, 2008. **371**(9608): p. 243-260.

152. Hibbeln, J.R., et al., *Healthy intakes of n-3 and n-6 fatty acids: estimations considering worldwide diversity.* Am J Clin Nutr, 2006. **83**(6 Suppl): p. 1483S-1493S.

153. Service, G.S., *Population and housing census, Upper Manya Krobo District. Data analytical reporting.* 2014.

154. Ahun, M.N., et al., *Child development in rural Ghana: Associations between cognitive/language milestones and indicators of nutrition and stimulation of children under two years of age.* Can J Public Health, 2018. **108**(5-6): p. e578-e585.

155. Nakamura, M.T. and T.Y. Nara, *Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases.* Annu Rev Nutr, 2004. **24**: p. 345-76.

156. Adjepong, M., et al., *Association of whole blood n-6 fatty acids with stunting in 2-to-6-year-old Northern Ghanaian children: A cross-sectional study.* PLoS One, 2018. **13**(3): p. e0193301.

157. Adjepong, M., et al., *Whole blood n-3 fatty acids are associated with executive function in 2 to 6-year-old Northern Ghanaian children.* Journal of Nutritional Biochemistry, 2018.

158. Andrew J. Sinclaira, Nadia M. Attar-Bashia, and Duo Lib, *What Is the Role of α-Linolenic Acid for Mammals?.* Lipids, 2013. **37**(12).

159. Nations, U. *Ghana Nutrition Profile: Nutrition and Consumer Protection Division, Food and Agriculture Organization* 2009; Available from: ftp://ftp.fao.org/ag/agn/nutrition/ncp/gha.pdf.

160. Burrows, T.C., C. E.Garg, M. L., *Omega-3 index, obesity and insulin resistance in children.* Int J Pediatr Obes, 2011. **6**(2-2): p. e532-9.

161. Montgomery, P.B., J. R.Sewell, R. P.Spreckelsen, T. F.Richardson, A. J., *Low blood long chain omega-3 fatty acids in UK children are associated with poor cognitive performance and behavior: a cross-sectional analysis from the DOLAB study.* PLoS One, 2013. **8**(6): p. e66697.

162. Wolters, M.S., H.Foraita, R.Galli, C.Rise, P.Moreno, L. A.Molnar, D. et al.,, *Reference values of whole-blood fatty acids by age and sex from European children aged 3-8 years.* Int J Obes (Lond), 2014. **38 Suppl 2**: p. S86-98.

163. Carlson, A.L.S.E., *Polyunsaturated Fatty Acids and Infant Growth.* Lipids, 2001. **36**(9).

164. Hosomi, R., M. Yoshida, and K. Fukunaga, *Seafood consumption and components for health.* Glob J Health Sci, 2012. **4**(3): p. 72-86.

165. Clarisse Vilain, E.B., Gloria Gallego and Saray Samadee, *Fish and the Nutrition of Rural Cambodians.* Asian Journal of Agriculture and Food Sciences 2016. **04**(01).

166. Jimbin Mai, J.E.K.e.a., *Protein and amino acid composition of select freshwater fish.* Journal of Agric and Food Chemistry, 1980. **28**.

167. Semba, R.D.S., M. Sakr Ashour, F. A. et al.,, *Child Stunting is Associated with Low Circulating Essential Amino Acids.* EBioMedicine, 2016. **6**: p. 246-252.

168. WHO, *Guideline:Updates on the management of severe acute malnutrition in infants and children.* . 2013, Geneva Switzerland: World Health Organization.

169. Khan, N.A., et al., *The relation of saturated fats and dietary cholesterol to childhood cognitive flexibility.* Appetite, 2015. **93**: p. 51-6.

170. Ross, A.C., et al., *Modern Nutrition in Health and Disease*. 11 ed. 2012: Lippincott Williams & Wilkins.

171. Ghana Statistical Service (GSS), G.H.S.G., and ICF Macro. , *Ghana Demographic Health survey, 2008.* Accra, Ghana: GSS, GHS, and ICF Macro, 2009.

172. Jumbe, T., Pickens, C.A., Valentini, K., Adjepong, M., Li, W., Kinabo, J., Fenton, J., *Evaluation of fatty acid and mineral content of Tanzanian seeds and oils.* Journal of Food Composition and Analysis, 2016. **50**: p. 108-113.

173. Authors, A., *Official methods of analysis Minerals Analysis Ca, Cu, Fe, Mn, P, K, Na and Zn Infant Formula - item 62.* Association of Analytical Communities, 2006.

174. Authors, A., *Official methods of analysis Minerals Analysis Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) - item 63.* Association of Analytical Communities, 2006.

175. Adu-Afarwuah, S., et al., *Acceptability of lipid-based nutrient supplements (LNS) among Ghanaian infants and pregnant or lactating women.* Matern Child Nutr, 2011. **7**(4): p. 344-56.

176. Arimond, M., et al., *Considerations in developing lipid-based nutrient supplements for prevention of undernutrition: experience from the International Lipid-Based Nutrient Supplements (iLiNS) Project.* Matern Child Nutr, 2015. **11 Suppl 4**: p. 31-61.

177. Hess, S.Y., et al., *Acceptability of zinc-fortified, lipid-based nutrient supplements (LNS) prepared for young children in Burkina Faso.* Matern Child Nutr, 2011. **7**(4): p. 357-67.

178. Resnick D., B., S. C., Haggblade, S., Hendricks, S. and Mather, D. , *Conceptualizing-Drivers-of-Policy-Change-in-Agriculture-Nutrition-and-Food-Security-The-Kaleidoscope-Model.* IFPRI Discussion Paper Washington DC: International Food Policy Research Institute, 2015. **1414.**

179. UNICEF, *UNICEF annual report 1990*. 1990: New York: United NAtion's Children's Fund.

180. UNICEF, *Iodizing salt: A Health Policy: The Costa Rica Experience. San Jose, .* 2013: Costa Rica: Minstry of health.

181. UNICEF, *Vitamin A supplementation: A decade of progress*. 2007: New York, USA.

182. Steven Haggblade, S.B., Jody Harris,Elizabeth Mkandawire, Dorothy Nthani and Sheryl L. Hendriks, *Drivers of Micronutrient Policy Change in Zambia:An Application of the Kaleidoscope*. 2015, IFPRI.

183. Multiple Indicator Cluster Survey: Monitoring the situation of child, w.a.m., *Multiple Indicator Cluster Survey:*. 2006, UNICEF: Ghana.

184. Service, G.H., *National Nutrition Policy.* 2016.

185. Ghartey, A.B., *Nutrition Policies and Programs in Ghana the limitation of a single sector approach.* The World bank 2010.

186. WHO., *Guideline: Vitamin A supplementation in postpartum women. World Health Organization.* 2011.