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Whole blood n-3 fatty acids are associated with executive function in 2–6-year-old Northern Ghanaian children

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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=.06), total n-3 (β=0.17, P=.06) and dihomo-gamma-linolenic acid (DGLA; β=0.60, P=.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.

Keywords: Cognition; Executive function; Fatty acids; Lipids; Essential fatty acids; DCCS; Ghana

1. Introduction

Essential fatty acids (EFAs) and their long chain metabolites have crucial roles in human growth, both in fetal and neonatal development [1–3]. They accumulate in the fetus during pregnancy and during early childhood [1]. Long chain polyunsaturated fatty acids (LCPUFA) are also concentrated in the central nervous system [4] playing significant roles in neuronal growth and differentiation of cells and have been associated with cognitive abilities [4–6]. In addition, the brain and retinal function are highly dependent on EFAs, especially for membrane fluidity and signal transduction [7]. Due to these crucial roles of LCPUFAs, poor PUFA status may affect brain development and cognitive abilities in children [6]. 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 1200 g [1]. Maximal cerebral volume is achieved between 10–15 years of age, but 95% is reached by six years of age [8]. Thus, LCPUFA should be included in the diets of infants and children to ensure optimal brain development [9–11].

EFAs can be found in foods such as peanut and soybean oil, walnuts, fish, eggs, poultry and whole grains ([12,13], 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 [14], 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 [15], verbal learning, memory [16], progress in myelination, mental and motor development [17] as well as influencing neurological development status [18]. 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

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; BAZ, BMI-for-age z-score; BMI, body mass index; CHP, Community health post; DBS, dried blood spot; DCCS, Dimensional change card sort; DGLA, Dihomo gamma linolenic acid; DHA, docosahexaenoic acid; EFA(s), essential fatty acid(s); EFAD, essential fatty acid deficiency/deficient; EPA, eicosapentaenoic acid; FA (s), fatty acid(s); Hb, hemoglobin; LA, linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; mos, months; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acid.

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EPA-rich fish oil correlated with higher plasma levels of DHA and EPA in 7–9 year olds [16]. 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 [19] 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 years old [20], 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 [21] is the conscious control of thoughts and actions. It develops in children between the ages of two and ten years [21]. The frontal and temporal lobes of the brain controls executive function [19]. These two regions of the brain contain high amounts of AA and DHA and continue to develop after the second year of life [22]. The dimensional change card sorting (DCCS) task is a validated method commonly used to provide a unitary measure of executive function in young children [21,23]. In this study, we utilized the DCCS task to assess executive function in Ghanian children age 2–6. Little is known about cognitive function assessment in the Ghanaian population as well as the association with FAs. In this study, we assessed 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.

2. Methods

2.1. 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 [24].

2.2. 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.

2.3. Anthropometric measurements

Heights of all participants were measured to the nearest 0.1 cm with a stadiometer (Seca, USA). Weight was measured using a digital bathroom scale to the nearest 0.1 kg (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 [25] and WHO AnthroPlus [26] software to calculate height-for-age (HAZ), weight-for-age (WAZ), and BMI-for-age (BAZ) z-scores.

2.4. Blood fatty acid assessment

Blood spots (40ul) were collected on a dried blood spot card (DBS) as previously described in Jumbe et al., 2016 [27]. 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 to the US lab was eight days. The samples were stored at −20°C and analyzed as previously described [28–30]. 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 min. After cooling, 40 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 [31–33]. Unless otherwise stated, whole blood FA proportions are expressed as a percent of total identified FAs.

2.5. 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).

2.6. Cognitive assessment: dimensional change card sort (DCCS)

The DCCS [21,34] 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 Supplementary Fig. 1). 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) [21,35]. 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 [20,35,36]. 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 Supplementary Fig. 1) [20]. The total number of DCCS test conditions passed was used as an index of executive function [20]. 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.

2.7. 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 [37] 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 [38] 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 [39]. 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, $P<.05$ was used to define statistical significance.

Table 1
Charateristics of all children who attempted the DCCS test (n=307).

	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)[a]	11.5	6.79	8.40-13.6
BAZ[b]	−0.38	0.79	−2.82-1.83
HAZ[c]	−1.34	1.13	−4.28-2.43
WAZ[d]	−1.12	0.86	−3.40-1.60

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

3. Results

In this study, 313 children between 2 and 6 years of age were enrolled. Six children with outlier myristic acid values were excluded from the study. Demographic information for all 307 participants is shown in Table 1. 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 13.9 kg. The mean hemoglobin level of 11.5 was within the normal range [40]. Z-scores were used to calculate the prevalence of stunting (HAZ), malnutrition (BAZ) and wasting (WAZ). According to WHO standards [41], 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 2). 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

Table 2
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) Mean ± SE	Fail (n=154)	*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)[a]	11.2±1.07	10.8±0.93	**0.001**
	BAZ[b]	−0.53±0.06	−0.23±0.06	**0.001**
	HAZ[c]	−1.21±0.09	−1.47±0.09	**0.013**
	WAZ[d]	−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[2]	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[3]	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[4]	3.67±0.09	3.54±0.08	0.196

The WHO definitions of moderate and severe stunting, wasting, underweight and malnutrition were applied to the data (ref). [2]Total SFA includes myristic, palmitic, arachidic, behenic, lignoceric; [3]Total n-6 includes linoleic, linolaidic, ϒ-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6; [4]Total n-3 includes alpha linolenic, eicosapentaenoic, docosapentaeonic n-3, and docosahexaenoic;
[a] HB, hemoglobin;
[b] BAZ, BMI-for-age z-score;
[c] HAZ, height-for-age z-score;
[d] WAZ, weight-for-age z-score.

Table 3
[1]Whole blood fatty acid proportions in Ghanaian children (Mean ± SE, n=307)

Class	Fatty acid	Mean ± SE	Range
	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
SFA	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
	Alpha-linolenic	0.18±0.01	0.04–0.78
	Eicosapentanoic	0.22±0.01	0.05–3.11
n-3 FA	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
	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
n-6 FA	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
	Mead acid	0.14±0.003	0.04–0.39
	Oleic	21.3±0.16	15.7–31.2
n-9 FA	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
	SCD n-7	0.02±0.001	0.003–0.05
	SCD n-9	1.61±0.02	1.01–3.40
Desaturases	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
	AA/EPA ratio	65.3±1.82	2.71–217.2

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.
[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, ϒ-linolenic, eicosadienoic, di-homo-gamma-linolenic, arachidonic, docosatetraenoic, docosapentaenoic n-6;
[5] Total n-9 includes oleic, elaidic, eicosanoic, Mead, nervonic.

Table 4
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)

Class	Fatty acid	Regression results for total pass (n=307)	
		B ± SE	*P*
	Myristic	−0.18±0.72	.80
	Lignoceric	−0.17±0.26	.50
	Palmitelaidic	−5.38±3.13	**.09**
SFA	Palmitic	−0.03± 0.05	.53
	Behenic	−0.43±0.44	.33
	Arachidic	−0.97±1.24	.44
	Total SFA[a]	0.00±0.07	.96
	Alpha-linolenic	1.04±0.70	.14
	Eicosapentanoic	0.27±0.32	.40
n-3 FA	Docosahexaenoic	0.25±0.13	**.06**
	Omega-3 Index	0.17±0.09	**.07**
	Total n-3[b]	0.17±0.09	**.07**
	Linoleic	−0.02±0.04	.60
	Arachidonic	0.02±0.05	.63
	GLA	0.94±1.21	.44
n-6 FA	DGLA	0.60±0.32	**.06**
	Docosatetraenoic	0.11± 0.22	.61
	Eicosadienoic	0.12±1.19	.92
	Total n-6[c]	0.01±0.03	.84
	Mead acid	−0.81±1.55	.60
	Oleic	−0.02±0.03	.55
n-9 FA	Eicosenoic	0.11±0.79	.89
	Nervonic	−0.15± 0.37	.69
	Total n-9[d]	−0.02±0.03	.44
	SCD n-7	−0.46±9.39	.96
	SCD n-9	−0.21±0.23	.35
Desaturases	D6D	10.04±5.62	**.08**
	D5D	−0.09±0.06	.10
	Palmitoleic	−0.02±0.41	.96
	Total MUFA	−0.02±0.03	.44
Other	Total PUFA	0.02±0.03	.43
	T/T ratio	−9.76±15.4	.53

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.
[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.

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 FAs (P=.02), but higher n-6 DGLA (P=.01) and arachidonic acid (P=.01), as well as higher n-3 DHA (P=.05).

Whole blood fatty acid levels of the 307 children whose data were analyzed are presented in Table 3. 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 4. 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≤.001). DHA (β=0.40, P=.02) and ALA (β=0.14, P=.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<.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=.02) and ALA (β=2.75, P=.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.

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 5. Multiple linear regression using the four factors showed no factor to be significantly associated with performance on the DCCS tasks (Table 6). 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.

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 7. Children with highest DHA levels per the category (DHA>4.0%) were four times more likely to pass the initial phase of the

Table 5
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
DPAn3	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.

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).

4. 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

Table 6
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	.41
Factor 2	0.002	0.001	.98
Factor 3	−0.09	−0.05	.27
Factor 4	0.08	0.05	.33
Age	0.08	0.56	<**.01**[*]
HB	0.02	0.01	.78
BMI-for-age (BAZ)	−0.22	−0.10	**.04**[*]

[*] All significant associations (P<.05).

[†] Model: total passes = factor 1 + factor 2 + factor 3 + factor 4 + Age + Hb concentration + BMI-for-age z-score; model P value, P<.001; r^2 0.369; adjusted r^2 0.354.

Table 7
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]**	

Polytomous logistic regression was used to regress BMI category on FA tertiles. All data is 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≤.05 or italicized is P≤.09 and >.05.

[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.

that contained DHA, AA and LA, there was an improvement in visual acuity and IQ maturation [15]. 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 [42]. Also, there was an improvement in short term memory when children 6 to 10 years old were supplemented with ALA, DHA, LA and micronutrients [43]. 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 [44]. 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 [45]. The mechanisms by which DHA and n-3 FA affects cognition include myelination of axons [17].

DHA and n-3 FAs influences membrane fluidity, neurotransmitter receptor activity and nutrition of nerve cells [46,47], as well as disruption of lipid rafts that affect signal transduction pathways [48]. 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 [48]. Rigid membranes do not allow adequate nutrients to get into the cells and the arrangement of the cellular domains is dependent of the presence of double bonds [49]. 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 [49], 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, 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 [45]. 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 [50], 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 [51], children with higher n-6 s and lower n-3 s performed poorly on the DCCS test. Sheppard and Cheatham, 2017 [51] 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 [51].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 [51]. In addition, in mice, supplementation with only DHA, (an n-3 FA) or AA (an n-6 FA) was insufficient for optimal development [52] 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; n-3 levels increasing over a lifetime while n-6 levels decrease [53]. While these results reflect changes in FA levels over decades, there was no significant difference in FA levels for different age categories within the small age range in 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 [54].

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 [55] 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 [20], USA [56] and Scotland [35]. This investigation builds off our prior work in the Tanzanian population [20], 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 [24]. 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.

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Conflict of interest

No authors report a conflict of interest except WSH whose laboratory (OmegaQuant Analytics, LLC) performed the DBS FA analyses.

Authorship

MA, MBP, RAA and JIF designed the research. MA and RAA conducted the research. WSH, MBP, and JIF provided essential materials necessary for the research. MA, WY, MBP and JIF analyzed the data. MA, WY and JIF wrote the manuscript. All authors made contributions to the manuscript, but JIF has primary responsibility for the final content. All authors contributed to the critical interpretation and writing of the paper and approved the final version.

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Figure S1

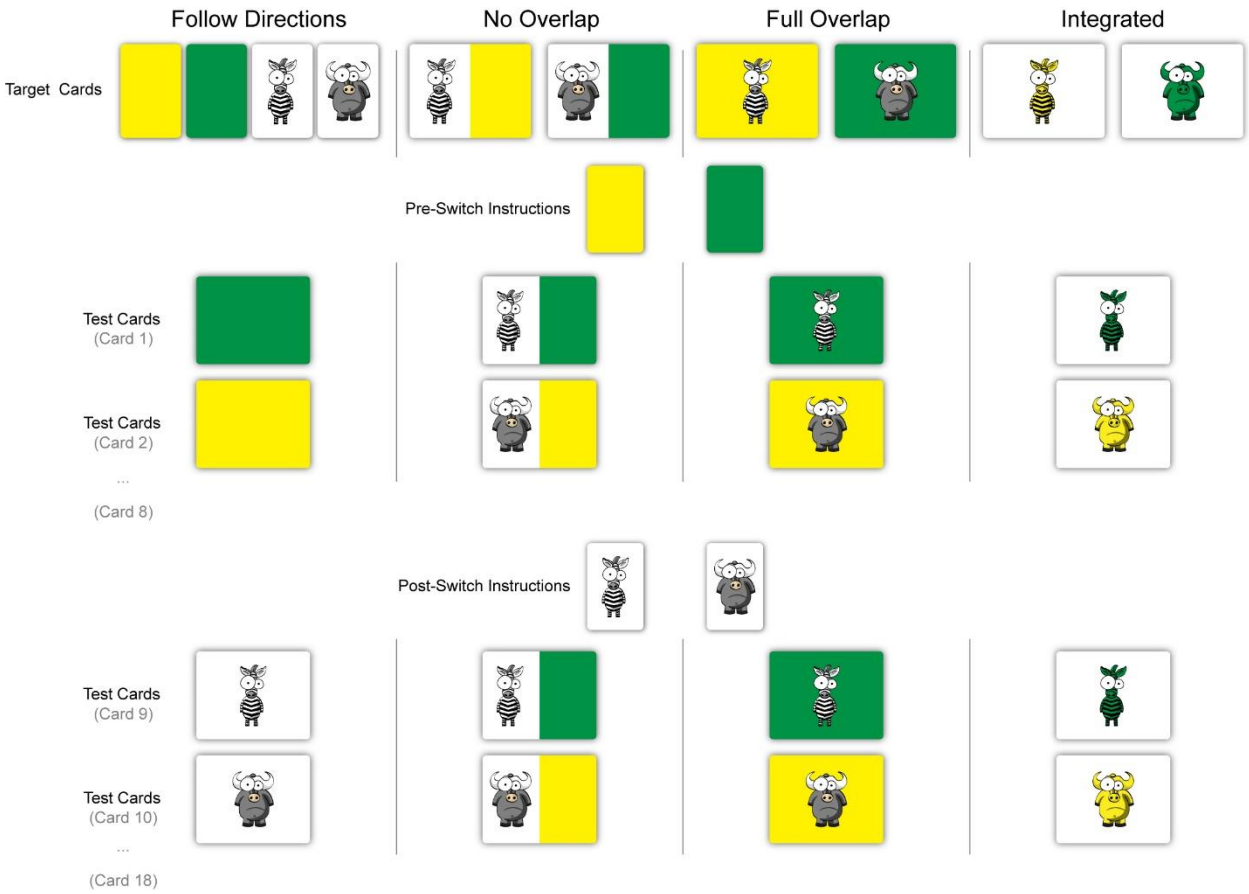

Figure. S1. Illustration of the dimensional change card sort test conditions.