



Randomized Control Trials

The effect of Atlantic salmon consumption on the cognitive performance of preschool children – A randomized controlled trial



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SUMMARY

Background and aims: Long chain polyunsaturated n-3 fatty acids (LC-PUFA) are of functional and structural importance for brain development. Observational studies have shown positive relations between fatty fish consumption and cognitive performance in children, but Results from intervention studies using supplementary n-3 LC-PUFA are conflicting. Salmon is a good source of n-3 LC-PUFA, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). We tested the hypothesis that an increased dietary salmon intake results in better cognitive outcomes than a meat based diet.

Methods: Children (n = 205, age 4–6 years) in this trial were individually randomized to eating meals containing farmed Atlantic salmon or meat three times weekly for 16 weeks. Pre- and post-intervention a cognitive test (Wechsler Preschool and Primary Scale of Intelligence, 3rd edition, WPPSI-III) and a fine-motor coordination test (Nine Hole Peg Test, 9-HPT) were performed. Biochemical analyses included glycerophospholipid fatty acid profiles in plasma and cheek cells, serum 25-hydroxyvitamin D, and urinary iodine concentration. Dietary intake before and during the study were determined using food frequency questionnaires.

Results: Intakes of EPA, DHA, vitamin D and iodine were higher in the salmon than the meat group, but on biomarker level only EPA and DHA increased significantly in the salmon group compared to the meat group (p < 0.001). In general linear models no significant differences between the intervention groups were found in the scale scores of the WPPSI-III tests and the 9-HPT. In analyses of the raw scores, the salmon group showed significantly better improvement in two of the eight raw scores compared to the meat group (symbol search p = 0.038, picture concepts p = 0.047).

Conclusions: Intake of farmed Atlantic salmon led to a greater increase of the raw scores of the picture concept and symbol search subtests, while in the six other subtests raw scores were not different between the groups. This might indicate a modest positive association of salmon intake with the performance of preschool children in some subtests evaluating fluid intelligence but does not suggest an influence on global IQ development.

Clinical trial registry number and website: [ClinicalTrials.gov](https://clinicaltrials.gov) registration number: NCT01951937.

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1. Introduction

Fish and dietary supplements are the dominant dietary sources of the n-3 long chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1]. EPA and DHA are considered important for neural development during

the perinatal period [2,3]. Especially DHA, the major fatty acid in the brain [4], is thought to be essential for normal brain function and for optimal visual and cognitive development in children [5,6]. Brain growth and development continue throughout childhood and adolescence, however Results from randomized intervention trials (RCTs) providing n-3 LC-PUFAs to children and young adults are conflicting and have not demonstrated consistent benefits of additional n-3 LC-PUFA provision for neurological or cognitive performance [7,8]. A variety of reasons have been discussed for the ambiguous findings, e.g. dosage and duration of interventions, baseline n-3 LC-PUFA status, threshold values and the influence of genetic variation [9]. Methodological issues might limit the ability to detect effects of n-3 LC-PUFAs supplementation on test performance [10]. In contrast, several observational studies have shown positive associations between fish intake and cognitive and behavioural performance [11–13]. These effects were still significant after adjusting for a series of confounders, suggesting that the beneficial effects of fish might at least partially be due to nutrients provided by fish intake. The importance of nutrients other than n-3 LC-PUFA in fish is supported by a recent finding in the ALSPAC cohort which showed a positive association of maternal iodine status in pregnancy with verbal IQ and reading comprehension at offspring age 8–9 years [14]. Even after adjusting for n-3 LC-PUFA intake from seafood, iodine levels were significantly related to the test outcome. It is interesting to note in this context that n-3 LC-PUFA supplementation in healthy children hardly influenced dexterity, but there are indications that maternal fish intake is positively associated with offspring motor skills [15].

In a systematic review on the effects of different diets on executive, cognitive functions integrating observational and interventional studies, fish was identified as one of the foods positively associated with executive functions [16]. As executive functions are related to several cognitive domains [17] this could indicate that the influence of fish intake on cognitive functions is stronger than the effect of dietary n-3 LC-PUFA alone and is supported by the findings of a recently published RCT (FINS-KIDS) comparing fatty fish and meat intake in Norwegian children in relation to its influence on cognitive function development [18]. Although main analyses did not show a significant difference of the change of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) scores during the intervention between the meat and the fish group, the total raw score and the processing speed raw score were positively associated with fish intake, but not with meat intake. Considering the lower habitual fish intake of children in Southern Germany compared to Norway [1] the present study aimed to compare with a similar study design effects of salmon and meat intake on the outcomes of WPPSI-III tests and tests of fine motor skills in preschool kids in Germany.

2. Methodology

2.1. Study design

In this two-armed non-blinded RCT, 221 children aged 4–6 years were randomly assigned to receive three prepared meals weekly for 16 weeks, containing either approximately 50 g Atlantic salmon or 50 g meat per meal. The primary outcome measure was a change in cognitive performance, and the secondary outcome measures were EPA and DHA in plasma and cheek cells before and after intervention. At baseline and the week after the intervention was completed, biological samples were taken, cognitive tests were performed and a food frequency questionnaire (FFQ) was filled out. During the intervention period, the parents filled out a food diary and were contacted by structured telephone calls. Ethical approval for the study was obtained from the Ethics review board of the

Klinikum der Universität München and from the Regional Committees for Medical and Health Research Ethics, North (2013/1471/REK Nord). The study was registered at [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT01951937).

2.2. Sample size and power calculation

In preparing for the current study, the estimation of sample size was based on the Results from a study on 4–5 year old US American children showing a mean general ability score of 96.3 with a standard deviation (SD) of 12.7 using the K-ABC II test [19], which was the test battery originally planned for the current study. Based on these data a group comparison using non-paired t-test showed that a group difference of 5.7 points in the IQ-score (corresponding to an effect size = 0.45) can be detected with a power of 80% and a significance level of $P = 0.05$ by studying 80 subjects per group. Thus, enrolling 100 subjects into each of the groups should enable the detection of a 5.7 points score group difference, with a dropout rate of 20%. Nevertheless, it was decided to use the WPPSI-III test battery, as it is the most frequently used test for pre-school children's general ability [20], which gave the study a power of 80% to detect a group difference of 6.7 points assuming the normed standard deviation of 15 for the WPPSI-II test, as well corresponding to an effect size of 0.45.

2.3. Study population, recruitment, and randomization

Children were recruited through public and private kindergartens in the area of Munich, Germany. By using the city registry primarily private, but randomly selected kindergartens were contacted with information letters. Some 90 kindergartens showed no interest in taking part, whereas 160 kindergartens agreed to support the study. From 71 kindergartens, families of 350 children indicated interest in participation, and after screening 221 children were found eligible for the study (Fig. 1). Inclusion criteria included age between 4 years; 0 months and 6 years; 0 months at baseline, apparently healthy and an omnivorous diet. Exclusion criteria were any food allergies, severe illness diagnosed by a paediatrician, weight below the 10th percentile or above the 90th percentile [21], reported dislike of fish, habitual fish intake greater than one meal per week, and use of n-3 supplements during the last three months.

Participating families were informed about the details of the study, and written consent was obtained from both parents prior to the pre-intervention testing. Families were randomized in a 1:1 ratio into the meat or the salmon group using a computer generated randomization list with stratification for gender and age (below or equal/above 5 years; 0 months) and a block size of 10.

2.4. Study meals

Atlantic salmon (*Salmo salar*) for the study meals was produced at Marine Harvest by Skretting Aquaculture Centre, Stavanger Norway from August 2012 to January 2014. The salmon was fillet (D-trim) and packed into 150 g individual vacuum-sealed portions at Rex Star Seafood AS (Lerøy, Norway). The content of selected nutrients and undesirables is given in supplementary Table S1.

The study meals were produced fresh and vacuum packed in containers of either 200 g (salmon burger, beef burger), 250 g (pasta filled with salmon pate, salmon fillet in paprika sauce, potato gratin with salmon, turkey fillet with pepper sauce, potato gratin with turkey fillet, Tortellini with ham filling) or 300 g (Bolognese sauce with salmon, Bolognese sauce with ham) by Feneberg Lebensmittel GmbH (Kempten/Allgäu, Germany). The meals were stored at 4 °C at Freshfoods GmbH (Munich, Germany) until weekly delivery to the participants' homes. To increase acceptance, meals

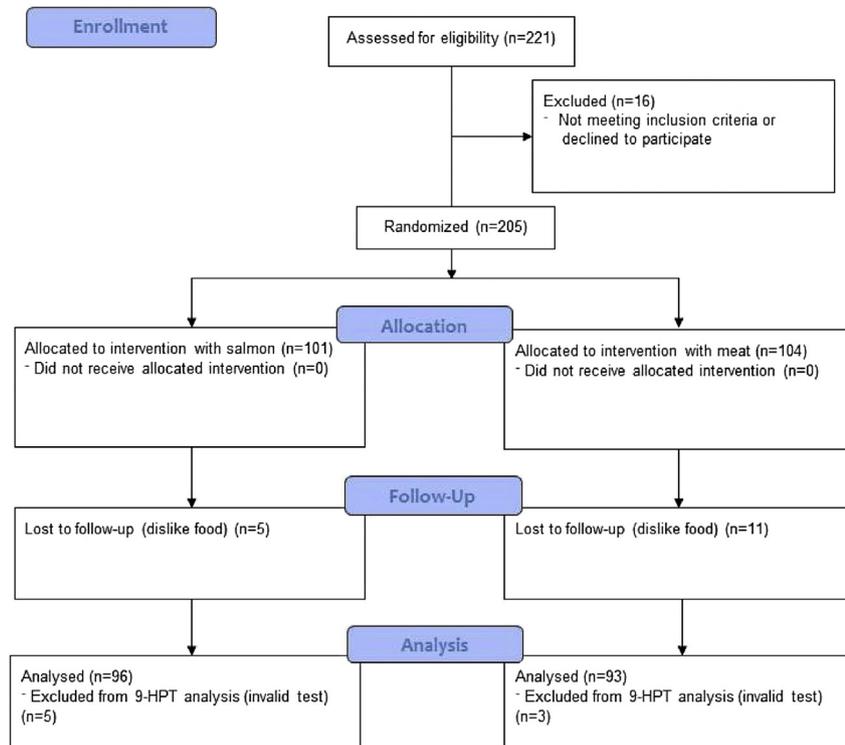


Fig. 1. Flow-chart showing the study population.

were provided for the whole family, and families randomized to the salmon group could choose among the five salmon meals, and those in the meat group among the five meat meals. No side dishes were offered and no recommendations to influence the families' choice of side dishes were given.

The nutrient composition of the salmon fillet and meals was analysed by ISO-certified (NS-EN-ISO 17025) routine methods at the Institute of Marine Research. Certified reference material was analysed to assess the accuracy and precision of the analytical methods used. Energy content was determined by bomb calorimetry and the gross energy was calculated following the manufacturer's instructions (Parr instruments, Moline, IL, USA). Total fat content was determined after extraction into n-heptane. Fatty acid composition was determined by gas chromatography with flame ionization detection [22]. Protein was analysed with a Leco N Analyzer [23]. Vitamin D was determined by HPLC [24], and iodine was analysed by ICP-MS [25]. Mercury was determined by ICP-MS after pressure digestion including microwave heating [26]. Dioxins and dioxin-like PCBs in the salmon were analysed using GC/MS [27].

Based on the analyses (Table S1) and assuming that the measured mercury and dioxin like polychlorinated biphenyls (PCB) content of the study salmon was representative of the total fish intake, the median fish intake of 45.3 g per day as observed in the salmon group corresponds to a weekly intake of about 3 µg Hg and 140 pg toxic equivalent (TEQ) dioxin-like PCB. For an average weight child in the study this corresponds to intakes of 0.2 µg/kg/week and 7 pg/kg/week, respectively. The CONTAM panel of the European Food Safety Authority (EFSA) has established a tolerable weekly intake for inorganic mercury of 4 µg/kg body weight and of 14 pg TEQ per kilo bodyweight for dioxin and dioxin-like PCB [28]. Thus, the estimated median Hg intake is only about 4% of the tolerable intake, and there is practically no risk of excessive mercury intake, but more care has to be taken in case of the PCB as median intakes could be as high as 50% of the acceptable intake.

2.5. Pre- and post-intervention measurements

2.5.1. Wechsler preschool and primary scale of intelligence, 3rd edition (WPPSI-III)

The primary outcome was cognitive function. We used the standardized HAWIVA-III (Testzentrale, Göttingen, Germany), which corresponds to the WPPSI-III test with norms for German children [29]. This test was chosen because it is a widely used and standardized test which provides normed scale scores by age of a German population. All seven core tests (information, vocabulary, word reasoning, block design, matrix reasoning, picture concepts and coding) that are mandatory for calculating the full scale IQ (FIQ) score were performed. Furthermore, the symbol search subtest was conducted in order to get the complete subscale processing speed (PSQ), which includes coding and symbol search. The other two subscales were the verbal IQ (VIQ), which includes information, vocabulary and word reasoning, and the performance IQ (PIQ), with block design, matrix reasoning as well as picture concepts. In addition, WPPSI-III raw scores including the eight sub-tests were used according to the following groupings: verbal sub test (information, vocabulary, word reasoning), performance sub-test (block design, matrix reasoning, picture concepts), processing speed sub-test (coding, symbol search), and total raw score (all eight sub-tests) following the suggestion of White et al [30]. The test was performed according to the WPPSI-III manual [29]. All tests were administered by nine testers, all females and all with academic training in education or psychology. Before data collection, testers were trained for at least two days and reviewed to ensure standardized data collection. Each tester videotaped a sample session and obtained feedback on her performance. In all cases test and test replication were performed by the same tester. If possible, tests were conducted in a kindergarten setting, otherwise tests were performed in a neutral room in the home of the child. Each child was tested individually. All testers were blinded for the child's group allocation (salmon or meat).

2.5.2. Nine whole peg test (9-HPT)

The 9-HPT was used for assessment of fine manual dexterity. Testing procedures were adapted from detailed explanations [31]. The test was explained to the children according to a standardized protocol. Each subject was provided the opportunity to practice exactly once before the timed test. All children started with the dominant hand (9-HPT1) and did the second round with the non-dominant hand (9-HPT2). The dominant hand was identified by the tester as the hand used in the WPPSI-III test for writing with the pen.

2.5.3. Food frequency questionnaire (FFQ)

For assessing nutrient intake before and during the intervention, the web based FFQ “European Food Propensity Questionnaire” developed by the Department of Epidemiology of the German Institute of Human Nutrition Postdam-Rehbrücke (revision 3528) was used [32].

2.5.4. Food diary and socioeconomic status

During the intervention period, the parents answered the following questions for each day: has the child eaten a study meal, which menu was chosen, how much of the study meal was consumed (nothing, ¼, ½, ¾, one portion, more than one portion), other seafood meals eaten, intake of supplements, has the child been sick. The total numbers of portion eaten was used as an estimate of dietary compliance. Questions regarding socioeconomic status (SES) were included in the food diary and it was measured by four questions according to the social class index of the KiGGS study (Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland) [33].

2.6. Biological samples

Non-fasted blood samples were collected by venipuncture in EDTA coated tubes (2.7 ml, Sarstedt, Nürmbrecht, Germany). Blood cells and plasma were separated by centrifugation at +4 °C with 1600 g for 10 min. Plasma samples were stored for up to 8 months at –80 °C until analysis. Cheek cells were collected after a mouth wash with an endocervical brush (Herenz Medizinalbedarf, Hamburg, Germany) and additional mouth rinse with 10 ml water. After collection brush and mouse rinse were centrifuged together in a 15 ml tube (Sarstedt, Nürmbrecht, Germany) with 1400×g for 10 min at +4 °C to obtain a mucosal cell pellet devoid of bacteria. Supernatant and brush were discarded and the cell pellet stored at –80 °C until analysis.

Urine was collected, using urine monovettes (Sarstedt, Nürmbrecht, Germany), by the parents at home and brought without refrigeration to the study center.

Quantification of glycerophospholipid fatty acids in plasma and determination of the composition of cheek cell glycerophospholipid fatty acids were performed as described elsewhere [34,35]. With both methods selective preparation of methyl ester derivatives of glycerophospholipid fatty acids is obtained by coprecipitation of triacylglycerols and cholesterol esters with proteins and base catalysed transesterification excluding methyl ester synthesis from non-esterified fatty acids. Serum 25-hydroxyvitamin D (s-25(OH)D) concentrations were determined by a liquid chromatographic-tandem mass spectrometric assay [36]. Urinary iodine concentration was determined in spot samples by ICP-MS [37].

2.7. Statistical analyses

Continuous variables are expressed as mean with standard deviation (SD), or if the distribution is highly skewed, as median with

interquartile range (IQR), and categorical variables as numbers and percentages. Independent samples t-test was used for continuous normal distributed variables. Independent samples Mann–Whitney U test was used for skewed distributed variables, for comparisons between the intervention groups at pre-intervention. Chi-square test (Fishers Exact Test) was used for comparisons of categorical variables between the intervention groups at pre-intervention. The correlations between numbers of study meals and total fish intake and plasma EPA + DHA, DHA, total fish intake, and WPPSI-III score changes were assessed using Spearman's bivariate rank order correlation (ρ). Paired-samples t-test was used for comparisons of pre-and post-intervention data within each group for the normal distributed variables, and related-samples Wilcoxon signed rank test was used for skewed distributed variables. In addition, general linear models with adjustments for the pre-intervention WPPSI-III variable, and age post-intervention for the raw scores (only appropriate when using raw scores) were used for the WPPSI-III scores and 9-HPT Results. All primary outcome analyses were repeated with stratification for child sex. In addition, analyses with adjustments for socioeconomic status and location of test performance (Kindergarten or at home) were done. Furthermore, sub-group analyses for the children who ate at least 34 study meals (70% of the allocated number of meals according to the protocol) were performed.

The analyses were performed using the Statistical Package for the Social Sciences (SPSS) for windows (IBM SPSS Statistics 23, Chicago, IL, USA). P-values below 0.05 were considered statistically significant and due to the explorative nature of the analyses, multiple testing was not considered in the statistical evaluation.

3.0. Results

The principal finding of the study is that the children improved outcomes in several WPPSI-III IQ scale scores from pre-to post-intervention, but no significant differences between the intervention groups were observed, but in the raw scores of two out of eight applied subtests the children in the salmon group showed greater improvements than the children in the meat group.

3.1. Study population

Families of 205 children were enrolled and randomized to salmon ($n = 101$) or to meat meals ($n = 104$) in March and April 2014. The intervention was completed in August 2014, prior to the summer holiday period in Bavaria. Sixteen children did not complete the intervention. Thus, 96 children in the salmon group and 93 children in the meat group completed the intervention including pre and post study testing and were included into the intention to treat analysis. The final sample for the 9-HPT analysis included 91 and 90 children in the salmon and meat group, respectively, because the test could not be performed adequately in five children in the salmon group and three children in the meat group, respectively (Fig. 1). The baseline description of the children completing the study is shown in Table 1. There were no differences between the study groups and the non-completers (data not shown) were not different from the completers.

3.2. Pre-to post-intervention

3.2.1. Study meals

According to the study diaries during the intervention period some 34.0 (median, IQR 16.9) of 48 study meals (16 weeks times three meals per week) were consumed. No significant difference of the number of consumed meals between the salmon (median 33.6, IQR 20.3) and meat group (34.3, 13.7) was observed ($p = 0.148$). The

Table 1
Baseline characteristics of study participants by intervention group. Data are median (IQR).

	N	Overall (n = 189)	Salmon group (n = 96)	Meat group (n = 93)	P-value ^a
Demographics					
Age (yr)	185	5.0 (0.8)	4.9 (0.8)	5.0 (0.8)	0.483
Weight (kg)	185	18.7 (3.2)	18.9 (4.0)	18.6 (2.9)	0.668
Height (cm)	183	110.6 (7.8)	110.9 (9.6)	110.1 (7.2)	0.695
Boys (n, %)	189	94 (49.7)	50 (52.1)	44 (47.3)	0.562
Girls (n, %)		95 (50.3)	46 (47.9)	49 (52.7)	
Handedness (n, %)					
Right	189	169 (89.5)	86 (89.6)	83 (89.2)	1.000
Left		20 (10.6)	10 (10.4)	10 (10.8)	
Test location (n, %)					
Kindergarten	189	150 (79.4)	74 (77.1)	76 (81.7)	0.475
Home		39 (20.6)	22 (22.9)	17 (18.3)	
Socioeconomic score					
Mother	170	16.0 (5.0)	17.0 (4.0)	16.0 (5.0)	0.292
Father	170	17.0 (5.0)	17.0 (5.3)	16.0 (5.0)	0.341

^a P-value for comparison for differences between intervention groups are given with Independent samples Mann–Whitney U test for continuous variables and Fisher's exact tests for categorical variables.

energy and nutrient content of the individual meat and salmon meals is shown in Table 2.

3.2.2. Nutritional intake

From the FFQ data we estimate a median habitual weekly fish intake of approximately 110 g in both groups. For the intervention period the FFQ indicates for the salmon and meat groups weekly fish intakes of approximately 315 g and 70 g, respectively (Table 3). A significant correlation between reported numbers of study meals and total fish intake was observed in the salmon group (ρ 0.302, p = 0.003), but not in the meat group (ρ 0.159, p = 0.136). The participants in the salmon group increased their dietary intake of EPA, DHA and vitamin D during the intervention, whereas the subjects in the meat group decreased their intake of EPA, DHA, vitamin D, iodine and also showed a significant decrease of total energy intake (Table 3). Both groups seemed to decrease their carbohydrate intakes. Significant intake differences between the groups were observed for fish, EPA, DHA, total polyunsaturated fatty acids, vitamin D, and iodine (Table 3).

3.2.3. Biological parameters

Plasma and cheek cell EPA and DHA increased in the salmon group, with significantly greater changes from pre-to post-intervention than in the meat group (Fig. 2). The omega-3 index (the content of EPA and DHA expressed as percent of total fatty acids) increased from $3.9 \pm 0.9\%$ to $5.2 \pm 1.3\%$ in the salmon group (p < 0.001) and was unchanged in the meat group ($4.1 \pm 1.1\%$ to $3.9 \pm 1.1\%$, p = 0.107), resulting in a significant group difference of the change (p < 0.001).

With the transition from spring to summer during the intervention period, s-25(OH)D increased significantly both in the salmon (from 46.9 ± 19.0 nmol/L to 74.2 ± 17.0 nmol/L, p < 0.001) and the meat group (from 44.4 ± 12.0 nmol/L to 72.5 ± 17.0 nmol/L, p < 0.001) from pre-to post-intervention. No significant differences between the intervention groups were observed for the change (p = 0.747).

Urinary iodine concentration decreased significantly both in the salmon (from median (IQR) 110.0 (127.5) μ g/L to 88.0 (90.5) μ g/L, p = 0.025) and the meat group (from 110.0 (118.5) μ g/L to 75.0 (69.0) μ g/L, p < 0.001) from pre-to post-intervention. No significant differences between the intervention groups were observed for the change (p = 0.241).

There was a significant correlation between reported total fish intake in grams and plasma omega-3 index post-intervention in the salmon group (ρ 0.24, p = 0.036), but not in the meat group (ρ 0.12, p = 0.278).

3.2.4. WPPSI-III

3.2.4.1. IQ scale scores. The children in the salmon group improved outcomes in the WPPSI-III FIQ, PIQ, and PSQ scores from pre-to post-intervention, and the children in the meat group improved their PSQ score (Table 4). However, no significant differences between the intervention groups were observed for the changes of any of the WPPSI-III IQ scale scores from pre-to post-intervention (Table 4).

Although the major part of dietary fish was derived from the background diet, we investigated the correlations between the number of consumed study meals and the change of plasma DHA

Table 2
Energy and nutrient contents of the individual salmon and meat meals. Data are mean \pm SD.

	Protein (g/100 g)	Total fat (g/100 g)	EPA (mg/g)	DHA (mg/g)	Vitamin D (mg/kg)	Iodine (mg/kg)
Salmon meals						
Pasta filled with salmon pate	11.05 \pm 0.21	10.10 \pm 0.28	1.11 \pm 0.04	2.03 \pm 0.05	0.010 \pm 0.001	0.070 \pm 0.003
Salmon fillet in paprika sauce	8.80 \pm 0.14	8.25 \pm 0.92	1.13 \pm 0.17	2.00 \pm 0.12	0.02 \pm 0.01	0.030 \pm 0.002
Pasta sauce with salmon ("Bolognese")	7.900 \pm 0.004	6.65 \pm 0.07	1.03 \pm 0.01	1.860 \pm 0.001	0.010 \pm 0.001	0.070 \pm 0.004
Potato gratin with salmon	9.75 \pm 0.21	11.65 \pm 0.35	1.410 \pm 0.003	2.38 \pm 0.04	0.020 \pm 0.002	0.040 \pm 0.002
Salmon burger	11.85 \pm 0.07	9.55 \pm 0.35	1.47 \pm 0.03	2.67 \pm 0.06	0.020 \pm 0.002	0.060 \pm 0.002
Meat meals						
Turkey fillet in paprika sauce	14.00 \pm 0.57	3.55 \pm 0.07	0.030 \pm 0.001	0.030 \pm 0.001	<0.01	0.020 \pm 0.001
Potato gratin with turkey fillet	11.85 \pm 0.35	5.20 \pm 0.14	0.02 \pm 0.01	0.030 \pm 0.001	<0.01	0.040 \pm 0.002
Beef burger	16.50 \pm 0.71	10.70 \pm 1.13	0.10 \pm 0.01	0.060 \pm 0.002	<0.01	0.060 \pm 0.004
Tortellini with ham filling	9.05 \pm 0.35	3.65 \pm 0.35	0.040 \pm 0.002	0.020 \pm 0.001	<0.01	0.060 \pm 0.003
Bolognese sauce	5.25 \pm 0.49	3.95 \pm 0.35	0.010 \pm 0.001	0.020 \pm 0.001	<0.01	0.07 \pm 0.02

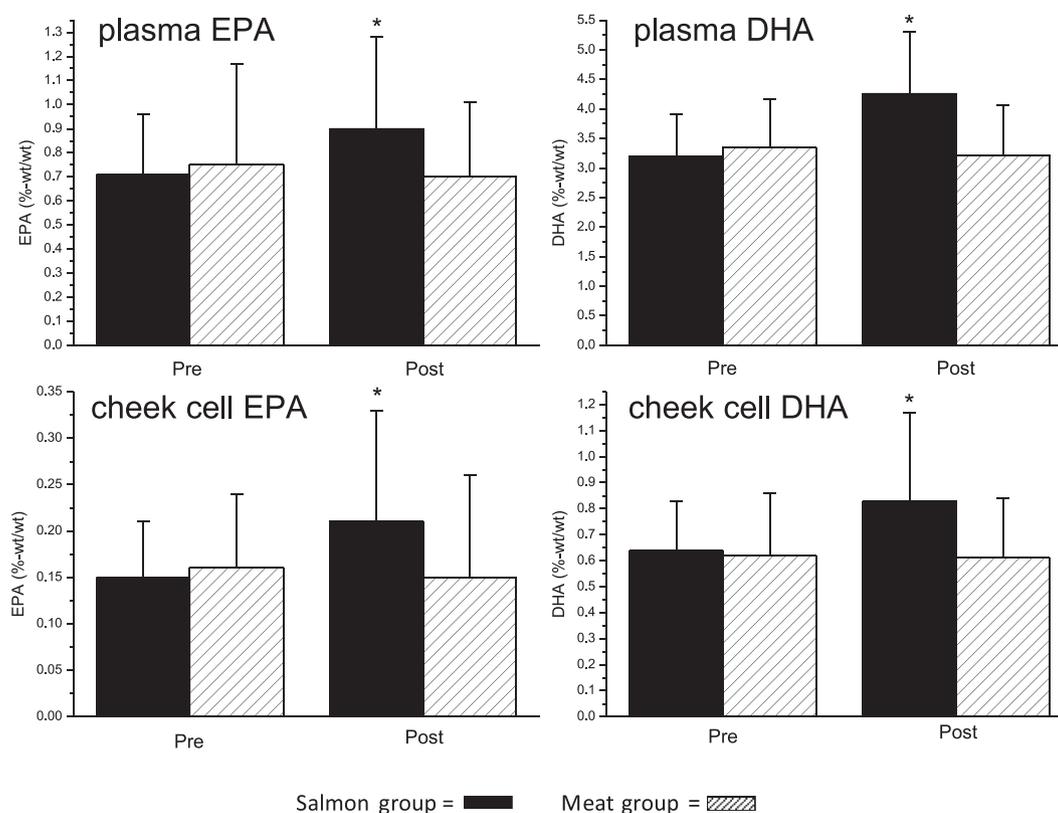
Abbreviations: DHA, docosahexaenoic acids; EPA, eicosapentaenoic acids.

Table 3

Pre- and post-intervention daily dietary intakes of the children in each intervention group. Data are median (IQR).

	Salmon group (n = 96)					Meat group (n = 93)					
	N	Pre	Post	Change	P-value	N	Pre	Post	Change	P-value ^a	P-value ^b
Total fish (g)	95	16.2 (15.2)	45.3 (29.7)	29.7 (38.1)	<0.001	92	15.6 (14.1)	9.7 (11.7)	-2.8 (12.6)	<0.001	<0.001
EPA (g)	91	0.07 (0.09)	0.31 (0.20)	0.24 (0.26)	<0.001	89	0.07 (0.09)	0.05 (0.04)	-0.01 (0.07)	<0.001	<0.001
DHA (g)	91	0.16 (0.11)	0.41 (0.24)	0.26 (0.30)	<0.001	89	0.16 (0.10)	0.12 (0.07)	-0.02 (0.10)	<0.001	<0.001
Total energy (kJ)	91	7612 (2434)	7210 (2344)	-338 (2051)	0.289	89	7552 (2418)	7040 (2311)	-364 (2196)	0.045	0.493
Total fat (g)	91	87.5 (29.4)	87.3 (26.3)	-1.2 (26.4)	0.992	89	83.6 (29.7)	83.4 (30.1)	-2.6 (26.5)	0.211	0.344
Saturated fat (g)	91	36.0 (10.5)	34.1 (10.9)	-1.0 (10.9)	0.254	89	33.4 (13.4)	33.8 (13.3)	-1.5 (10.9)	0.216	0.896
Monounsaturated fat (g)	91	32.2 (11.0)	32.1 (10.3)	0.3 (9.7)	0.489	89	31.6 (11.1)	30.8 (11.8)	-1.1 (10.2)	0.317	0.226
Polyunsaturated fat (g)	91	13.8 (4.6)	13.7 (5.2)	0.6 (3.2)	0.060	89	13.1 (4.4)	12.8 (3.8)	-0.5 (4.4)	0.099	0.017
Total carbohydrate (g)	91	184.7 (72.6)	175.5 (56.6)	-15.3 (49.7)	0.004	89	192.4 (70.7)	177.4 (58.0)	-10.8 (66.1)	0.010	0.965
Total protein (g)	91	62.2 (21.4)	62.8 (19.0)	1.8 (16.5)	0.223	89	60.4 (21.2)	59.9 (20.9)	-0.1 (19.8)	0.429	0.149
Vitamin D (mg)	91	0.003 (0.002)	0.008 (0.005)	0.006 (0.010)	<0.001	89	0.003 (0.002)	0.002 (0.002)	-0.001 (0.002)	<0.001	<0.001
Iodine (mg)	91	0.076 (0.025)	0.081 (0.029)	0.002 (0.030)	0.181	89	0.073 (0.028)	0.070 (0.026)	-0.009 (0.030)	0.008	0.004

Abbreviations: DHA, docosahexaenoic acids; EPA, eicosapentaenoic acids; IQR, interquartile range.

^a P-value for comparison within the intervention groups are given with related-samples Wilcoxon signed rank test.^b P-value for comparison of the changes between the intervention groups are given with Independent-samples Mann–Whitney U test.**Fig. 2.** Pre- and post-intervention percentages (mean + SD) of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in plasma and cheek cell phospholipids. While pre-intervention values were not different between the salmon group and the meat group, all post-intervention values were significantly different between groups ($p < 0.001$, t-test). Correspondingly changes from pre-to post-intervention were only in the salmon group statistically significant ($p < 0.001$, paired sample t-test).

and the WPPSI-III FIQ. Fig. 3 shows that after stratification according to study group only the DHA change in the salmon group was significantly associated with the intake of study meals and that in none of the two groups an association of the WPPSI-III FIQ change with the intake could be found.

In sub-group analyses including the children who ate at least 70% of the study meals (considered as per protocol participants) the estimates were similar as for the whole population with available data (considered as intention to treat population) without differences between the intervention groups (Table S2).

3.2.4.2. Raw scores. In both intervention groups the WPPSI-III raw scores improved in almost all scores (total scale, sub-scale and sub-test level) from pre-to post-intervention (Table 5). Interestingly, the WPPSI-III Performance sub-scale score improved more in the salmon group than in the meat group from pre-to post-intervention, which was mainly due to a difference in the picture concepts sub-test. Furthermore, in the symbol search sub-test the salmon group improved more than the meat group from pre to post testing. No further significant group differences were observed and if adjustment for multiple testing according to Bonferroni would

Table 4

Pre- and post-intervention WPPSI-III IQ scale scores and predicted change after study meals with salmon (n = 96) and meat (n = 93). Pre- and post-intervention data are mean \pm SD, and changes are mean (95% CI).

	Pre	Post	p-value ^a	Adjusted for pre-score	
				Change	p-value ^b
FIQ					
Salmon	104.7 \pm 12.9	106.5 \pm 12.3	0.011	1.2 (0.6, 3.1)	
Meat	103.5 \pm 11.7	104.6 \pm 10.9	0.087	1.0 (-0.2, 2.2)	0.334
VIQ					
Salmon	105.4 \pm 13.7	105.0 \pm 13.7	0.545	-0.4 (-1.8, 1.0)	
Meat	103.7 \pm 14.1	103.5 \pm 12.9	0.799	-0.3 (-1.6, 1.1)	0.923
PIQ					
Salmon	104.3 \pm 13.5	107.8 \pm 11.8	<0.001	3.5 (1.8, 5.2)	
Meat	104.2 \pm 10.7	105.6 \pm 12.3	0.139	1.4 (-0.3, 3.1)	0.082
PSQ					
Salmon	102.9 \pm 15.7	106.1 \pm 13.7	0.006	3.4 (1.3, 5.6)	
Meat	101.5 \pm 14.9	105.1 \pm 15.5	0.009	3.3 (1.1, 5.5)	0.934

Abbreviations: FIQ, full scale IQ; PIQ, performance IQ; PSQ, processing speed; VIQ, verbal IQ.

^a P-value for comparison of individual pre- and post-intervention values within each intervention group with paired-samples T-test.

^b P-value are given with general linear model with adjustment for pre-intervention score for comparison between the intervention groups.

have been performed (0.05/12 tests = 0.004) none of the differences would have been considered statistically significant (Table 5). The analyses of the per protocol sub-group indicated similar findings as for the intention to treat population (Table S3).

3.2.4.3. 9-HPT. For the 9-HPT evaluation Results of children with test behaviour rated as not adequate by the testers, were excluded (five in the fish and three in the meat group). Considering this, both intervention groups improved their time for 9-HPT1 and 9-HPT2 from pre-to post-intervention, but without a significant group difference (Table 6).

3.2.4.4. Confounding factors. All described analyses were repeated with stratification for child sex. This yielded basically the same Results for boys and girls and did not indicate gender difference in the outcome (data not shown). Similarly inclusion of socioeconomic status and location of test performance (kindergarten or at

home) did not appreciably influence the results with the exception of 9-HPT1, where the group difference of the change tended towards significance with a p-value after adjustment of 0.08.

4.0. Discussion

The dietary intervention with meat or salmon, respectively, did not change the development of the total IQ score, but the salmon meals induced a slightly stronger improvement in two out of 8 performance IQ sub-test raw scores relative to children eating meat meals. On the other hand, the 16-week intervention comprising three meals per week containing 50 g salmon each increased plasma DHA and EPA in a group of 4–6 year old children with a low fish intake compared to the corresponding meat intake.

The present findings align well with the outcome of studies randomizing children and adolescents to n-3 LC-PUFA supplementation or a placebo, which frequently showed no overall benefit of the active intervention compared to placebo, but some advantages in specific cognitive domains as reviewed in 2010 [7]. Although different cognitive tests have been performed or different age groups have been studied, the present study could be compared to the FINS-TEENS study, the OPUS school meal study [38,39] and specifically with the FINS-KIDS study, where the same tests were applied in the same age group, and thus comparisons can focus on the intervention and the dietary background [18].

The recently published FINS-KIDS study found no overall effects on cognitive function in the main analyses comparing herring/mackerel intake to chicken/lamb/beef in children aged 4–6 years [18]. While this is in agreement with the present RCT, a higher improvement of the total WPPSI-III raw scores in the fish compared to the meat group was found after taking dietary compliance into account in the FINS-KIDS study. This difference in the findings might be related to the significantly higher measured compliance in the meat group compared to the fish group in the FINS-KIDS study, while according to parental report the participants in the trial presented here showed similar compliance in both groups. In contrast to the FINS-KIDS trial we could not find a significant association between the change of the WPPSI-III scores and the number of study salmon meals consumed although it was significantly associated with the DHA increase (Fig. 3). Thus, the findings

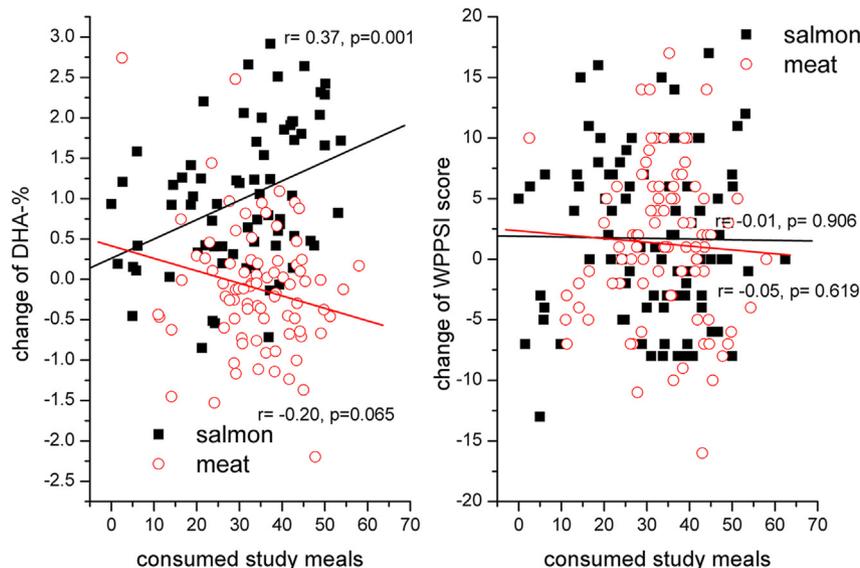


Fig. 3. Association between the number of consumed study meals after stratification for the salmon and the meat group and the change of the plasma DHA percentage and the WPPSI-III full scale IQ (FIQ).

Table 5

Pre- and post-intervention WPPSI-III raw scores and predicted change after study meals with salmon (n = 96) and meat (n = 93). Pre- and post-intervention data are mean ± SD, and change are mean (95% CI).

WPPSI-III	Pre	Post	p-value ^a	Adjusted for pre-score and age	
				Change ^b	p-value ^c
Total raw score ^d					
Salmon	123.6 ± 29.7	140.8 ± 30.8	<0.001	17.4 (14.8, 20.1)	
Meat	118.7 ± 29.0	133.6 ± 27.0	<0.001	14.6 (11.9, 17.3)	0.143
Verbal raw score					
Salmon	40.8 ± 10.6	39.1 ± 10.4	<0.001	2.4 (1.5, 3.4)	
Meat	39.1 ± 10.4	41.1 ± 9.9	0.001	1.9 (0.9, 2.9)	0.444
Information					
Salmon	19.2 ± 3.4	20.3 ± 3.5	<0.001	1.1 (0.6, 1.5)	
Meat	19.1 ± 3.4	19.7 ± 3.2	0.014	0.6 (0.2, 1.0)	0.142
Vocabulary					
Salmon	11.6 ± 5.6	12.4 ± 5.0	0.055	0.9 (0.2, 1.6)	
Meat	10.9 ± 5.5	11.4 ± 5.3	0.240	0.4 (−0.3, 1.1)	0.329
Word reasoning					
Salmon	10.0 ± 3.0	10.4 ± 3.2	0.053	0.6 (0.1, 1.0)	
Meat	9.1 ± 3.1	10.1 ± 3.2	<0.01	0.8 (0.4, 1.3)	0.407
Performance raw score					
Salmon	45.6 ± 9.3	50.5 ± 9.3	<0.001	5.0 (3.8, 6.2)	
Meat	44.6 ± 8.7	47.9 ± 9.4	<0.001	3.2 (2.1, 4.4)	0.039
Block design					
Salmon	30.2 ± 5.2	32.4 ± 5.9	<0.001	2.3 (1.4, 3.2)	
Meat	29.0 ± 5.9	30.7 ± 5.7	0.002	1.5 (0.6, 2.4)	0.222
Matrix reasoning					
Salmon	7.0 ± 3.1	8.1 ± 3.0	<0.001	1.1 (0.7, 1.6)	
Meat	6.6 ± 2.5	7.7 ± 2.7	<0.001	1.0 (0.6, 1.5)	0.718
Picture concepts					
Salmon	8.3 ± 3.6	10.0 ± 3.2	<0.001	1.5 (1.0, 2.1)	
Meat	9.0 ± 3.2	9.5 ± 3.5	0.149	0.7 (0.1, 1.3)	0.038
Processing speed raw score					
Salmon	37.3 ± 16.4	47.3 ± 17.5	<0.001	10.1 (7.9, 12.3)	
Meat	35.1 ± 16.6	44.6 ± 17.0	<0.001	9.4 (7.1, 11.6)	0.640
Coding					
Salmon	23.3 ± 10.2	28.3 ± 12.0	<0.001	5.2 (3.3, 7.0)	
Meat	21.7 ± 10.7	27.3 ± 11.9	<0.001	5.4 (3.6, 7.3)	0.833
Symbol search					
Salmon	14.0 ± 7.6	19.0 ± 7.6	<0.001	5.0 (4.1, 6.0)	
Meat	13.3 ± 7.7	17.0 ± 7.5	<0.001	3.6 (2.6, 4.6)	0.047

Abbreviations: WPPSI, Wechsler Preschool and Primary Scale of Intelligence.

^a P-value are given with Paired-samples T-test for comparison of individual pre- and post-intervention values within each intervention group.

^b Change as estimated by the General linear model applied.

^c P-value are given with general linear model adjusted for pre-intervention score and age for comparison between the intervention groups.

^d Total raw score here refers to the sum of the raw scores of all performed subtests including the symbol search subtest, which is not included in the IQ calculation.

Table 6

Pre- and post-intervention nine hole peg test (9-HPT) Results and predicted change after study meals with salmon (n = 91) and meat (n = 90). Pre- and post-intervention data are mean ± SD, and change are mean (95% CI).

	Unadjusted		p-value ^a	Adjusted for pre 9-HPT	
	Pre	Post		Change	p-value ^b
9-HPT1					
Salmon	29.9 ± 5.7	28.2 ± 4.2	0.004	−2.0 (−2.9, −1.1)	
Meat	30.8 ± 6.3	27.6 ± 5.5	<0.001	−3.0 (−3.8, −2.1)	0.149
9-HPT2					
Salmon	34.2 ± 7.1	31.0 ± 4.2	<0.001	−3.6 (−4.8, −2.4)	
Meat	35.0 ± 7.8	31.1 ± 7.3	<0.001	−3.6 (−4.8, −2.4)	0.976

Abbreviations: 9-HPT1, Nine Hole Peg Test dominant hand; 9-HPT2, Nine Hold Peg Test non-dominant hand.

^a P-value are given with Paired-samples T-test for comparison of individual pre- and post-intervention values within each intervention group.

^b P-value are given with general linear model adjusted for pre-intervention score for comparison between the intervention group.

of both trials are not fully in line, which could be related to the unsupervised intake of the study meals at home by the German children, which prohibited a similarly precise estimation of compliance as in the FINS-KIDS study and the lower amount of fish per meal applied in the current study (50 g) compared to FINS-KIDS (50–80 g) [18]. Furthermore, a higher fish intake of the Norwegian children already at baseline could be a factor [18] and the fish tested in FINS-KIDS are different from salmon with a higher n-3 LC-PUFA

content in mackerel and a higher vitamin D content in herring [18]. Differences in the effects between different fish, would be in line with the assumption of effects of fish intake beyond the provision of n-3 LC-PUFA.

FINS-TEENS compared the effects of fatty fish (herring, mackerel, salmon), meat and n-3 LC-PUFA supplements in a RCT with adolescents in a 12 week school meal intervention. The d2 test and the strength and difficulties questionnaires performed pre- and

post-intervention did not reveal significant differences between the groups [40,41]. The OPUS School Meal Study also investigated the influence of a three months intervention with meals according to the New Nordic Diet, of which fatty fish comprised a substantial part, in comparison to habitual diets on concentration and school performance in 8–11 year old children using a crossover design. It turned out that the New Nordic Diet was significantly associated with improved school performance and reading comprehension, but better reading comprehension was also associated with a higher error rate in the d2 test for concentration [39]. In our study, in OPUS, and in FINS-TEENS higher intake of salmon or other fatty fish was evaluated with a randomized study design, but besides the difference in outcome measures also the evaluated nutrients could have been different due to different kinds of fish tested and additional dietary changes introduced [39,42,43].

Based on the FFQ data we estimated a clearly higher daily EPA + DHA intake in the salmon group than in the meat group, mainly due to increased intake in the salmon group, but also partially explained by lowered intake in the meat group. This suggests that our study design with three salmon meals per week, providing about 200 mg EPA + DHA per day, introduced a higher group difference than 100 mg/day, as reported for the OPUS study [39]. We found a slightly, but significantly, higher iodine intake in the salmon group compared to the meat group, although the iodine contents in the study meals were similar. However, both intervention groups showed a decrease of urinary iodine concentrations from pre- to post-intervention, which suggests that overall iodine status of the groups were similar. The intake data showed a higher vitamin D intake in the salmon group, but both groups had increased and not significantly different s-25(OH)D levels post-intervention, which seems to be due to a seasonal effect which overrides the small dietary intake difference [44]. Thus, our intervention seems comparable with specific n-3 LC-PUFA supplementations in healthy children which used typically dosages from about 100 to 1200 mg per day [45–50] and even higher doses in studies with patients suffering from various disorders [51]. In these n-3 LC-PUFA supplementation trials beneficial effects were only found in individual subtests and/or in subgroups, but no general improvement of cognitive function was observed [45–50]. This is in agreement with our Results, as we did not observe a general improvement, but some aspects of the WPPSI-III test seemed to be positively influenced by salmon compared to meat.

In the OPUS study, fish intake was associated with whole-blood EPA + DHA, the omega-3 index [52]. In the present trial, the association between plasma omega-3 index and reported fish intake was significant in the salmon group post-intervention. A close correlation between seafood intake and blood EPA + DHA has also been found in other studies [53,54]. The omega-3 index determined from whole blood has also been found associated with two of nine cognitive performance scores in typically developing Dutch adolescents [55]. The 3.9% pre-intervention omega-3 index of our subjects was similar to Results from other studies in European children [52,55,56], but lower than in the FINS-KIDS study [18]. Taken together with the observational studies, which showed positive associations between fish consumption and cognitive and behavioural performance [11–13], this suggests that n-3 LC-PUFA are good biomarkers for fatty fish intake and fish intake and cognitive function are positively associated. However, it does not demonstrate a primary importance of n-3 LC-PUFA intake for mediating this association.

In our study, the raw scores of the sub-tests picture concepts and symbol search improved significantly more in the salmon group compared to the meat group. This is interesting as both subscales are indicators of non-verbal fluid intelligence, i.e. the capacity to solve novel problems, in contrast to crystallized

intelligence, which is described as the ability to use already learned knowledge [57]. In their systematic review on the effects of multiple micronutrient supplementation on cognitive performance in children Eilander et al. found that there seems to be a small effect on measures of non-verbal fluid intelligence, but not on measures of verbal crystallized intelligence [58]. Thus, although there seems to be no effect of salmon intake on global IQ, this leaves the possibility that certain aspects of cognitive performance are positively affected.

The 9-HPT provided no indications of an effect of fish intake on fine manual dexterity. This is in agreement with an observational study in 7 year old children, which did not find an association between current DHA status and motor skills [59], and an intervention study in children with developmental coordination disorders comparing a DHA, EPA, γ -linolenic acid supplement to a placebo and reported no significant group differences of motor skills [60]. On the other hand, in children suffering from phenylketonuria, who had due to their dietary constraints an extremely low EPA and DHA intake, some benefit of supplementation could be shown [61], and in the above mentioned study by Bakker et al. a positive association between cord blood DHA and motor skills at age 7 could be identified [59]. Interestingly, in the coordination disorder children and in the studied healthy children beneficial effects of supplementation on cognitive function or a positive association of current motor skills with umbilical cord DHA, respectively, have been reported [59,60]. This might indicate that effects on motor skills are more difficult to detect, or motor skills are less sensitive to n-3 LC-PUFA status than cognitive functions, or that the status very early in life is considerably more influential than the status later in childhood.

A major strength of this trial is the individual randomized intervention design, the provision of defined meals, and the collection of biological samples and chemical analysis of the study meals. The study compared the effects of salmon intake to meat intake, thus we compared food items rather than specific nutrients. While this precludes the allocation of effects to a specific nutrient, it enables a better comparison of the Results to observational studies and effects of not quantified nutrients in fish and meat including eventual synergistic effects are studied.

The 16 week intervention period was chosen based on the recommendation by Stonehouse [62]. This intervention period could be criticized as too short, but a study in non-human primates showed that brain fatty acid composition adapts within 12 weeks to an increased EPA + DHA intake with changes already detectable after one week [63]. If this applies to humans as well, a reasonable amount of time was left for differences in cognitive performance to establish. As the retesting has been performed 16 weeks after the initial test, it has to be assumed that the retest Results are influenced by the initial test, but there are no indications that this influence is different between randomized groups.

A limitation of this study is the inaccuracy of the dietary data obtained with the FFQ where portion size definitions varied among children and where no validation was available for estimating quantitative nutrient intakes in children. The intake of study meals was estimated according to parental report, while in the FINS-KIDS study the intake was exactly quantified by weighing the not consumed part of the study meals.

Our study population tended to have a relatively high socioeconomic status as compared to the population in the representative KiGGS study [33]. Thus, generalizability of the findings might be limited, although socioeconomic score as a confounder in the statistical models did not substantially change the Results.

Our Results are based on the participants who completed the intervention with cognitive measures at pre- and post-intervention. Thus, not all recruited participants could be studied, but as there were no difference between completers and non-

completers at baseline, including WPPSI-III scores, we assume that completers with available data are representative of the whole population. Furthermore, a strict per protocol analysis was not possible, as only 15 children consumed the allocated 48 study meals. Alternatively the sub-group of 46 and 50 children in the salmon and meat group, respectively, who consumed at least 70% meals was analyzed and this yielded the same findings as the main analyses, which agrees with the assumption that the whole dietary intake is of importance rather than the study meals only.

In conclusion, the Results obtained with randomizing children to salmon or meat meals are comparable to findings in randomized trials comparing n-3 LC-PUFA to a placebo and partially agree with findings after intervention with herring and mackerel. Increased salmon intake did not improve IQ significantly more than meat intake in preschool children after four months of intervention, but improvement of two sub-test raw scores related to non-verbal fluid intelligence indicates a possibility that specific developmental aspects of cognitive functions might benefit from increased salmon consumption.

Statement of authorship

The authors' responsibilities were as follows: HD, TP, MK, ARP, KMS, IEG, ØL, and BK designed the research; HD, TP, MK, ARP conducted the research; JØ performed the statistical analyses; HD, JØ, MK, KMS, and BK prepared the manuscript; all authors read and critically revised the manuscript and approved the final manuscript, and had primary responsibility for final content. None of the authors had any financial or personal relationship with the companies, outside of sponsoring the research, and the authors declare no conflict of interest.

Conflict of interest statement and funding sources

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2018.11.031>.

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