Diet-induced changes in iron and *n*-3 fatty acid status and associations with cognitive performance in 8–11-year-old Danish children: secondary analyses of the Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet School Meal Study

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Abstract

Fe and *n*-3 long-chain PUFA (*n*-3 LCPUFA) have both been associated with cognition, but evidence remains inconclusive in well-nourished school-aged children. In the Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet (OPUS) School Meal Study, the 3-month intervention increased reading performance, inattention, impulsivity and dietary intake of fish and Fe. This study investigated whether the intervention influenced *n*-3 LCPUFA and Fe status and, if so, explored how these changes correlated with the changes in cognitive performance. The study was a cluster-randomised cross-over trial comparing school meals with packed lunch (control). At baseline and after each treatment, we measured serum ferritin, whole-blood *n*-3 LCPUFA and Hb, and performance in reading, mathematics and d2-test of attention. Data were analysed using mixed models (*n* 726) and principal component analysis of test performances (*n* 644), which showed two main patterns: 'school performance' and 'reading comprehension'. The latter indicated that children with good reading comprehension were also more inattentive and impulsive (i.e. higher d2-test error%). The intervention improved 'school performance' (*P* = 0·015), 'reading comprehension' (*P* = 0·043) and EPA + DHA status 0·21 (95 % CI 0·15, 0·27) w/w % (*P* < 0·001), but it did not affect serum ferritin or Hb. At baseline, having small Fe stores was associated with poorer 'school performance' in girls, but with better 'reading comprehension' in both boys and girls. Both baseline EPA + DHA status and the intervention-induced increase in EPA + DHA status was positively associated with 'school performance', suggesting that *n*-3 LCPUFA could potentially explain approximately 20 % of the intervention effect. These exploratory associations indicate that increased fish intake might explain some of the increase in reading performance and inattention in the study.

Key words: School meals: Iron: *n*-3 PUFA: Cognitive performance: School performance

Fe deficiency (ID) is one of the most prevalent nutrient deficiencies in the world, but it is mainly seen in high-risk groups – that is, infants, preschool-aged children and women[1]. Similarly, there is a potential risk of insufficient *n*-3 long-chain PUFA (*n*-3 LCPUFA) status in otherwise well-nourished children, as dietary intake is lower than recommended and endogenous synthesis is likely to be insufficient[2,3]. However, Fe and *n*-3 LCPUFA are important nutrients for optimal brain development in childhood[4]. DHA (22 : 6*n*-3) is accumulated in the brain, and it has been shown to have an important role in neuronal growth, differentiation, myelination and monoamine neurotransmission[5,6]. Fish is the main source of *n*-3 LCPUFA, and maternal fish intake and blood DHA status during pregnancy has been associated with offspring neurological and cognitive development[7–10]. Trials investigating maternal fish oil supplementation during pregnancy and lactation indicate

that early *n*-3 LCPUFA intake may have beneficial effects on visual and cognitive development in early life[11]. In adolescents, fish consumption has been shown to correlate with better school grades[12,13]. Nonetheless, evidence is limited in school-aged children, and studies including biomarkers of *n*-3 PUFA status in children are few[11,14]. Fe is required for neuronal growth, for the synthesis of neurotransmitters and myelin, as well as for the formation of Hb to ensure adequate oxygen transport in the body[15,16]. As Fe is preferentially directed towards synthesis of Hb when the body is short of Fe, the brain may become Fe-depleted when intake is marginal, even if the individual is not anaemic[17]. In school-aged children, cognitive function is impaired by having ID with anaemia (IDA), but the effects of ID without anaemia are uncertain[18]. Fe supplementation may improve attention and concentration irrespective of baseline Fe status, whereas intelligent quotient (IQ) only seems to improve in children who were initially anaemic, and there is no effect on memory, psychomotor function and scholastic achievement[19]. However, the influence of Fe status on cognitive performance has not been studied in populations with low prevalence of ID.

School meal programmes have the potential to improve the diet of children from various socio-economic backgrounds. We have previously shown that a 3-month school meal intervention based on a Nordic Diet increased dietary intake of Fe and fish[20], improved reading performance and increased the percentage of errors in the d2-test of attention (i.e. increased inattention and impulsivity) in 8–11-year-old Danish children in the Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet (OPUS) School Meal Study[21]. In this paper, we will identify patterns in test performance and perform a secondary outcome evaluation of the effect of the school meal intervention on Fe and *n*-3 LCPUFA status, as well as the identified test performance patterns. In addition, we will explore whether baseline Fe and *n*-3 LCPUFA status were associated with cognitive test performance, and when possible examine whether the changes in Fe and/or *n*-3 LCPUFA status correlated with the concurrent changes in test performance.

Methods

The OPUS School Meal Study was a cluster-randomised trial with cross-over design comparing 3 months of school meals based on the New Nordic Diet with packed lunch (control) on dietary intake, nutrient status, cognitive performance, early disease risk markers, growth, well-being and school attendance in Danish third- and fourth-grade children (i.e. age 8–11 years). The design and methods of the study has been described previously[22]. Briefly, the study took place at nine schools, which were located in the eastern part of Denmark, had available kitchen facilities and ≥4 classes at the third- or fourth-grade level. Schools were allocated to the order of intervention and control in clusters of year group within each school. The computer-generated randomisation was performed so that at each school either third- or fourth-grade pupils had the intervention in the first study period, whereas the other year-group had the

intervention in the second study period. The allocation order was not blinded. All 1021 children from third and fourth grade at the participating schools were invited to participate. Individual children were only excluded if the child (1) had diseases or conditions that might obstruct the measurements or put the child at risk when eating the intervention meals or (2) participated in other scientific studies that involved radiation or blood sampling. Participants were recruited in May–October 2011 and data were collected during August 2011–June 2012. 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 Committees on Biomedical Research Ethics for the Capital Region of Denmark (H-I-2010–124) and registered at www.clinicaltrials.gov (NCT 01457794). Written informed consent was obtained from the custody holders of all participating children (82 % of invited)[22].

Intervention

During the school meal intervention period, an *ad libitum* lunch meal, a mid-morning snack and an afternoon snack were served on each school day. The recipes for the school meals were in line with the Nordic recommendations for a healthy dietary intake[23] and contained energy corresponding to 40–44 % of the daily energy requirement of an 11-year-old boy. A 3-week lunch menu was developed for each season in line with the guidelines for the New Nordic Diet[24]. Each menu had soup on Mondays, meat on Tuesdays, vegetarian food on Wednesdays, fish on Thursdays and food from the first 4 d were served in a buffet on Fridays. Hence, fish was served twice a week. In each season, the 3-week menu included one fatty and two lean fish types (autumn: sea trout, ling, cod/winter: salmon, hake, cod/spring: salmon, pollock, cod). The specific fish served during the intervention period was not the same for all children because schools started successively and the menu depended on the season. The intervention meals were prepared at each school by kitchen staff employed for the study. In small teams, children participated in cooking, tasting, presenting and serving the lunch meal to their peers two to three times during the intervention period. Children were encouraged to taste all parts of the meal and to keep a reasonable plate distribution. The lunch meal was served in a common dining area at seven schools and in the classroom at two schools, and the lunch break was increased to 20–25 min. In the control period, children continued their usual lunch habits, which is usually a lunch pack from home that was consumed in the classroom during a 15-min break.

Data collection

Socio-demographic information. Information on parental education was obtained during an interview with the custody holder(s) before the start of the study. Household education was based on the parent with the highest education in the household and categorised as follows: (1) lower secondary education or less (≤10 years); (2) upper secondary education or equivalent; (3) vocational education; (4) short academic

education; (5) Bachelor's degree or equivalent; and (6) Master's degree or higher (≥17 years). A child was categorised as immigrant/descendant if his/her grandparents and ≥1 parent were born outside Denmark. Pubertal stage was based on Tanner stages for breast development in girls and pubic hair in boys[25].

Physical activity and dietary intake. Children's physical activity and food and beverage intake were assessed for a 7-d period at baseline, and at the end of each period (visits 2 and 3). Physical activity was measured for a 7-d period by an Actigraph™ accelerometer (GT3X+ or GT3X). Data on total physical activity (counts/min) were expressed as total vertical counts divided by measured wear-time[26] and were only included in this paper if valid data were available from at least one weekday and one weekend day. Dietary intake was assessed using a validated Web-based Dietary Assessment Software for Children[27] and estimated as an average of the days recorded. Under-reporters and over-reporters were identified using mean reported energy intake (EI) divided by BMR[28,29], and only acceptable reporters ($1{\cdot}05 < EI/BMR < 2{\cdot}29$) with data from at least 4 d were included in analyses of dietary intake. We included the estimated dietary intake of EPA and DHA even though intake might be underestimated because the Danish Food Composition Databank lacks fatty acid data on a few fish types (squid, kippered herring, turbot, canned tuna in water, sea trout and hake), of which especially tuna, sea trout and hake are commonly consumed in Denmark. Each day the child was asked about any use of supplements, and the use of supplements containing *n*-3 LCPUFA was categorised as ≥1 time/week or 0 times/week. Dietary intake did not include supplements.

Anthropometry. At baseline and at the end of each period (visits 2 and 3), weight was measured after an overnight fast to the nearest 0·1 kg (Tanita BWB 800 S; Tanita) and the mean height was calculated from three consecutive measurements to the nearest 0·1 cm (Tanita; CMS Weighing Equipment Ltd). Age- and sex-specific cut-offs were used to categorise children as underweight, normal weight, overweight or obese[30,31].

Biomarkers of iron and n-3 *PUFA status*. A 35-ml fasting blood sample was taken by venepuncture on the same morning as the anthropometric measurements. Serum was obtained by centrifugation at 2500 ***g*** at 4°C for 10 min after 30 min of coagulation at room temperature. Whole-blood samples with antioxidant and serum samples were stored at −80°C for later analysis.

Whole-blood Hb was analysed immediately after sampling on a Hemocue Hb 201+ (HemoCue Danmark). Concentrations are presented in g/l (nmol/l × 16·114). Serum ferritin and plasma C-reactive protein (CRP) were analysed at Klinisk Biokemisk Afdeling, Gentofte Hospital. Ferritin concentration was assessed using the ADVIA Centaur XP chemiluminescence immunoassay (Siemens Healthcare). High-sensitivity CRP was analysed with an immunochemical assay using Vitros 5.1 FS (Ortho-Clinical Diagnostics, Johnson & Johnson Medical). Values for serum ferritin were not used in analyses if CRP > 5 mg/l

(baseline: *n* 55, visit 2: *n* 80, visit 3: *n* 76). ID was defined as serum ferritin <15 µg/l and anaemia was defined as an Hb concentration < 115 g/l[32]. Children were classified with IDA if serum ferritin was <15 µg/l and Hb was <115 g/l. The serum ferritin level correlates with relative total body Fe stores[32], and as few children were ID we additionally present the baseline proportion of children with ferritin values in the lower range: 15–25 µg/l. In line with this, the associations between small Fe stores and cognitive scores were explored by comparing children with ferritin ≤25 µg/l with children with ferritin >25 µg/l. The inter- and intra-assay CV were 3·1 and 4·5 % for ferritin and 1·3 and 2·9 % for CRP, and the inter-assay variation for Hb was 1·2 %.

Whole-blood fatty acid composition was analysed within 3 months of blood sampling at Department of Kinesiology, University of Waterloo, Canada using high-throughput GC. As previously described, fatty acid methyl esters were prepared from whole-blood samples by direct *trans*-esterification with convectional heat[33]. Briefly, whole-blood fatty acids including an internal standard (22 : 3*n*-3 ethyl ester; Nu-Check Prep) were *trans*-methylated by boron trifluoride in methanol (Pierce Chemicals) in the presence of 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene; Sigma-Aldrich) for 60 min at 90°C. Separation was achieved by the addition of water and hexane, and the fatty acid methyl esters were collected for analysis on a Varian 3900 GC equipped with a DB-FFAP capillary column (15 m × 0·10 mm i.d. × 0·10 µm film thickness; J&W Scientific from Agilent Technologies). Mean total whole-blood fatty acids amounted to 226 (SD 38) µg/100 µl (range 44–365 µg/100 µl; *n* 758) at baseline. Individual fatty acids were expressed as w/w % of total fatty acids in whole blood. The inter- and intra-assay CV were 4·5 and 1·2 % (EPA) and 6·4 and 2·4 % (DHA).

Cognitive performance. As previously described[21], the d2-test of attention and Danish standard tests in reading and maths were administered at baseline and at the end of each period (visits 2 and 3) on three separate weekdays during the same week as assessment of physical activity and dietary intake. Each test was administered on the same weekday and time of day at each occasion. Briefly, the d2-test consists consists of fourteen rows that each contains forty-seven test items; each test item was either a 'd' or a 'p' with zero to four dashes above and/or below[34,35]. Project staff instructed the children to mark as many target items (a 'd' with a total of two dashes) as possible within 20 s for each line. Concentration performance (CP) was defined as the number of correctly marked target items minus errors of commission (incorrectly marked distractor items). Processing speed was defined as the total number of items processed. Inattention was defined as the percentage of errors of omission (unmarked target items divided by processing speed), impulsivity was defined as the percentage of errors of commission[36] and inattention and impulsivity were summarised to the total percentage of errors (d2-error%). The sentence reading test and maths test were administered by the teacher in Danish and maths, respectively. The test consists of twenty-seven drawings of a situation, each accompanied by four sentences[37,38]. The children had 8 min to evaluate as many sentences as possible

for whether the statement matched with the situation in the drawing. Outcome parameters were the total number of sentences read (reading speed), the number of correct sentences (number correct) and the percentage of sentences that were correct out of the total number of sentences read (%correct). In mathematics, there was a specific test for third-grade students, which consisted of fifty problems, and a fourth-grade test that had sixty-nine problems[37,38]. Children were given one lesson (45 min including instructions) to solve as many problems as possible. The outcome parameter was the number of correctly solved problems.

Sample size

The sample size of the study was derived from the sample size calculation for the primary outcome 'metabolic syndrome score' reported by Damsgaard *et al.*[39]. Specifically, the detectable difference was obtained using the standard paired *t* test equation assuming a between-child correlation coefficient of 0·5. This calculation resulted in a required sample size of 673 children in order to detect a relevant difference of 0·11 SD between the intervention and control diets ($\alpha = 0.05$, $\beta = 0.80$)[22,40].

Statistical methods/analyses

The data are presented as mean values and standard deviations for continuous variables that were approximately normally distributed and as median and interquartile range for skewed variables. Correlations between continuous variables were evaluated using Pearson's ρ or Spearman's rank correlation. Student's *t* test was used to test for group differences in continuous variables and a rank-sum test was used for ordinal variables.

A principal component analysis (PCA) was used to derive patterns (principal components (PC)) in test performance, on the basis of outcomes from the d2-test (CP, processing speed, error%), the reading test (number read, number correct, %correct) and the maths test (number correct). PCA was performed on baseline data, as well as on data from the three time points using LatentiX 2.11. The number of components was chosen on the basis of the scree plot. A score for each of the identified components was generated for each child at each time point. The components were named after the most negative loading, and a lower score thus indicates that the child's performance was characterised by this type of performance. To focus on the overall effects of the intervention, the PC scores were considered as main outcome variables of the present paper and analysed along with the individual outcome scores.

Hierarchical mixed-effects models were applied for statistical analyses of intervention effects and of correlations between biomarkers and cognitive test outcomes. Linear mixed models were used for continuous outcomes (biomarkers, dietary intake, CP, processing speed and overall test performance patterns (PCA components)) and logistic mixed-effects models were used for binary outcomes and discrete outcomes with an upper limit (all other outcomes). In cross-sectional baseline analyses, school, year group (within each school) and class were included as random effects in order to account for nonindependence (clustering). In analyses of intervention effects, individual was additionally included as random effect to consider repeated measurements. The effects of the intervention on dietary intake, biomarkers and test performance patterns were evaluated in a model in which the dependent variable was the respective outcome at visit 2 and/or 3. The intervention effect model was adjusted for baseline value of the dependent variable, visit, the order of the intervention and control period, sex, household education, year group, baseline age and month of baseline test. Analyses of intervention effects on biomarkers also evaluated potential interactions between sex, order-of-treatment and baseline biomarker value in quartiles.

For cross-sectional analyses of associations between baseline test performance (dependent variable) and serum ferritin status (≤ 25 or above 25 µg/l) or *n*-3 LCPUFA status (continuous variable), the model was adjusted for sex, age, year group, household education, month of baseline test, immigrant/descendant (yes or no), baseline total physical activity and baseline BMI, and potential interactions with sex were evaluated. Besides this, cross-sectional analyses of outcomes from the d2-test were additionally adjusted for weekday and lesson of test.

The correlation between changes in *n*-3 LCPUFA status and changes in a cognitive test outcome was evaluated in a model in which the dependent variable was the respective cognitive test score at visit 2 and/or 3 and the independent variable of interest was the respective biomarker value at visit 2 and/or 3. Potential interactions with sex were evaluated, and the model was adjusted for the same variables as baseline analyses, but additionally for baseline cognitive test score, baseline *n*-3 LCPUFA status and visit. All regression analyses that included fatty acid status were adjusted for total fatty acid concentration (µg/100 µl whole blood), which to some extent will adjust for the variation in the size of plasma lipid pool (which is more likely to be influenced by the last meal) relative to the size of the more stable blood cell membrane pool. The underlying assumptions for the models were investigated by visual inspection of residual and normal probability plots. When interactions were significant, results were also reported separately for the relevant subgroups. OR were translated back to the original scale when presented in text[41]. Data pre-processing, descriptive analyses and linear mixed models were performed using STATA 12.0 (StataCorp LP), whereas logistic mixed-effects models were performed using R[42] and the extension package lme4. $P < 0.05$ was considered statistically significant.

Results

Of the 834 children enrolled in the study, sixty-nine withdrew (8·3 %) (Fig. 1). A total of 773 children had data from baseline and visit 2 and/or 3 on at least one of the three cognitive tests. Blood samples were taken at baseline and visit 2 and/or 3 in 747 children. Of these, whole-blood fatty acid composition, serum ferritin (excluding twenty-five children with elevated CRP) and Hb were determined for 734, 678 and 747 children, respectively. Hence, a total of 726 children (87 % of the original study population) had sufficient data to be included in the
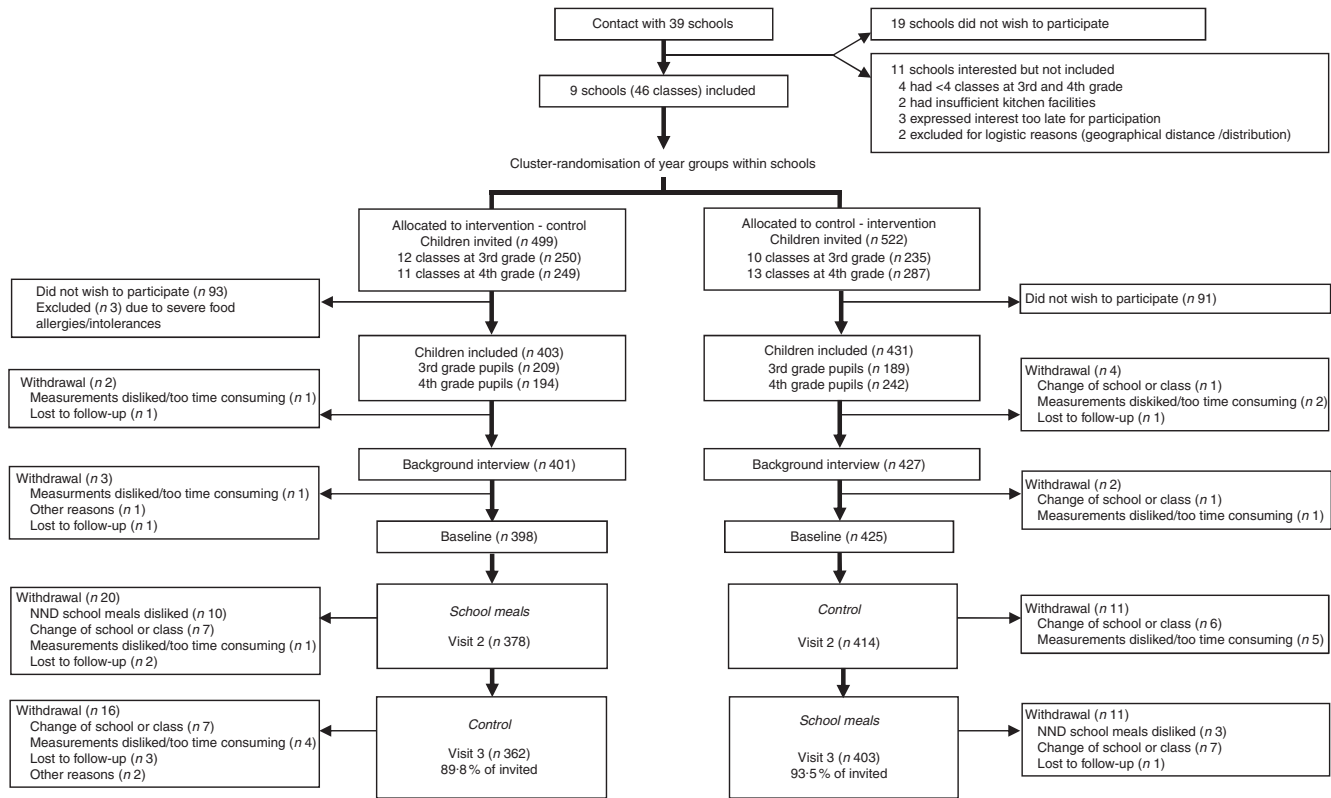
Fig. 1. Flowchart for the Optimal Well-Being, Development and Health for Danish Children through a Healthy New Nordic Diet School Meal Study. NND, New Nordic Diet.

Table 1. Baseline characteristics of the study population
(Mean values and standard deviations; percentages)

	All (n 726)		Girls (n 352)		Boys (n 374)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	10·0	0·6	9·9	0·6	10·1*	0·6
F:M ratio	49:51					
3:4 grade ratio	47:53		48:52		47:53	
Puberty						
Have entered puberty (%)†	34·6		46·1		23·7*	
Have had first menstruation (%)‡			0·6			
Immigrant/descendant (%)§	11·6		10·5		12·6	
Household education level‖						
Lower secondary education or less (%)	5·5		5·7		5·4	
Upper secondary education or equivalent (%)	3·4		4·3		2·7	
Vocational education (%)	31·5		33·0		30·2	
Short academic education (%)	9·6		9·4		9·9	
Bachelor's degree or equivalent (%)	28·8		28·4		29·1	
Master's degree or higher (%)	21·1		19·3		22·7	
Total physical activity (cpm)	488	130	454	117	521*	133
BMI (kg/m²)	17·2	2·4	17·1	2·4	17·3	2·4
Weight status¶						
Underweight (%)	10·2		11·9		8·6	
Normal weight (%)	76·5		75·0		77·8	
Overweight (%)	11·4		11·7		11·2	
Obese (%)	1·9		1·4		2·4	

F, female; M, male; cpm, counts per min.
† Puberty entered was determined based on Tanner[25] and defined as Tanner stage ≥2. Girls, n 343; boys, n 359.
‡ Two girls out of 342.
§ Immigrant or descendant was defined as participants whose grandparents and ≥1 parent were born outside Denmark.
‖ Households were categorised according to the parent/guardian with the highest education level.
¶ Based on age- and sex-specific cut-offs defined to pass through BMI at 18·5, 25 and 30 kg/m² at the age of 18 years[30,31].
* Significantly different from girls (P < 0·05) according to t test or χ² test, as appropriate.

present study. The baseline data of these children are presented in Table 1 in total and for girls and boys separately.

Patterns in test performance

In the PCA, two PC were found to describe 48·1 and 17·6 % of the overall test performance pattern, respectively (Fig. 2). The first test pattern was named 'school performance', as this component was primarily driven by CP, processing speed and all variables related to reading and maths (negative loadings relatively far from 0), whereas d2-error% and %correct in reading only had a small influence (loadings close to zero). The second test pattern was named 'reading comprehension', as it was primarily driven by %correct in reading at the negative end of the axis (loading furthest from zero). However, this component was also influenced by number correct in reading and d2-error% (positioned at the negative end of the axis) and by CP and processing speed (positioned at the positive end of the axis). According to this naming, a more *negative score* should be regarded as *better performance* for both test performance patterns. For example, a negative estimate for 'school performance' means that children move towards better school performance (indicating higher values for CP, processing speed, reading and maths, as well as lower d2-error% (i.e. less inattention/impulsivity)). Conversely, a negative estimate for 'reading comprehension' means that children move towards better reading comprehension (indicating higher values for reading number and %correct and d2-error% (i.e. more inattention/impulsivity), as well as lower CP and processing speed). The PCA loadings illustrate a close correlation between CP and processing speed, as well as a close correlation between maths performance and reading speed. Number correct in reading was correlated with %correct, and d2-error% was opposite of CP/processing speed (Fig. 2).

The baseline 'school performance' scores were fairly normally distributed with a mean of 1·12 (sd 1·52), whereas the 'reading comprehension' scores were slightly skewed to the right with a median of −0·49 (interquartile range (IQR) −0·84; 0·03) and a tail of children with poor reading accuracy (online Supplementary Fig. S1). Baseline correlations showed that 'school performance' worsened with increasing error% and improved with all other test outcomes. Contrary to this, 'reading comprehension' improved with increasing error% and reading outcomes, but worsened with CP and processing speed, and it was not correlated with maths performance. Fourth-grade students had higher baseline 'school performance' than third-grade students, whereas there was no difference in 'reading comprehension' scores. Except for d2-error%, all the individual test outcomes differed between grades, with better performance in the fourth grade (data not shown). The baseline PC scores did not differ between girls and boys (data not shown). However, for the individual outcomes, girls had lower error% in the d2-test (1·91 (IQR 0·93; 3·13) *v.* 2·25 (IQR 1·18; 3·77) %; *P* = 0·011), more correct answers in reading (53 (sd 17) *v.* 50 (sd 18) sentences; *P* = 0·027) and tended to read faster (57 (sd 15) *v.* 55 (sd 17) sentences; *P* = 0·057). In contrast, boys performed better in the maths test (33 (sd 12) *v.* 31 (sd 11) correct answers; *P* = 0·004).

Nutritional status at baseline

Mean serum ferritin and Hb are presented in Table 2, and they did not differ between boys and girls (data not shown). The estimated dietary intake of Fe at baseline was 9 (sd 2) mg/d and lower in girls than in boys (8 (sd 2) mg/d and 10 (sd 2) mg/d; *P* < 0·001). Serum ferritin and Hb were not correlated with Fe intake at baseline (data not shown). The baseline ferritin concentration was <15 µg/l in ten children (10 (sd 3) µg/l), 15–25 µg/l in seventy-seven children (21 (sd 3) µg/l) and >25 µg/l in 570 children (47 (sd 18) µg/l). The baseline prevalence of ID and anaemia was very low (1·5 and 0·8 %, respectively) (Table 2). Most of the children with low values were
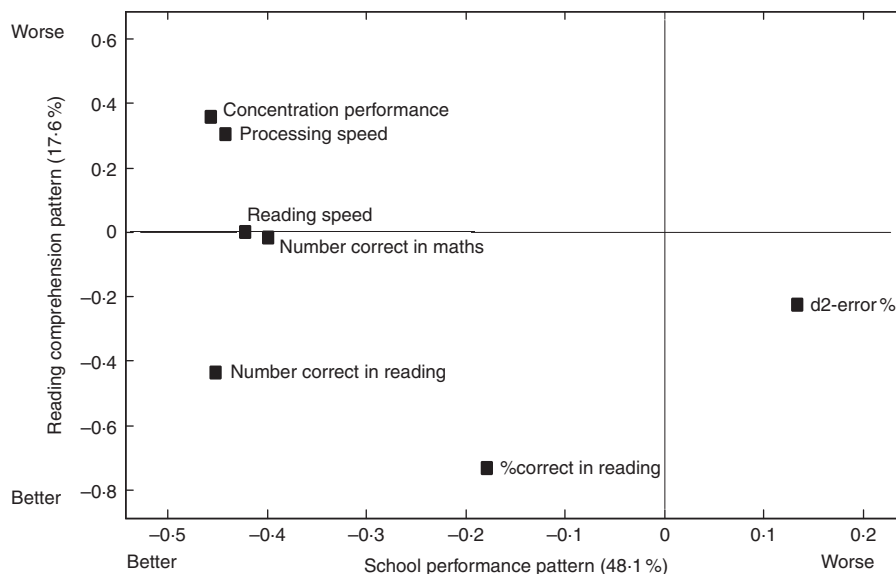

Fig. 2. Loading plot from principal component analysis on overall cognitive test performance.

Table 2. Iron status and whole-blood fatty acid composition at baseline, after the control and intervention periods and evaluated as differences between intervention and control*
(Mean values and standard deviations; odds ratios and 95 % confidence intervals; β coefficients and 95 % confidence intervals)

	Crude values									Estimates			
	Baseline			Control			Intervention			Difference between intervention and control†			
	$\%_{yes}$	n_{yes}	n_{total}	$\%_{yes}$	n_{yes}	n_{total}	$\%_{yes}$	n_{yes}	n_{total}	OR	95 % CI	P	n
Fe deficiency (ferritin <15 μg/l)	1·52	10	660	2·76	17	617	2·30	14	610	0·17	0·01, 1·96	0·16	660
Anaemia (Hb <115 g/l)	0·82	6	729	3·01	21	698	2·87	20	696	0·29	0·05, 1·68	0·17	729
Fe deficiency anaemia (IDA)‡	0·16	1	644	0·32	2	617	0·49	3	610	–		–	–
	Mean	SD	n	Mean	SD	n	Mean	SD	n	β	95 % CI	P	n
Serum ferritin (μg/l)	43·7	19·6	660	39·9	19·3	617	40·0	19·3	610	0·49	−0·80, 1·78	0·46	660
Hb (g/l)	132·3	7·4	729	130·4	7·4	698	130·2	7·4	696	−0·13	−0·54, 0·27	0·53	729
Total SFA	41·84	1·98	716	42·37	2·32	685	42·10	2·26	683	−0·17	−0·38, 0·04	0·11	716
Total MUFA	21·06	1·71	716	21·31	1·84	685	21·27	1·78	683	−0·01	−0·18, 0·16	0·90	716
Total PUFA	33·88	2·87	715	32·82	2·83	684	33·12	2·78	682	0·17	−0·06, 0·40	0·16	715
n-6 PUFA	28·78	2·53	716	27·94	2·48	685	27·99	2·39	683	−0·06	−0·25, 0·13	0·54	716
18 : 2n-6 (LA)	16·04	1·96	716	15·37	2·01	685	15·42	1·91	683	−0·0003	−0·16, 0·15	0·99	716
20 : 4n-6 (AA)	9·20	1·23	716	9·05	1·25	685	9·10	1·22	683	0·004	−0·09, 0·10	0·93	716
n-3 PUFA	5·09	1·07	715	4·88	1·02	684	5·13	1·08	682	0·23	0·15, 0·30	<0·001	715
18 : 3n-3 (ALA)	0·30	0·09	716	0·28	0·15	685	0·28	0·09	683	−0·001	−0·01, 0·01	0·77	716
20 : 5n-3 (EPA)	0·61	0·30	716	0·57	0·26	685	0·64	0·30	683	0·06	0·04, 0·08	<0·001	716
22 : 5n-3 (DPA)	1·19	0·18	716	1·16	0·18	685	1·18	0·18	683	0·02	0·01, 0·03	0·007	716
22 : 6n-3 (DHA)	2·96	0·74	716	2·82	0·71	685	2·98	0·73	683	0·15	0·10, 0·20	<0·001	716
EPA + DHA	3·57	0·96	716	3·39	0·90	685	3·62	0·95	683	0·21	0·15, 0·27	<0·001	716
n-6:n-3 PUFA ratio	5·90	1·31	715	5·96	1·24	684	5·69	1·24	682	−0·26	−0·34, −1·19	<0·001	715

LA, linoleic acid; AA, arachidonic acid; ALA, α linolenic acid; DPA, docosapentaenoic acid.

* The table includes children with data from baseline and visit 2 and/or 3 on at least one of the cognitive outcomes (n 726). All fatty acid values are presented as percentage of whole-blood total fatty acids (w/w %).

† Analyses of intervention effects were performed by linear or logistic mixed-effects models with random effects (school, year group within school, class and subject). The model included baseline values, visit, diet (intervention or control), the order of intervention and control period, sex, year group, baseline age, household education and month of baseline test. Analyses of fatty acid composition were furthermore adjusted for total fatty acid concentration.

‡ Children were classified with IDA if serum ferritin was <15 μg/l and Hb was <115 g/l[32]. Because of the low prevalence of IDA, regression analysis of intervention effects could not be performed.

PUFA, iron status and cognitive performance

just below the cut-off points – that is, low ferritin values were between 10 and 14 µg/l for six of ten children with ID, and none of the children had Hb concentration <110 g/l. One child had IDA at all three measurements, another child had IDA at visits 2 and 3 and a third child had IDA only at visit 3. There was a weak positive correlation between ferritin and Hb (r 0·09; $P = 0.026$).

Baseline whole-blood EPA (20 : 5n-3) concentrations ranged from 0·1 to 4·6 % and DHA from 1·0 to 5·7 % (Table 2). During the baseline dietary assessment, 26 % of children reported no fish intake, and among those who had consumed fish the median fish intake was 20 (95 % CI 8–32) g/d. Estimated dietary intake of EPA + DHA was 0·12 (95 % CI 0·04; 0·38) g/d at baseline. The reported fish intake correlated positively with EPA and DHA status (Pearson's $\rho = 0.34$ and 0·40, respectively, both $P < 0.001$). Moreover, those who had consumed fish had higher EPA and DHA status (EPA: 0·7 (sd 0·3) v. 0·5 (sd 0·2) %, DHA: 3·1 (sd 0·7) v. 2·6 (sd 0·7) %, both $P < 0.001$) and lower n-6:n-3 PUFA ratio (5·8 v. 7·3; $P < 0.001$) than children who did not eat fish. A fish oil supplement was consumed ≥1 time during the dietary assessment by 15·5 % of children. Fish intake was not different between children who did and did not take fish oil supplements ($P = 0.48$), but children who took fish oil supplements had higher concentrations of whole-blood EPA relative to children who did not consume fish oil supplements (0·8 v. 0·6 %; $P < 0.001$) and DHA (3·3 v. 2·9 %; $P < 0.001$). Fish consumption, use of fish oil supplements and n-3 PUFA status did not differ between boys and girls (data not shown).

EPA + DHA status in children with ID (3·3 (sd 0·9) %; n 9) or with low Fe status (ferritin 15–25 µg/l; EPA + DHA: 3·5 (sd 1·0) %; n 77) did not differ from that of children who were Fe sufficient (ferritin > 25 µg/l; EPA + DHA: 3·6 (sd 1·0) %; n 564). The whole-blood concentration of EPA + DHA did not correlate with ferritin or Hb ($P = 0.14$ and $P = 0.21$).

Effects of the school meal intervention

Over time (visits 1–3), children improved their 'school performance' (baseline: 1·12 (sd 1·52); visit 2: −0·32 (sd 1·59); visit 3: −0·99 (sd 1·70)) but worsened their 'reading comprehension' (baseline: −0·49 (95 % CI −0·84, 0·03); visit 2: −0·13 (95 % CI −0·52, 0·38); visit 3: −0·003 (95 % CI −0·41, 0·52)). In accordance with previously reported effects on reading performance and d2-error%[21], the intervention influenced children towards better 'school performance' (β −0·08; 95 % CI −0·15, −0·02; $P = 0.015$; n 644) and better 'reading comprehension' (−0·09; 95 % CI −0·17, −0·003; $P = 0.043$; n 644). Hence, the improvement in 'school performance' was larger during the intervention period compared with the control period. Similarly, the deterioration in 'reading comprehension' was less marked in the intervention period than in the control period.

The intake of Fe was 0·5 mg/d higher in the intervention period compared with the control period (95 % CI 0·3, 0·7; $P < 0.001$), but there was no effect of the intervention on ferritin status, Hb status or the prevalence of ID and anaemia (Table 2). Moreover, there were no significant interactions with baseline status, sex or order-of-treatment (data not shown). Yet, over time there was a decrease in ferritin status (from 44 (sd 19) µg/l at baseline to 38 (sd 16) µg/l at visit 3; $P < 0.001$) and Hb

concentration (from 132 (sd 7) µg/l at baseline to 131 (sd 7) µg/l at visit 3; $P < 0.001$).

The proportion of children eating fish was higher in the intervention period than in the control period (91 v. 73 %; $P < 0.001$), and among those eating fish the median fish intake was 9·8 (95 % CI 7·0, 12·6; $P < 0.001$) g/d higher during the intervention period. In line with this, the estimated intake of EPA + DHA was 0·10 (95 % CI 0·07, 0·12; $P < 0.001$) g/d higher during the intervention period. The proportion of children taking n-3 LCPUFA supplement was not influenced by the intervention ($P = 0.30$), but the proportion decreased over time (from 15·5 % at baseline to 9·0 % at visit 3) The school meal intervention increased EPA and DHA concentrations in whole blood and decreased the n-6:n-3 PUFA ratio (Table 2). There were no significant interactions with baseline status, sex or order-of-treatment for intervention effects on whole-blood fatty acid composition (data not shown). In the subsequent correlation analyses, we use whole-blood EPA + DHA as a marker of n-3 LCPUFA status, as both EPA and DHA were strongly associated with each other and with dietary intake of fish, EPA and DHA, and reflected the increase in fish intake during the intervention.

Baseline associations between iron status and cognitive test performance

The baseline association between serum ferritin (≤25 or >25 µg/l) and the 'school performance' pattern was modified by sex, indicating that lower Fe status was associated with worse 'school performance' in girls, but not in boys (Table 3). This interaction was in accordance with the associations for the individual reading test outcomes, which showed opposite associations for girls and boys. Girls with small Fe stores had lower reading speed and number correct (corresponding to −4·9 and −4·2 sentences, respectively), whereas boys with small Fe stores had higher reading speed and number correct (corresponding to 5·2 sentences and 6·7 sentences, respectively). Small Fe stores were also associated with 1·6 %-point higher % correct in reading, showing that the relative improvement in reading accuracy was of similar size in boys and girls (Table 3). Baseline ferritin status was also associated with 'reading comprehension', but the association indicated better performance in children with small Fe stores and the association was not different between boys and girls (Table 3). This association was in accordance with the results for boys' reading speed and number correct. Furthermore, having small Fe stores was associated with higher d2-error% with an effect size that corresponded to 0·6 %-point, and tended to be associated with poorer CP. Findings were similar when the group with small Fe stores was defined as serum ferritin from 15–25 µg/l, that is, excluding children with ID (data not shown). As the intervention did not influence Fe status, we did not investigate the associations between concomitant changes during the study.

Baseline correlations between n-3 LCPUFA status and cognitive test performance

Baseline EPA + DHA status was correlated with 'school performance', but not with the 'reading comprehension' pattern,

Table 3. Baseline test performance depending on iron status and the estimated difference between groups
(Mean values and standard deviations; medians and interquartile ranges (IQR); β coefficients and 95 % confidence intervals; odds ratios and 95 % confidence intervals)

	Fe sufficient*			Small Fe stores†			Small Fe stores v. Fe sufficient‡ Estimate			
	Mean/median	SD/IQR	n	Mean/median	SD/IQR	n	β	95 % CI	P_{group}	$P_{group \times sex}$
Overall test performance patterns§										
School performance										
Girls	0·92	1·43	256	1·63	1·26	34	0·52	0·02, 1·02		0·033
Boys	1·18	1·62	278	1·27	1·77	35	−0·24	−0·74, 0·25		
Reading comprehension	−0·49	−0·83; 0·03	534	−0·67	−1·03; −0·19	69	−0·23	−0·45, −0·02	0·035	0·37
D2-test of attention										
Concentration performance (items)	131·3	22·6	529	123·2	23·8	70	−5·2	−10·5, 0·2	0·061	0·43
Processing speed (items)	332·1	57·9	529	318·9	57·0	70	−7·8	21·3, 5·7	0·26	0·34
							OR	95 % CI		
Total error%	2·01	1·05; 3·27	529	2·45	1·09; 3·77	70	1·26	1·16, 1·37	<0·001	0·14
Inattention error%	1·56	0·82; 2·86	529	1·82	0·76; 3·38	70	1·27	1·16, 1·39	<0·001	0·23
Impulsivity error%	0·27	0·0; 0·58	529	0·30	0·0; 0·78	70	1·23	1·01, 1·50	0·037	0·23
Sentence reading test										
Reading speed (number of sentences)										
Girls	57·9	15·8	255	52·0	13·4	36	0·83	0·77, 0·89		<0·001
Boys	54·4	17·3	288	57·6	15·7	37	1·21	1·13, 1·31		
Number correct (sentences)										
Girls	54·1	16·8	255	48·3	15·0	36	0·85	0·79, 0·91		<0·001
Boys	49·7	18·4	288	53·38	18·9	37	1·29	1·20, 1·39		
% correct (of read)	97·0	93·3; 98·8	543	96·7	93·3; 98·4	73	1·32	1·12, 1·55	<0·001	0·072
Maths test										
Number correct (sentences)	32·3	11·2	530	30·1	11·0	69	0·96	0·90, 1·01	0·141	0·93

* Fe sufficient defined as serum ferritin >25 µg/l. Normal distributed variables are presented as mean values and SD, whereas skewed variables are presented as median and IQR.
† Small Fe stores defined as serum ferritin ≤25 µg/l. Normal distributed variables are presented as mean values and SD, whereas skewed variables are presented as median and IQR.
‡ The difference in performance between children with small Fe stores (serum ferritin ≤25 µg/l) and children who were Fe sufficient (serum ferritin >25 µg/l) was estimated using linear or logistic mixed-effects models including random effects (school, year group within school and class) and adjusted for sex, household education, immigrant/descendant, grade and baseline age, month of baseline test, BMI and total physical activity (counts/min). Besides this, analyses of outcomes from the d2-test of attention were adjusted for weekday and lesson of test. Estimates are presented separately for girls and boys when the interaction term was significant ($P < 0.05$).
§ Test performance patterns were derived from all test outcomes (except from inattention and impulsivity error%) at all available time points using principal component analysis. The two chosen components were named by the most negative variables in the loading plot – that is, a negative estimate indicates an improvement and a positive estimate indicates a deterioration.

Table 4. Correlations between whole-blood EPA + DHA status and cognitive test performances at baseline and between changes
(β Coefficients and 95 % confidence intervals; odds ratios and 95 % confidence intervals)

		Estimate*			
	n	β	95 % CI	$P_{EPA+DHA}$	$P_{sex \times EPA+DHA}$
Correlations at baseline†					
Overall test performance patterns‡					
School performance	634	−0·14	−0·26, 0·03	0·015	0·75
Reading comprehension	634	0·0002	−0·07, 0·08	0·99	0·55
D2-test of attention					
Concentration performance (items)	650	1·98	0·25, 3·72	0·025	0·36
Processing speed (items)	650	5·31	1·01, 9·62	0·015	0·98
Total d2-error%					
Girls	317	1·04§	0·999, 1·09		<0·001
Boys	333	0·94§	0·90, 0·97		
Inattention error%					
Girls	317	1·05§	1·003, 1·10		0·005
Boys	333	0·96§	0·92, 1·004		
Impulsivity error%					
Girls	317	0·99§	0·88, 1·10		0·015
Boys	333	0·82§	0·75, 0·91		
Sentence reading test					
Reading speed (number of sentences)					
Girls	327	1·06§	1·03, 1·08		<0·001
Boys	343	1·002§	0·98, 1·03		
Number correct (sentences)					
Girls	327	1·07§	1·04, 1·09		0·002
Boys	343	1·01§	0·99, 1·04		
% Correct (of read)	670	1·09§	1·04, 1·15	<0·001	0·50
Maths test					
Number correct (sentences)	654	1·07§	1·04, 1·09	<0·001	0·97
Correlations between changes‖					
Overall test performance patterns‡					
School performance	633	−0·07	−0·13, −0·01	0·027	0·90
Reading comprehension	633	−0·03	−0·10, 0·05	0·46	0·59
D2-test of attention					
Concentration performance (items)	650	0·09	−1·09, 1·27	0·88	0·95
Processing speed (items)	650	1·42	−1·46, 4·30	0·33	0·57
Total d2-error%	650	1·09§	1·04, 1·13	<0·001	0·51
Inattention error%	650	1·10§	1·05, 1·15	<0·001	0·87
Impulsivity error%	650	1·03§	0·91, 1·16	0·68	0·107
Sentence reading test					
Reading speed (number of sentences)	669	1·05§	1·03, 1·08	<0·001	0·109
Number correct (sentences)	669	1·04§	1·02, 1·07	0·001	0·44
% Correct (of read)	669	0·97§	0·90, 1·04	0·42	0·40
Maths test					
Number correct (sentences)	650	1·01§	0·97, 1·05	0·63	0·79

* Estimates represent the correlation between EPA + DHA (w/w %) and the respective outcome and are presented as β coefficients and 95 % CI or odds ratios and 95 % CI, as appropriate.
† Baseline correlations were assessed using linear or logistic mixed-effects models including random effects (school, year group (within school) and class), adjusted for age, sex, grade, household education, month of baseline test, immigrant/descendant, BMI, total physical activity (counts/min) and total fatty acid concentration (µg/100 µl whole blood). Besides this, analyses of outcomes from the d2-test of attention were adjusted for weekday and lesson of test.
‡ Test performance patterns were derived from all test outcomes (except from inattention and impulsivity error%) at all available time points using principal component analysis. The two chosen components were named by the most negative variables in the loading plot; that is, a negative estimate indicates an improvement and a positive estimate indicates a deterioration.
§ OR.
‖ Analyses were performed using linear or logistic mixed-effects models with school, year-group (within each school), class and individual as random effects. The model included baseline values of test outcome, baseline EPAz + DHA, visit, sex, baseline age, grade, household education, month of baseline test, immigrant/descendant, baseline BMI, baseline total physical activity (counts/min) and total fatty acid concentration (µg/100 µl whole blood).

and this correlation was not affected by sex (Table 4). In line with this, maths performance, processing speed and CP were all positively correlated with EPA + DHA status. Moreover, there was a positive correlation with reading speed and number correct, but only in girls (Table 4). In boys, the total d2-error% and impulsivity error% decreased with increasing EPA + DHA status, whereas inattention error% increased with increasing EPA + DHA status in girls (Table 4). Results from the separate baseline correlations for whole-blood EPA and DHA showed a similar overall pattern as EPA + DHA (online Supplementary Table S1).

Correlations between changes in n-3 LCPUFA status and cognitive test performance

During the study, the change in EPA + DHA status correlated negatively with the change in the 'school performance' score,

indicating better 'school performance' with increasing EPA + DHA status (Table 4). The correlation corresponded to an improvement of 0·07 for each 1 % increase in EPA + DHA status, which translates to a 0·015 %-point improvement for a 0·21 w/w% increase in EPA + DHA status (i.e. the intervention effect on EPA + DHA status). The increase in whole-blood EPA + DHA was also associated with increases in the individual test scores on reading speed, number correct in reading and the error% in the d2-test, specifically errors relating to inattention (Table 4). For a 1 % increase in EPA + DHA status, the correlations for reading speed, number correct and inattention error% corresponded to 1·2 sentences, 1 sentence and 0·15 %-point, respectively, which was equivalent to 0·25, 0·2 sentences and 0·03 %-point, respectively, for a 0·21 % increase in EPA + DHA status. The change in whole-blood EPA + DHA could potentially explain 19, 36, 11 and 21 %, respectively, of the effect of the school meal intervention on 'school performance' (−0·08), reading speed (0·7 sentences), number correct (1·8 sentences) and inattention error% (0·14 %-point)[21]. None of the associations were significantly influenced by sex. Apart from a few exceptions, the separate analyses of EPA and DHA status showed the same pattern (online Supplementary Table S2).

Discussion

The 3-month school meal intervention did not influence Fe status, but it increased whole-blood concentration of EPA and DHA. The increase in whole-blood EPA + DHA was correlated with the concomitant changes in test performances, suggesting that as EPA + DHA status increased children had a better overall 'school performance', which seemed to be driven specifically by outcomes related to reading performance. EPA + DHA status also increased with increasing inattention error%. The results indicate that the 0·21 % change in whole-blood EPA + DHA could potentially explain 19 % of the effect of the school meal intervention on an overall measure of 'school performance'. In line with this, the change in whole-blood EPA + DHA could explain 11–36 % of the intervention effect on reading speed, number correct in reading and inattention.

Interestingly, the 'reading comprehension' pattern separated CP from number and %correct in reading, whereas errors in the d2-test to some extent coincided with reading performance. This suggested that children who had a good sentence understanding and were able to correctly evaluate whether the content matched the depicted situation also made more d2-errors. This indicates that the interpretation of errors in the d2-test as impulsivity/inattention and as a barrier to performance in school might not be accurate. Perhaps, to some extent, d2-errors reflect an inclination to focus on the overall pattern, rather than on details.

Iron status and cognition

In line with the hypothesised adverse effect of ID on cognitive function, we found that girls with small Fe stores had a poorer overall 'school performance' and poorer reading performance compared with girls with larger Fe stores. This is in agreement

with previous studies in which ID without anaemia was shown to be associated with poorer school performance[43–45]. Furthermore, an randomised controlled trial (RCT) found that Fe supplementation to nonanaemic but ID adolescent girls improved verbal learning and memory, which are considered important for academic performance[46]. A similar trial found no differences between treatment groups, but a positive correlation between changes in ferritin and changes in reading span performance, as well as between changes in Hb and changes in working memory[47]. As results were similar with or without inclusion of children with ID, this study showed that non-ID girls with small Fe stores (ferritin 15–25 μg/l) also performed poorer than girls with larger Fe stores (>25 μg/l). This suggests that suboptimal Fe stores might inhibit school performance, even in the absence of ID. The results for error% and CP also pointed in the direction of a poorer performance in children with small Fe stores. Contrary to this, we found indications of better 'reading comprehension' in children with small Fe stores, which was probably related to better reading performance in boys with small Fe stores. We have only been able to identify one study with a similar finding in which children with serum ferritin ≤20 μg/l, but high Hb (>125 g/l), had higher IQ, language and mathematics score[48]. The authors speculated whether this was related to adverse effects of high Fe levels when Hb is high. It is possible that the opposite association in boys and girls was a chance finding caused by few children with small Fe stores and/or residual confounding.

n-3 Long-chain PUFA status and cognition

As expected, the increase in n-3 LCPUFA status was correlated with the improvement in 'school performance', and this appeared to be driven by the improvement in reading speed. Contrary to this, n-3 LCPUFA was not correlated with improved 'reading comprehension' pattern, although it was associated with changes in the number correct in reading and d2-error%. However, the 'reading comprehension' pattern was primarily influenced by %correct in the reading test, which was not correlated with the change in n-3 LCPUFA. Nevertheless, in the separate analyses of EPA and DHA status, a 1 % increase in EPA correlated with increased 'reading comprehension', whereas a 1 % increase in DHA was correlated with increased 'school performance'. As DHA makes up most of the whole-blood EPA + DHA, this may explain why EPA + DHA status only correlated with 'school performance'.

As opposed to baseline correlations, we found no significant associations between the change in EPA + DHA status and change in CP, d2-processing speed, %correct in reading or number correct in maths. Such inconsistencies between correlations at baseline and for intervention-induced changes suggest that the baseline correlations may have been confounded or that the increase in n-3 LCPUFA status might have been too small to influence these outcomes. Similarly, the interactions with sex on the baseline associations for reading speed, number correct and d2-errors were not confirmed by the post-intervention dose–response analyses. Previous studies have, however, also reported sex differences in association with test performance. A cross-sectional analysis of 6–16-year-old

American children found a stronger association between *n*-3 PUFA intake and cognitive performance (based on tests in maths, reading, block design and digit span) in girls than in boys[49]. Sex differences have also been seen in relation to early language development both in an observational study of DHA status in 3-year-old children[50] and in maternal DHA supplementation trials[51,52], although these results are conflicting with regard to in which sex the association was most pronounced. Our results suggested that girls with higher *n*-3 LCPUFA status were more inattentive (higher percentage of errors of omission) and better readers (higher reading speed and number correct), whereas boys with higher *n*-3 LCPUFA-status were less impulsive (lower percentage of errors of commission). This is interesting, because girls were better readers, more attentive and less impulsive at baseline, which means that the sex difference in reading increased with higher *n*-3 LCPUFA status, whereas the sex difference in inattention and impulsivity decreased with higher *n*-3 LCPUFA status. Nevertheless, there is a substantial risk of bias in observational studies in this field because of factors such as culture, upbringing, lifestyle and temperament that may influence test performance of boys and girls differently. In our study, the baseline correlations observed in girls seemed to be consistent with the correlations between intervention-induced changes (i.e. increased inattention error% and better reading performance with increased EPA + DHA-status). We therefore hypothesise that the contradictory baseline correlations in boys could be the result of residual confounding.

Although *n*-3 LCPUFA, especially DHA, is regarded as beneficial for neurodevelopment from pregnancy and into infancy[11], little research has been done on the effects of *n*-3 LCPUFA in school-aged children. In line with our results, an RCT providing a bread spread with 42 % fish flour found an improvement in spelling and a marginal improvement in reading among 7–9-year-old South African children[53]. Contrary to this, an Australian study found no effect of fish oil supplementation on reading or spelling in 3–13-year-old predominantly Indigenous children[54]. Recently, another South African study found no overall effect of fish oil supplementation on memory and visuospatial cognition in 6–11-year-old children, but identified adverse effects on memory in subgroups of children with non-anaemic ID and IDA[55]. The relatively high prevalence of malnourished children in these previous study populations makes the results difficult to compare with our study and evidence in well-nourished children is sparse. In line with our results, RCT have found that DHA/EPA supplementation improved reading, spelling and behaviour in 5–12-year-old children with developmental coordination disorder[56], behaviour and learning in 8–12-year-old children with dyslexia and above-average attention deficit hyperactivity disorder (ADHD) ratings[57], and parent-rated symptoms of inattention and hyperactivity/impulsivity in children with above-average ADHD ratings[58]. However, to our knowledge, only five RCT have investigated the effect of *n*-3 LCPUFA supplementation on cognition and school performance in a mainstream population of healthy school children from high-income countries[59–63]. As the school meal intervention only improved *n*-3 LCPUFA status through fish served twice a week,

the dose of *n*-3 LCPUFA in our study was markedly lower than in most of the supplementation trials (100–1200 mg/d). Our study is most comparable in design with the Australian Nutrition Enhancement for Mental Optimization (NEMO) study, which had a relatively high number of participants (*n* 396) and provided a low *n*-3 LCPUFA dose (DHA 88 mg/d and EPA 22 mg/d)[59]. However, there were no indications of improved cognitive outcomes in that study, in spite of a four times longer intervention period than in the present study. Interestingly, the NEMO study did not seem underpowered to detect an effect of nutritional intervention, as the micronutrient treatment increased scores on verbal learning and memory[59]. Similar results were seen in children from Indonesia[59] and in a study from India[64]. The remaining four studies applied a higher dose for a shorter period (8–16 weeks) to fewer children and all identified a positive effect of the supplement on cognitive outcomes[60–63]. Two of the trials found an effect of DHA on outcomes related to reading ability[60,63], but in both studies this effect was only one out of a large number of outcome measures. In the study by Kirby et al.[61], there was no effect on outcomes related to reading, but in opposition to our result they found improved impulsivity. Yet, this effect had a small effect size, was only present in per-protocol analyses and was not associated with changes in cheek cell *n*-3 LCPUFA. Interestingly, McNamara et al.[62] showed improved neurophysiological brain activation, but found no effect on sustained attention after 8 weeks of DHA supplementation (400 or 1200 mg/d) in 8–10-year-old American boys. Similar results have been observed in an RCT in sixty-five young adults[65] and in a prospective study of 154 Inuit children aged 10–13 years[66]. This brings into question the sensitivity and appropriateness of the cognitive tests commonly administered for assessing the effects of *n*-3 LCPUFA. Possibly some tests do not assess the specific cognitive aspects that are affected by *n*-3 LCPUFA or the effect size in healthy well-nourished children is small and requires larger group sizes in order to achieve sufficient statistical power.

The conclusions from the present analyses are limited by the study design. Because of the school integrated meal intervention, the design did not allow us to keep participants blinded to the study periods. Thus, expectations and attitudes might have influenced test performance. Moreover, dietary aspects of the school meal intervention cannot be separated from the environmental differences between the control and intervention period. However, we adjusted statistically for a number of potential confounders. Even though we did not adjust for school attendance, this is unlikely to confound the results, as school attendance was not influenced by the school meal intervention[67]. Similarly, the whole-diet approach means that we cannot make a final conclusion on whether intake of *n*-3 LCPUFA was responsible for the intervention effect on reading and inattention. DHA and EPA status both reflected the increase in fish intake and estimated EPA + DHA intake. Therefore, the discrepancies between DHA and EPA in separate analyses should be interpreted with caution. Besides, fish contains other nutrients (e.g. amino acids, Se, I and vitamin D) and the intervention also increased the intake of several other micronutrients, which have been associated with cognitive performance[20,68]. On the other hand, an increased intake of

vitamins and minerals does not necessarily imply that nutrient status will increase, as was the case for Fe status. We do not have biomarkers for all nutrients that could be of relevance to cognitive performance, and even if we did the whole-diet approach would most likely cause co-linearity between the different nutrients. In spite of the limited evidence in healthy school-aged children, the plausibility of an effect of *n*-3 LCPUFA on cognition is substantiated by evidence regarding potential mechanisms, especially for DHA[6].

In the OPUS School Meal Study, the increased fish intake was one of the major dietary differences between the intervention period and the control period. In this study, we confirmed that the intervention resulted in an increased whole-blood *n*-3 LCPUFA status. Furthermore, we identified two patterns in test performance that were influenced by the school meal intervention. However, only the improvement in the 'school performance' pattern appeared to be associated with the increase in *n*-3 LCPUFA status. The dose–response relationship suggested that approximately 20 % of the intervention effect on 'school performance' could be related to the increase in *n*-3 LCPUFA status. In agreement with this, *n*-3 LCPUFA status was positively associated with the previously reported intervention effects on reading performance (i.e. improved reading speed and number correct). Furthermore, our results suggested that about 20 % of the intervention effect on inattention (increased d2-error%) could be related to *n*-3 LCPUFA status. Although we cannot determine causality with the results, this study contributes to the limited evidence regarding *n*-3 LCPUFA and cognition in school-aged children and supports that *n*-3 LCPUFA may be involved in cognitive function in school age. The possible effect of *n*-3 LCPUFA intake on school performance in otherwise healthy children has great potential. However, it is important to investigate the role of *n*-3 LCPUFA further, especially in relation to inattention and whether this should be interpreted as a potential adverse effect. Given the evidence regarding the potential effects of *n*-3 LCPUFA on brain monoaminergic systems, it would be useful to include assessments of behavioural and emotional aspects in future studies. Moreover, it would be relevant to qualify the knowledge regarding the effects of specific nutrients that are present in fish – for example, the effects of fish oil compared with fatty fish and lean fish.

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Supplementary material

For supplementary material/s referred to in this article, please visit http://dx.doi.org/doi:10.1017/S0007114515003323

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