European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1734-x

ORIGINAL CONTRIBUTION

Effects of fortified milk on cognitive abilities in school-aged children: results from a randomized-controlled trial

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Received: 29 September 2017 / Accepted: 25 May 2018

Abstract

Background Micronutrients such as vitamins and minerals and long-chain polyunsaturated omega-3 fatty acids (PUFAs) are essential for children's brain development and cognitive functions. The current study investigated whether milk fortified with micronutrients and PUFA can result in improved cognitive function in mainstream school children.

Methods One-hundred-and-nineteen children (age 8–14, 58 boys) were randomly allocated to a fortified milk group or a regular full milk control group. Participants consumed 0.6L/day of the milk for 5 months. We recorded relevant biochemical, anthropometric, and cognitive measures (working memory and processing speed) at the start of the study and at follow-up after 5 months.

Results The fortified milk significantly increased docosahexaenoic acid (DHA) (change from baseline of 28% [95% CI 17–39%] vs. −6% [95% CI − 13 to 0%] in the control group) and serum 25OH-vitamin D concentrations (41% [95% CI 30–52%] vs. 21% [95% CI 11–30%] in the control group). The fortified milk improved working memory on one of two tests (32% [95% CI 17–47%] vs. 13% [95% CI 6–19%] in the control group). The fortified milk also indirectly increased processing speed on one of two tests; this effect was small and completely mediated by increases in 25OH-vitamin D concentrations.

Conclusions These results suggest that fortifying milk with micronutrients and PUFA could be an effective and practical way to aid children's cognitive development.

Keywords Fortified milk · Omega-3 · PUFA · Micronutrients · Cognitive abilities · Working memory

Introduction

School-aged children are in a life stage of considerable cognitive and physical development. Not meeting the nutritional demands required for this intensive development can have negative effects on their physical state and cognitive performance and can influence health and productivity later in life [1]. The modern Western diet is generally poor in certain important micronutrients (mainly vitamin D, iron, iodine, and minerals) and essential fatty acids like long-chain polyunsaturated omega-3 fatty acids (PUFAs) that are important for brain development and cognitive function [2–5]. For instance, docosahexaenoic acid (DHA) is the most abundant fatty acid in the brain and is found in high concentrations in the membranes and vesicles of neuronal synapses [6]. It is involved in basic processes that influence cognitive development including synaptic efficacy, rate of transmission, and myelination [7]. The generally low levels of such important nutrients in the modern Western diet suggest that a change

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Published online: 07 June 2018

in diet would be beneficial. Children of lower socioeconomic status could be at risk of deficiencies because they have lower access to micronutrient- and PUFA-rich foods like fruits, vegetables, fish, meat, and dairy [8].

Micronutrient deficiencies can have a negative impact on children's cognitive development [9] and supplementation with micronutrients like iron, vitamin A, iodine, and zinc could be beneficial for children's cognitive function. It has been suggested that combined supplementation could achieve better results than single-nutrient supplementation [1]. Trials of micronutrients supplementation have often used fortified foods and drinks. The majority of these studies have been conducted in children with poor nutritional status (e.g., anemia, zinc deficiency, iodine deficiency). A systematic review showed that micronutrient-fortified food interventions generally showed mixed results on cognitive performance [1]. Improvements were most often observed in working memory (four of six studies showing significant results) (e.g., [1, 10, 11]). One study in particular tested the effectiveness of a fruit drink fortified with a micronutrient mix, PUFA, or both, in Australian (high socioeconomic status) and Indonesian (low socioeconomic status) children [11]. The micronutrient mix contained iron, zinc, folate, and vitamins A, B-6, B-12, and C, and the PUFA supplementation consisted of 88 mg/day of DHA and 22 mg/day of EPA (eicosapentaenoic acid) lasting for 12 months. Results showed that after 2 months the micronutrient mix increased verbal learning and memory in Australian children and Indonesian girls; however, neither the PUFA treatment, nor its combination with the micronutrient mix had effects on cognitive scores.

Consistent with these results, a recent meta-analysis of randomized controlled trials (RCTs) concluded that PUFA supplements could improve cognitive development in infants; however, no overall significant effects were observed in children, adults, or the elderly on common cognitive measures including memory, executive function, and processing speed [12]. In particular, research on the effects of PUFA supplementation in mainstream, well-nourished school-age children is relatively scarce and the results are inconclusive [4, 12]. For instance, a RCT in 450 mainstream school children (8–10-years-old) in the UK tested the effectiveness of daily supplementation with 200 mg of DHA, 28 mg of EPA, and vitamins (vs. placebo) for 16 weeks; however, results showed no PUFA-related improvements in reading, memory, or attention [13]. In contrast, a more recent trial in schoolchildren in Sweden showed that 3 months of PUFA supplementation (daily 558 mg of EPA, 174 mg of DHA, and 60 mg gamma-linolenic acid vs. placebo) resulted in better reading skills [14, 15]. Another UK-based RCT focused on 7–9-year-old children underperforming in reading found that daily 600 mg of DHA supplementation for 16 weeks resulted in improved reading skills [15]; however, a recent replication attempt failed to reproduce this effect [16]. Most of these and other published trials on PUFA supplementation have relied on capsules as method of delivery [12]. However, delivering essential micronutrients and PUFA by fortifying foods that are part of children's regular diet can be a more practical and effective intervention [1], increasing adherence and facilitating absorption [17].

The aim of the current study was to test the effectiveness of a fortified milk beverage in improving cognitive skills in mainstream school-aged children in a developed country (Spain). The fortified milk contained high levels of various added micronutrients (e.g., vitamins A, B complex, C, D, and E, calcium, phosphorus, zinc) and PUFA (DHA + EPA), and was compared to regular full milk. The primary cognitive outcomes of the study were working memory and processing speed scores. These cognitive skills are the main building blocks of fluid intelligence—the ability to recognize patterns, reason, and solve problems [18] and are strong predictors of children's academic achievement [19, 20]. Secondary outcomes included relevant biochemical indicators and anthropometric measures. We also aimed to investigate if improvement in the primary outcomes (cognitive measures) was explained by changes in the secondary outcomes (biochemical indicators).

Method

The study protocol was approved by the Ethics Committee of the Foundation of the University Hospital Virgen de la Nieves, and written informed consent was obtained from the parents of the participating children. The study was conducted in accordance with the ethical standards of the Helsinki Declaration (Hong Kong Review, September 1989), following the guidelines of the EEC Good Clinical Practices (Document 111/3976/88 of July 1990), and the Spanish law regulating clinical research in humans (Royal Decree 561/1993 regarding clinical trials).

Participants

Participants were recruited at three schools in Granada, Spain. A total of 234 children were approached and 119 (51%) agreed to participate in the study. Fifty-eight (49%) were male with mean age 11.40 (SD = 2.17) and 61 (51%) were female with mean age 11.15 (SD = 2.30).

The inclusion criteria were being between 8- and 14-years-old, agreeing to participate in the study, having parental consent, and not having abnormal hematological and biochemical tests. The exclusion criteria were taking long-term medication, suffering a chronic disease or injury, having a developmental or learning disorder, developing a metabolic or acute disease during the 5 months of treatment,

having taken medication during the past 3 months, being allergic to milk protein, having lactose-intolerance, and showing little intent for collaboration and completing the study (based on the teacher's opinion).

No a-priori sample size calculation was done. Previous research on supplementation with similar enriched milk products had shown that a sample size of 30 per group would be sufficient to detect significant changes in plasma concentrations after a few weeks of supplementation [21, 22]. Of the 119, 103 (87%) children participated in the cognitive assessment (see Fig. 1). Post hoc sensitivity analysis with G*power (gpower.hhu.de) showed that the achieved sample size was sufficient to detect a medium-sized effect $d = 0.56$ (critical $t = 1.98$) with alpha = 0.05 and power of 0.80.

Study design

The study used a randomized, controlled double-blind design. Children were randomly allocated to a Fortified group or a Control group. Children in the Fortified group ($n = 60$) consumed 0,6 L/day of a fortified milk beverage containing vitamins (A, B complex, C, D and E), minerals (calcium, phosphorus, zinc), fish oils (with high levels of DHA and EPA), oleic acid, and carbohydrates (sugar and honey) (Puleva Max®). Children in the Control group ($n = 59$) consumed 0.6 L/day of regular full milk. Table 1 shows that exact content of the two beverages. Both groups consumed the beverages every day during 5 months. Measures were taken at the start of the study (pre: cognitive tests, a fasting blood sample, anthropometric measures) and after 5 months (post: cognitive tests, a fasting blood sample, anthropometric measures). The cognitive tests were administered by trained personnel during the first assessment session. During a second assessment session, anthropometric measures were taken and blood was drawn, directly after which the intervention started.

Intervention

Randomization was done with the program SIGESMU [23]. The two beverages were labeled Product A and Product B and all persons involved in the execution of the study were blind to their true content. The beverages were supplied in vacuum-sealed tetrabrik containers with blank surfaces, without any trademarks or identification.

In both groups the beverages were distributed in three portions – 0.2 L during breakfast, 0.2 L during teacher-supervised recess, and 0.2 during the afternoon. On school days the first two portions were consumed at school under teacher supervision and the third portion was distributed to drink at home. On weekends, the drinks were consumed at home. Both groups received the same dietary advice (to

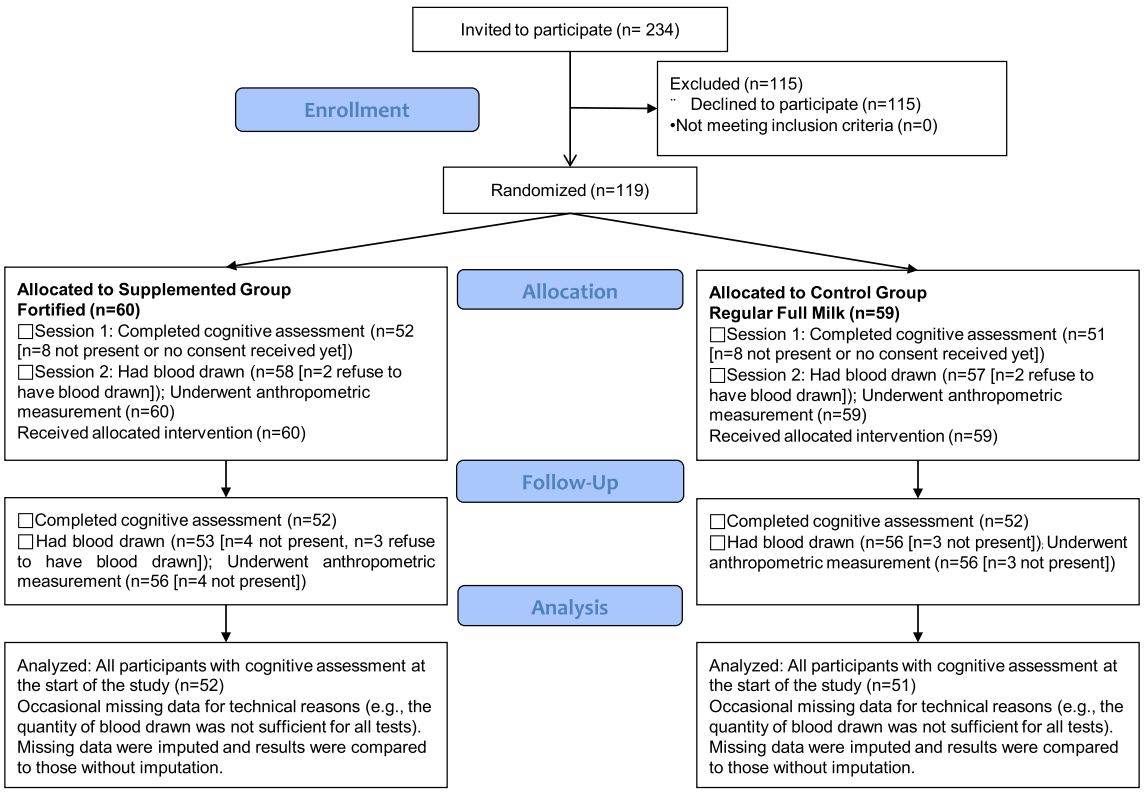

Fig. 1 CONSORT flow chart for reporting randomized controlled trials

Table 1 Composition of the beverages consumed by the control group (Regular Full Milk) and the Fortified group (Puleva Max®)

Quantity/100 mL	Control	Fortified
Energy (Kcal/kJ)	64/266	69/288
Proteins (g)	3.1	3.0
Carbohydrates (g)	4.7	7.4
Total fat (g)	3.6	3.0
Saturated fatty acids (g)	2.4	1.2
Monounsaturated fatty acids (g)	1.1	1.5
Polyunsaturated fatty acids (g)	0.1	0.3
Omega-3 (mg)	0	35
Docosahexaenoic acid (DHA) (mg)	0	20
Eicosapentaenoic acid (EPA) (mg)	0	10
Vitamin A (Retinol) (mg)	28	120
Vitamin B1 (mg)	0.04	0.21
Vitamin B2 (mg)	0.18	0.24
Vitamin B3 (mg)	0.09	2.7
Pantothenic acid (mg)	0.35	0.9
Vitamin B6 (mg)	0.04	0.3
Biotin (mg)	3.5	22.5
Folic acid (mg)	6.4	30.0
Vitamin B12 (mg)	0.38	0.15
Vitamin C (mg)	1.7	9.0
Vitamin D (mg)	0.17	0.75
Vitamin E (mg)	0.07	1.5
Calcium (mg)	120	140
Zinc (mg)	0.36	2.25

keep to their regular diet) and consumed the beverage for 5 months in addition to their regular diet. The study was conducted during the school year between January and June and the duration of the intervention (5 months) was chosen to avoid the long school holidays during the winter and summer, when ensuring adherence would have been more difficult. All participants were instructed not to change their lifestyle or diet during the study.

Adherence to the intervention was confirmed by teacher observation (regarding consumption at school) and by interviews with parents, in person or by telephone, once a week (regarding consumption at home). All participants reported regularly drinking the beverages and hence no participants were dropped due to systematic non-adherence.

Measures

Cognitive skills (primary measures)

These included components from the Wechsler Intelligence Scale for Children (WISC IV) measuring working memory capacity (Digit Span and Letter-Number Sequencing) and processing speed (Coding and Symbol/Animal Search) [24]. The WISC IV is among the most frequently used instruments to assess children's intelligence and an adapted Spanish version is available [25]. The working memory tests measure children's ability to memorize new information, hold it in short-term memory, and manipulate it to arrive at a solution (e.g., repeat several digits backwards). The processing speed tests measure children's abilities to focus attention and quickly scan, identify, and order visual information (e.g., identify whether a target symbol is among a row of several symbols). Higher scores identify superior performance on all measures.

Biochemical indicators (secondary measures)

These were obtained from fasting blood samples and included levels of serum total cholesterol, High-Density Lipoprotein (HDL) cholesterol, Low-Density Lipoprotein (LDL), triglycerides, iron, ferritin, plasma DHA, serum calcium, total serum 25OH-vitamin D, vitamin E, and a complete blood count (see [26] for further details on measurement). Blood cell count results are reported elsewhere [26].

Anthropometric measures (secondary measures)

These included Body-Mass Index (BMI = weight/height^2) based on height in cm and weight in kg, and waist circumference in cm. Participants were measured barefoot and wearing only underwear. Body weight was measured using a standard weight balance scale using a precision stadiometer (Seca; ± 0.1 cm) attached to the weight scale. Height was measured while participants adopted a standard posture (e.g., feet together, straight knees, etc.). Waist circumference was measured with a non-elastic and flexible tape.

Analysis

The data analyst was initially blind to the composition of the two beverages. Scores that were skewed were transformed for analyses (square root transformation).

We checked for statistical differences between groups at the start of the study using *t* tests and tested whether participants who did not participate in the cognitive assessment were more likely to be from the Fortified or the Control group. We investigated whether the type of beverage had an effect on biochemical indicators, anthropometric measures, and cognitive tests using analyses of covariance with post-measures as dependent variables and pre measures, age, and gender as covariates. We then investigated whether any of the biochemical indicators that were affected by the type of beverage were also related to the cognitive test results, and could thus be potential mediators of the effect (i.e., type of beverage → biochemical indicator → cognitive results). In case of such variables, we conducted mediation analyses in a

multiple linear regression framework, controlling for gender, age of participants, and the other biochemical indicators or anthropometric measures that had shown significant differences ($p < .10$). For the mediation analyses for each measure we calculated a difference score based on each participant's value at the start of the study. This was done using the formula % difference score = (Post − Pre)/Pre, where negative values indicate % relative decrease with time and positive values % relative increase with time. The mediation analyses were conducted using the Process SPSS macro suitable for small samples [27]. Analyses were based on 5000 bootstrap samples and 95% CI were computed to assess the significance of indirect effects.

Occasional missing values were imputed using multiple imputation [28, 29]. Analyses were conducted using the imputed data set and results were compared to those using complete cases only. Below we report pooled results based on 40 imputations. There were no significant differences between these results and the results based on the complete cases analyses besides minor changes in coefficients and p values. To the best of our knowledge no validated method exists to pool indirect effect estimates across multiple imputations. Hence, we report indirect effects and associated confidence intervals based on the complete cases analyses and a simple average of the indirect effect across imputations.

Results

The intervention was delivered to 119 participants (see Fig. 1), of which 103 completed the cognitive assessment before the start of the intervention. The remaining 16 children were either not present during the days of cognitive assessment or parental consent was only obtained later. All participants who completed the cognitive measures before the intervention were also assessed after the intervention (children who were not assessed at the start were not assessed after). Participants who completed the cognitive measures were 52 (51%) boys and 51 (49%) girls, who were on average 11-years-old at the start of the study (SD = 2.14). Of the 16 participants who did not participate in the cognitive assessment, 8 were in the Fortified group and 8 were in the Control group, showing that participants were not more likely to be missing from one particular group, $Chi^2(1) = 0.001$, $p > .999$. There were no significant differences between the Fortified and Control groups at the start of the study, with the exception of larger Ferritin concentrations in the Control group. There was also a tendency towards larger iron concentrations in the Fortified group and a larger BMI in the Control group compared to the Fortified group (see Table 2).

Table 2 shows that there were significant differences between the Fortified and the Control group for the following biochemical indicators: On average, after 5 months there was an increase in DHA levels in the Fortified group, while these levels stayed relatively the same in the Control group, $F(1, 5953) = 41.43$, $p < 0.001$, $\eta^2 = 0.36$. Similarly, vitamin D levels increased to a larger extent in the Fortified group than in the Control group, $F(1, 8380) = 11.54$, $p < 0.001$, $\eta^2 = 0.12$. There was a tendency for a decrease of Calcium levels in the Control group, $F(1, 1655) = 3.23$, $p = .072$, $\eta^2 = 0.03$. There were no differences on the anthropometric measures. Regarding cognitive skills, there were larger increases in Digit Span in the Fortified compared to the Control group, $F(1, 98) = 5.04$, $p = 0.027$, $\eta^2 = 0.05$. Among the significant effects, the largest effect size was observed on DHA levels with partial $\eta^2 = 0.36$, followed by vitamin D with $\eta^2 = 0.12$, and finally digit span with $\eta^2 = 0.05$ (Table 2).

Next, based on these results, we investigated correlations between changes in DHA and vitamin D levels and cognitive skills. Change in DHA was not related to any of the cognitive measures ($p > 0.1$), so DHA was no longer considered as a potential mediator. Increases in vitamin D were marginally related to increases in Digit Span ($r = 0.183$, $p = 0.078$) and Symbol/Animal Search ($r = 0.201$, $p = 0.063$); hence, we went on to test vitamin D as a potential mediator of the effect of beverage on cognitive scores.

Mediation analyses

Because vitamin D concentration increased to a larger extent in the Fortified compared to the Control group, it is possible that there are indirect effects of beverage on cognitive skills via vitamin D. To test this possibility, we fitted two mediation models (one for each cognitive test to which vitamin D was related: Digit Span and Symbol/Animal Search), controlling for age, gender, and the rest of the indicators that had shown a significant difference as a function of group (calcium, iron, ferritin, DHA, and BMI). First, the candidate mediator (vitamin D) was regressed on all independent variables. Then each of the cognitive tests was regressed on all independent variables and vitamin D.

In multiple regression, being in the Fortified group continued to be significantly related to a higher vitamin D increase, $B = 0.20$, $p = 0.030$. Vitamin D increase was not related to changes in Digit Span, $B = 0.09$, $p = 0.239$. However, Vitamin D increase was (marginally) related to a higher increase in Symbol/Animal Search scores, $B = 0.25$, $p = 0.078$. Consequently, there was a significant indirect effect of Group on Symbol/Animal Search, effect = 0.06, 95% CI [0.01, 0.15] (based on complete cases only, $N = 91$; effect = 0.05, 95% CI [0.00, 0.15], $N = 103$, average across imputations), such that vitamin D concentration increased to a larger extent in the Fortified group compared to the Control group, which was then related to a higher increase

Table 2 Comparisons ($N = 103$) between the Fortified and the Control group

Measure	Group	Mean pre (SE)	Mean post (SE)	Comparison pre			Comparison post	
Biochemical indicators				*t*	*p*	*F*	*p*	η^2
HDL mg/dL	Control	52.10 (1.80)	48.65 (1.11)	0.17	0.859	0.42	0.517	0.01
	Fortified	51.65 (1.75)	49.69 (1.16)					
LDL mg/dL	Control	102.37 (3.33)	101.09 (2.04)	− 0.04	0.972	1.35	0.245	0.02
	Fortified	102.54 (3.53)	97.97 (2.08)					
TG mg/dL	Control	58.79 (3.48)	61.50 (3.27)	0.32	0.746	0.42	0.518	0.01
	Fortified	57.07 (4.03)	58.48 (3.23)					
DHA	Control	2.02 (0.09)	1.88 (0.07)	0.45	0.654	41.43	< 0.001	0.36
	Fortified	1.97 (0.09)	2.43 (0.07)					
Ferritin ng/mL (sqrt)	Control	5.72 (0.25)	5.10 (0.20)	2.45	0.014	1.26	0.262	0.01
	Fortified	4.94 (0.20)	4.78 (0.20)					
Iron mcg/dL (sqrt)	Control	8.67 (0.29)	9.51 (0.31)	− 1.68	0.093	0.15	0.701	0.01
	Fortified	9.45 (0.38)	9.33 (0.33)					
Calcium mg/dL	Control	10.17 (0.11)	9.92 (0.11)	0.41	0.686	3.21	0.073	0.03
	Fortified	10.11 (0.10)	10.22 (0.12)					
25OH vitamin D ng/mL (sqrt)	Control	5.21 (0.08)	5.64 (0.08)	0.60	0.552	11.54	< 0.001	0.12
	Fortified	5.14 (0.09)	6.01 (0.08)					
Vitamin E mcg/mL	Control	11.40 (0.41)	11.80 (0.35)	0.31	0.759	0.06	0.803	0.00
	Fortified	11.19 (0.59)	11.92 (0.35)					
Anthropometric measures								
BMI (sqrt)	Control	4.51 (0.06)	4.43 (0.01)	1.90	0.058	1.68	0.194	0.03
	Fortified	4.34 (0.06)	4.41 (0.01)					
Waist circumference	Control	73.77 (1.55)	72.43 (0.50)	1.64	0.101	1.14	0.287	0.01
	Fortified	69.97 (1.71)	71.69 (0.49)					
Cognitive tests								
Digit span (sqrt)	Control	2.78 (0.06)	2.96 (0.04)	− 0.82	0.412	5.04	0.027	0.05
	Fortified	2.87 (0.08)	3.10 (0.04)					
Letter-number sequencing (sqrt)	Control	2.71 (0.10)	2.72 (0.07)	− 0.77	0.441	1.16	0.281	0.02
	Fortified	2.81 (0.09)	2.83 (0.07)					
Coding	Control	8.49 (0.36)	10.12 (0.30)	− 1.57	0.115	2.59	0.111	0.03
	Fortified	9.37 (0.42)	9.46 (0.29)					
Symbol/animal search	Control	9.33 (0.33)	9.75 (0.38)	− 0.65	0.513	0.06	0.811	0.00
	Fortified	9.69 (0.44)	9.88 (0.38)					

The comparison at the start of the study (comparison pre) was based on *t* tests. The comparison at the end of the study (comparison post) was based on analysis of covariance with post-measure as dependent variable, group as a predictor, and pre measures, age, and gender as covariates

sqrt squared-root transformation, *SE* standard error

in the Symbol/Animal Search score (see Fig. 2). The effect size of this indirect effect was small-to-medium with $\kappa^2 = 0.07$, 95% CI [0.01, 0.14] [30]. Other significant results included an effect of age such that younger participants accumulated more vitamin D, $B = 0.05$, $p = 0.015$, and hence improved more on the Symbol/Animal Search test compared to older participants, indirect effect = 0.01, 95% CI [0.03, 0.002]. There were no further significant effects. Results from multiple regressions with change scores are displayed in Figs. 2 and 3.

Effect sizes

To illustrate the sizes of all significant effects we computed the mean percentage change from baseline and the associated 95% confidence intervals for each group. Mean observed change from baseline in DHA levels was 28% [95% CI 17–39%] in the Fortified group vs. − 6% [95% CI − 13 to 0%] in the Control group. Mean observed change from baseline in serum 25OH-vitamin D concentrations was 41% [95% CI 30–52%] in the Fortified vs. 21% [95% CI 11–30%] in the Control group. Mean observed change from baseline

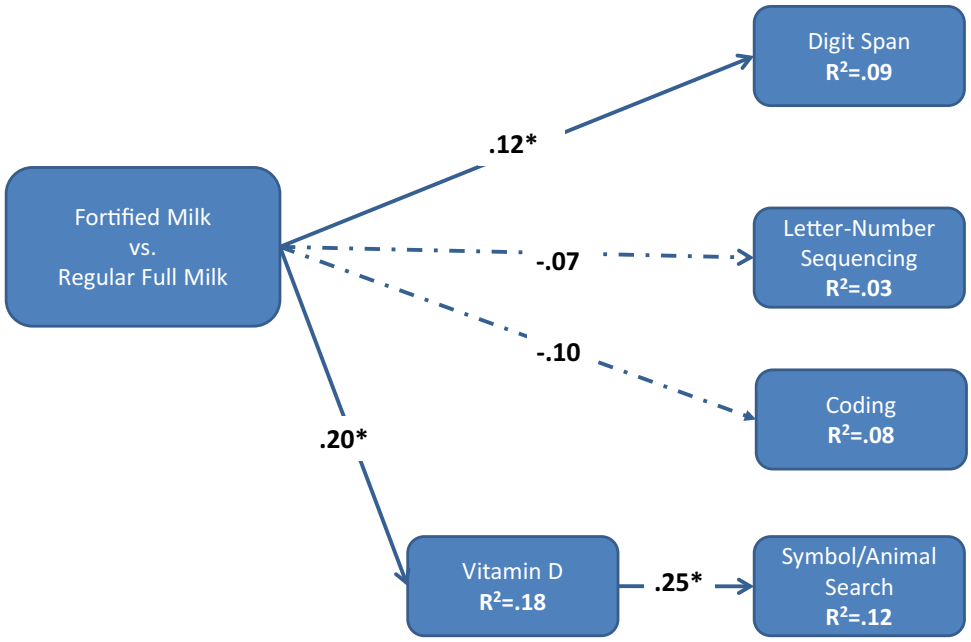

Fig. 2 Multiple regression results on % change scores. The models controlled for age, gender, and changes in calcium, iron, ferritin, DHA, and BMI levels. Coefficients are unstandardized B. R^2 = percentage of variance explained (average across imputations). Dashed lines indicate non-significant effects

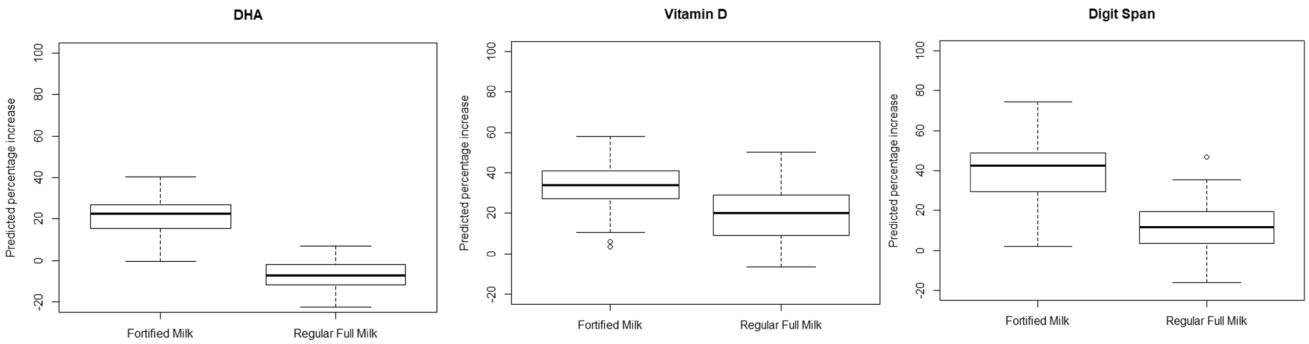

Fig. 3 Predicted percentage increase in DHA, vitamin D, and digit span levels according to group (Fortified vs. Control) based on multiple regression analyses

in Digit Span was 32% [95% CI 17–47%] in the fortified group vs. 13% [95% CI 6–19%] in the control group.

Discussion

Children who drank fortified milk during 5 months showed larger improvement in working memory than children who drank regular full milk during the same period. In particular, the increase in Digit Span scores observed over time in the Fortified group was more than double that of the increase observed in the control group (32% vs. 13%). The improved performance overall could be due to practice effects, progress in cognitive development during these months, and regularly drinking milk. However, no similar effects of the fortified milk were observed on the other cognitive tests (Letter-Number Sequencing, Coding, and Symbol/Animal Search).

The Fortified group also showed a larger indirect increase in processing speed than the control group (Symbol/Animal Search task); however, this effect was small and was entirely driven by increases in 25-hydroxy vitamin D concentration (i.e., there were no direct effects of group on the symbol/animal search task). A mediation analysis indicated that the fortified milk increased circulating vitamin D concentrations to a larger extent, which was in turn related to a larger improvement in processing speed. This is not surprising given that the fortified milk had larger vitamin D concentration (see Table 1). While the benefits of vitamin D for physical health are well-established, its role in cognitive function has only been addressed recently. It has been suggested that vitamin D plays a role in important processes including antioxidation, regulation

of neuronal calcium, immunomodulation, and enhanced nerve conduction, and thus has a neuroprotective function in both the developing and the adult brain [31]. Some studies show positive associations between serum 25OHD concentrations and cognitive performance of adults; however, the results are generally mixed and studies with children are needed to confirm its role in the developing brain [31, 32]. The current study is the first to our knowledge to show that vitamin D could have a beneficial effect on cognitive function in school-aged children. However, these results are at best suggestive and the role of vitamin D should be further researched.

Although the supplemented milk increased DHA concentration, DHA levels were not significantly related to cognitive performance. Similar studies of PUFA supplementation in school-aged children are still scant and have shown mixed results, possibly due to differences in dosage or measurement [12]. For instance, the supplemented DHA dosage in the current study (about 60 mg daily) was smaller than the dosage administered with capsules in some previous studies [2, 14, 16].

Results regarding PUFA supplementation during pregnancy, lactation, and early childhood have shown more consistent positive results. For instance, 8.5-year-old children born to mothers who received prenatal PUFA supplementation have better scores on tests measuring attention and demonstrate different patterns of brain activation compared to children born to mothers who received placebo [3]. Similar results are observed when children's formula is supplemented with PUFA during the first year of life [33]. On one hand, this suggests that PUFAs may be more beneficial in earlier compared to later stages of development [12, 34]. However, it should also be noted that in the present study DHA levels were measured in plasma samples, whereas it is well-established that measuring DHA in erythrocytes is much more representative of DHA status [34]. Hence, DHA in erythrocytes could be a more suitable and sensitive measure to detect relationships with cognitive performance (e.g., see [2, 34–36]). Thus, the possible relationship between erythrocyte DHA concentration and cognitive performance after an intervention with fortified milk remains to be investigated.

Given the correlation and mediation results, and previous studies showing that micronutrient supplementation improves working memory capacity [1, 11], it is possible that positive results observed are due to the micronutrient supplementation (e.g., vitamin D). We should also note that the fortified beverage contained higher levels of carbohydrates and lower levels of saturated fatty acids, which could also have influenced the results. Overall, because the fortified milk contained a mixture of various micronutrients and PUFA, it is not possible to determine what was the exact cause of the observed effects, which may very well be driven by a synergistic effect of the mix of the various compounds [1].

The current results also highlight the utility of milk as a supplement delivery vehicle. Milk has both an aqueous and an oil phase, making it an excellent vehicle for supplementation with water-soluble and fat-soluble nutrients. For instance, milk fat is highly dispersed in very small micelles. This facilitates fat absorption, whereby supplementing only a small amount of PUFA can result in significant increases in plasma levels [17]. Besides its molecular properties, compared to capsules, milk as a delivery method has the advantage of being part of children's regular diet, ensuring greater adherence and not creating the impression of "being medicated". The latter may be positively perceived by parents and can have advantages for children themselves. For instance, the current trial achieved remarkable adherence, also thanks to the acceptance and support of teachers and parents. Nevertheless, it is possible that children who did not like to drink milk regularly or (whose parents) knew they were intolerant declined participation. These children could benefit from an alternative method of supplement delivery (e.g., fruit drink). Finally, no children agreeing to participate were excluded based on the list of exclusion criteria (e.g., having chronic diseases or taking medication), suggesting that these children or their parents might have self-selected them out of the study.

On one hand, the current results suggest that supplementing milk with essential nutrients could significantly aid children's cognitive development. However, we observed effects (one direct and one indirect) only on two out of the four administered measures and the sample size was sufficient to only detect medium (or large) changes in cognitive skills. Hence, the current study adds some suggestive results to the already generally mixed literature on supplementation-related cognitive benefits in mainstream school children [1, 11, 13]. Given the high heterogeneity of the available studies and the mixed results, more evidence is needed to understand under what conditions, in what populations, and through what mechanisms micronutrient and PUFA supplementation may improve cognitive abilities. Among other things, differences in outcomes may be due to measurement differences, dosage of the supplements, or the method of delivery (capsules, drink, or food).

Given the limitations of the current study, further research is needed to corroborate and extend our findings. Overall, the results suggest that milk could be an effective way to supplement children's nutrition and observe tangible cognitive benefits and its capability to deliver important nutrients should be investigated further.

Acknowledgements The study was funded by Lactalis Puleva SL. JF is currently employed and ELH was employed by Biosearch Life, which is part of Lactalis. Dafina Petrova is supported by a Juan de la Cierva

Fellowship (FJCI-2016-28279) from the Spanish Ministry of Economy, Industry, and Competitiveness. We would like to thank the schools in Granada that participated in the study: I.E.S. Albayzín de Granada, Colegio Diocesano Virgen del Espino de Chauchina (Granada), and C.E.I.P. Cardenal Cisneros de Villanueva Mesía.

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