Effect of a 12-mo micronutrient intervention on learning and memory in well-nourished and marginally nourished school-aged children: 2 parallel, randomized, placebo-controlled studies in Australia and Indonesia[1–4]

The NEMO Study Group

ABSTRACT

Background: Little is known about the combined effect of micronutrients and essential fatty acids on cognitive function in school-aged children.

Objective: We assessed the effect of micronutrients, long-chain n−3 fatty acids, or both on indicators of cognitive performance in well-nourished and marginally nourished school-aged children.

Design: Two 2-by-2 factorial randomized controlled double-blind trials were performed home-based in Adelaide, South Australia, and at 6 primary schools in Jakarta, Indonesia. A total of 396 children (aged 6-10 y) in Australia and 384 children in Indonesia were randomly allocated to receive a drink with a micronutrient mix (iron, zinc, folate, and vitamins A, B-6, B-12, and C), with docosahexanoic acid (DHA,88 mg/d) and eicosapentaenoic acid (EPA, 22 mg/d), or with both or placebo 6 d/wk for 12 mo. Biochemical indicators were determined at baseline and 12 mo. Cognitive performance was measured at baseline, 6 mo, and 12 mo.

Results: The micronutrient treatment significantly improved plasma micronutrient concentrations in Australian and Indonesian children. DHA+EPA treatment increased plasma DHA and total plasma n−3 fatty acids in both countries. The micronutrient treatment resulted in significant increases in scores on tests representing verbal learning and memory in Australia (estimated effect size: 0.23; 95% CI: 0.01, 0.46). A similar effect was observed among Indonesian girls (estimated effect size: 0.32; 95% CI: −0.01, 0.64). No effects were found on tests measuring general intelligence or attention. No effects of DHA+EPA on the factors of cognitive tests were observed.

Conclusion: In well-nourished school-aged children, fortification with multiple micronutrients can result in improvements in verbal learning and memory. *Am J Clin Nutr* 2007;86:1082–93.

KEY WORDS Micronutrients, fatty acids, cognition, school-aged children

INTRODUCTION

Nutrition is one of the many factors that influence cognitive development in infants and children, particularly in undernourished children in developing countries. Micronutrient deficiencies, such as iron and iodine deficiencies, are associated with impaired cognitive development in infants and young children (1–3), and there is emerging, conflicting evidence that deficiencies in zinc, folate, and vitamin B-12 are also linked to compromised development in children (3, 4). More recent publications indicate a possible role for the long chain n−3 fatty acids, in particular, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), on child cognitive development (5, 6), although most evidence in school-aged children is limited to studies in children with specific neurodevelopmental disorders (7).

Most studies on the effect of nutrient interventions on cognition have focused on the role of single nutrient interventions in children with deficiencies in the younger age groups when brain development is at its peak and is particularly sensitive to insults due to dietary deficiencies (1, 3, 4, 8). However, micronutrient deficiencies are known to often coexist, and multiple micronutrient interventions may be more effective (2). The brain continues to grow and develop during childhood and adolescence, and relatively little is known about the role of nutrition after 2 y of age and how much the role of nutrition in cognitive development differs in children in poorer versus well-off environments. Very few randomized controlled intervention studies assessing the efficacy of a multiple-micronutrient intervention on cognitive function in schoolchildren have been reported in the literature so far. Beneficial effects on verbal and nonverbal abilities, including short-term memory, attention, and concentration, were observed in some (9–11) but not all (12) of these studies.

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We are not aware of any studies comparing the effect of multiple micronutrients with or without long-chain n−3 fatty acids on cognitive function in healthy children. It is known that certain micronutrients, such as iron and zinc, may play a role in fatty acid bioavailability and metabolism (13), and it can be argued that combined supplementation with micronutrients and fatty acids may enhance the potential beneficial effects of both on cognition.

We performed 2 randomized, double-blind, controlled intervention studies [the NEMO (Nutrition Enhancement for Mental Optimization) studies] to assess the single and combined effects of 12 mo of supplementation with selected multiple micronutrients and DHA+EPA on cognitive performance in school-aged children. One trial was conducted in South Australia among well-nourished children and the other trial in urban Jakarta, Indonesia, in marginally nourished children. Our hypothesis was that the marginally nourished children would benefit more from the intervention. Both trials followed the same design, and we will present the methods of the 2 studies combined. However, because the countries differed substantially in nutritional status and other important characteristics, the results will be presented and discussed separately for Australia and Indonesia.

SUBJECTS AND METHODS

Study population

The trials were conducted from August 2003 to April 2005 in children aged 6–10 y from South Australian government metropolitan schools of higher socioeconomic status in Adelaide and from schools in the central district of Jakarta of middle to low socioeconomic status. Children were eligible for entry in the study if they met the entry criteria for age, did not have any severe physical or neurologic health problems that could affect their performance on the cognitive tests, and had not consumed any micronutrient, mineral, or fatty acid supplements during the 2 wk preceding the study start and were not intending to use these supplements during the course of the study. In addition, children who were severely malnourished [defined as a weight-for-height z-score ≤ -3 SDs (14)] or severely anemic [defined as a hemoglobin concentration ≤ 8.0 g/dL (15)] were excluded. In Indonesia, the study population consisted of marginally nourished children, and for this reason, children with a weight-for-height z-score above 0.44 SD were also excluded from participation. The cutoff of 0.44 SD was selected arbitrarily. In both countries, written informed consent was obtained from the parents of the children, and oral assent was obtained from the children.

In Australia, the intervention was home-based, with the children being recruited through invitations distributed either through the schools or through an additional media drive. A general, unpersonalized invitation to the parents of children in the appropriate age range was distributed through the schools. Thereafter, the parents contacted the researchers if they wished to have their child participate in the trial. A total of 434 children from 42 different schools followed up on the opportunity to participate, and their parents provided informed consent. Of these 434 children, 1 child was not eligible for inclusion in the study because birth data were missing. Initially, 329 Australian children were randomly assigned in the study between August and October 2003. In March-May 2004, it was necessary to conduct a further recruitment drive ($n = 67$) to compensate for the children who had withdrawn from the study before being randomly assigned. A total of 37 children had withdrawn from the study before the start because of the time lag between recruitment and the actual start of the study.

In Indonesia, a total of 498 children from 6 public schools located in an urban poor area of central Jakarta were screened for eligibility. Out of these children, 105 were not eligible because they either had incomplete birth data, had chronic health problems, or did not fulfill the nutritional status criteria. A total of 9 children refused to participate; 384 children were randomly assigned in the study.

Power calculations before the start of the study had identified a required sample size of 60 children per treatment group to enable detection of an effect size of 0.4–0.7 SD for most of the cognitive test scores with 80% power and a type I error of 5%.

The CSIRO Human Ethics Committee and the Department of Education and Children's Services Ethics Committee in Adelaide, Australia, and the Committee on Medical Research Ethics, Faculty of Medicine, University of Indonesia, Jakarta, gave ethical approval for the study.

Study design

The studies in Australia and Indonesia both used a 2-by-2 factorial design in which the children were individually randomly allocated to 1 of 4 intervention groups: *1*) a mix of micronutrients, *2*) n−3 fatty acids, *3*) both, or *4*) none. A fruit-flavored drink (soy 0.6%) was used as the vehicle for all treatments, which were added as powders. In Australia, the children were randomly assigned to intervention groups on entry in the study. In Indonesia, the children were stratified by school before being randomly assigned. Random assignment was done by means of a computer-generated list in both countries. Before the randomization step, baseline data for anthropometry, micronutrient status, fatty acid status, cognitive performance, parental education, and child's medical history and current health and behavior were collected. The interventions began immediately after baseline data collection and continued for 1 y.

Intervention

The intervention products used consisted of 4 powdered fortificants that were added to a base powder containing 8 g protein, 12 g sugar, and 4 g maltodextrin to be dissolved in 100 mL of a soy-based fruit drink (AdeS; Unilever, Buenos Aires, Brazil) in a plastic shaker with a screw top and then shaken for ≥20 s. The powders were produced by Unilever Netherlands BV (Vlaardingen, Netherlands). The micronutrient mix consisted of iron, folate, vitamin B-6, and vitamin B-12 at one times the Recommended Dietary Allowance or Recommended Daily Intake (RDA/RDI; 16) and zinc at one-half the RDA/RDI, because all these nutrients have been associated with cognitive performance in children, with the association being strongest for iron in this age group. Zinc was added at one-half the RDA to avoid potential interference with iron absorption (17). In addition, vitamin A and vitamin C at one times the RDA were added, because of their role in enhancing iron absorption and metabolism. The DHA+EPA mix consisted of 88 mg DHA and 22 mg EPA, at a concentration based on intake recommendations for fatty fish in children (18) (**Table 1**).

The supplement powders were indistinguishable in color and taste and were color-coded. The codes remained unknown to both investigators and participants until the study was completed, all data had been entered, and initial analyses had been performed.

TABLE 1
Composition of the intervention products[1]

Nutrient	Concentration in intervention product	RDI, Australia (8–11 y)	RDA, Indonesia (7–9 y)
Iron as NaFeEDTA	10 mg	6–8 mg	10 mg
Zinc as zinc sulfate	5 mg	9 mg	10 mg
Vitamin A as retinol acetate	400 μg RE	500 μg RE	400 μg RE
Folate	150 μg	150 μg	80 μg
Vitamin B-6	1 mg	1.5 mg	1.0 mg
Vitamin B-12	1.5 μg	1.5 μg	0.9 μg
Vitamin C	45 mg	30 mg	45 mg
DHA	88 mg	NA	NA
EPA	22 mg	NA	NA

[1] DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NA, not available; RDI, Recommended Daily Intake; RDA, Recommended Dietary Allowance (16).

The powder drinks were to be consumed daily. The study participants in Australia were required to prepare and consume the drink at home under parental supervision. Compliance assessment was calculated from records of sachets issued and returned, along with self-report calendars. In Indonesia, the children received the drink prepared by their teachers at school every school day, that is, 6 d/wk. Two trained compliance officers undertook compliance assessment at the schools by using a marked stick to indicate the percentage of the drink left over. Additionally, in Indonesia only, the children in all 4 intervention groups received 3 biscuits (Flora Maria; Unilever, Barcelona, Spain) together with the intervention products, delivering ≈100 kcal as a protein-energy supplement. During school holidays and the fasting month (Ramadan), the supplement was consumed at home, and the parents were instructed that the supplement and biscuits were to be consumed 6 d/wk.

Cognitive tests

Measures of cognitive and school performance were administered at baseline and at 6 and 12 mo. The cognitive assessment battery is described in more detail in **Table 2** and consisted of a series of standardized neuropsychological tests applicable for use in school-aged children and selected for their good reliability and sensitivity to nutrition effects as described elsewhere (19, 20).

For Indonesia, the English language assessment battery was translated and back-translated into the local language, Bahasa Indonesia, for verification. Some items were changed according to cultural sensitivity.

The cognitive assessment battery was administered to children by psychologists registered with the South Australian Psychological Board and by licensed psychologists in Indonesia. The psychologists in Indonesia were trained in a 2-d workshop conducted by the Australian psychologists to ensure standardization in the assessment and scoring procedures.

In Australia, the tests were administered in separate rooms free from distraction at schools or at the CSIRO center. In Indonesia, 3 individual assessment sessions took place in the same room at schools with groups separated by partition boards. The duration and order of the tests were similar in Australia and Indonesia, and each assessment session took ≈60–90 min.

Biochemical indicators

In Australia, nonfasting venous blood samples were obtained at the Women's and Children's Hospital, Adelaide; in Indonesia, they were obtained at school. Separation of red blood cell and of serum or plasma was conducted by centrifuging the blood immediately (3000 × *g*, 15 min, room temperature), after which the serum was divided into aliquots. In Indonesia and Australia, the hemoglobin concentration of the blood samples was assessed by absorption spectrophotometry (Cell-Dyn 3700; Abbott, Abbott Park, IL). For all other analyses, Indonesian samples were stored at −70°C until their transport on dry ice to Australia. All assays were performed in accredited laboratories in Adelaide with the use of standard quality control measurements.

Serum ferritin, serum vitamin B-12, and red blood cell folate were measured by a Microparticle Enzyme Immunoassay [Abbott AxSYM analyzer (21)]. Serum transferrin receptor was determined by immunoturbidimetric assay (Roche Hitachi 904, Roche Diagnostics 2003-05, V4, Indianapolis, IN). Plasma zinc was analyzed by measuring zinc from plasma lithium-heparinized tubes by use of flame atomic absorption spectrophotometry [Perkin Elmer 5100PC (22); Waltham, MA)]. Serum C-reactive protein was analyzed by a turbidimetric method [Beckman SYNCHRON CX System (21); Brea, CA]. Fatty acid determination was done in serum phospholipids that were separated by thin-layer chromatography and evaporated to dryness under nitrogen. Fatty acid methyl esters were separated and quantified by using a Hewlett-Packard 6890 gas chromatograph equipped with a 50-m capillary column (0.33 mm internal diameter) coated with BPX-70 (0.25-m film thickness; SGE Pty Ltd, Victoria, Australia). Fatty acid methyl esters were identified on the basis of retention time to authentic lipid standards obtained from Nuchek Prep Inc (GLC-463, Elysian, MN; 23).

Data handling

All data collection forms were checked for inconsistencies, and cognitive tests were double-scored by independent psychologists in both countries. Data forms were shipped from Indonesia to Australia, where all cognitive data were again checked and double-scored by the Australian psychologists to ensure standardization of scoring. All data were double-entered in Indonesia and again in Australia, and cross-checks were performed. Body iron stores were calculated according to the method described by Cook et al (24). We performed a correction on our serum transferrin receptor values as described by Pfeiffer et al (25) to correct for the Roche assay that was used in our study. A Rey Auditory

TABLE 2
Overview of measures of cognition and school performance administered in Australia and Indonesia[1]

Test	Description	Cognitive abilities
Both countries		
Digits backwards (WISC-III)	Strings of digits (1 to 9) are read by the examiner. The child is asked to repeat the number strings in reverse order.	Working memory, transformation of information, and mental manipulation
Visual attention 2 (NEPSY)	An array of different faces (drawings) is presented simultaneously on paper. The child has to identify the 2 target faces in the array.	Visual selective attention
Coding (WISC-III)	Symbols paired with digits are presented. The child has to draw as many symbols as possible under corresponding digits within 120 s.	Visual-motor processing speed and coordination, short-term memory, visual perception, visual scanning, cognitive flexibility, attention
Block design (WISC-III and WAIS)[2]	The child has to reproduce designs of increasing complexity from a stimulus booklet by using 2 to 9 bicolor cubes.	Visuospatial problem solving, visual nonverbal reasoning, visual perception and organization
Fluency structured and random (NEPSY)	The child has 60 s to rapidly generate nonrepresentational designs in squares containing 5 regularly or randomly positioned dots by connecting 2 or more dots in each square.	Components of executive functions: initiate, generate, shift, self-regulate, and self-monitor (planning ability)
Rey Auditory Verbal Learning Test (RAVLT)	A list of 15 unrelated, concrete nouns is read aloud. The child has to repeat any words recalled (the order has no importance). There are 8 free recall trials in total (5 trials of list A; then one interference trial: list B; followed immediately by recall of list A; a final recall of list A after a 20-min interval) and 2 visually cued recall tests (recognition list A and recognition list B).	Provides different measures of immediate and long-term verbal learning and verbal memory
Vocabulary (WISC-III)	The child is asked to define words of increasing difficulty. (Adapted into Bahasa in Indonesia.)	Acquired knowledge and verbal concept formation
Mathematical reasoning (WIAT Screener)	Written mathematical problems are presented in a stimulus booklet and orally. (Examples were adapted to Indonesia.)	Mathematical achievement and school performance
Australia		
Reading (WIAT Screener)	The child has to read aloud words of increasing difficulty.	Reading achievement and school performance
Spelling (WIAT Screener)	Words are read aloud by the examiner, then repeated in a sentence, then isolated again. The child then writes the word.	Spelling achievement and school performance
Factor 1 (general or fluid intelligence)[3]	Tests loading on this factor: design fluency, block design, coding, vocabulary, digits backwards, and mathematical reasoning.	
Factor 2 (verbal learning and memory)	Tests loading on this factor: RAVLT-A3, RAVLT-slope, RAVLT delayed recall.	
Factor 3 (attention and concentration)	Tests loading on this factor: visual attention.	
Indonesia		
Reading comprehension (Neale Analysis of Reading)	The child had to read a series of short stories of increasing length and difficulty. Questions of comprehension were asked. (Original test was translated into Bahasa and modified.)	Verbal comprehension, memory, language development
Factor 1 (fluid intelligence)[3]	Tests loading on this factor: design fluency, block design	
Factor 2 (verbal learning and memory)	Tests loading on this factor: RAVLT-A3, RAVLT-slope, delayed recall, vocabulary.	
Factor 3 (attention and concentration)	Tests loading on this factor: visual attention, coding, digits backwards, mathematical reasoning.	

[1] WISC-III, Wechsler Intelligence Scale for Children, third edition; NEPSY, Developmental Neuropsychological Assessment; WAIS-III, Wechsler Adult Intelligence Scale, third edition; RAVLT, Rey Auditory Verbal Learning Test; WIAT Screener, Wechsler Individual Achievement Test.
[2] WISC-III was augmented with designs from WAIS-III to avoid ceiling effects and increase variability.
[3] More detailed description of factor loadings will be described in a future publication (C Wilson, C Transler, H van der Knaap; 2007).

Verbal Learning Tests (RAVLT) learning slope was calculated as the linear regression coefficient of the individual RAVLT tests (A1 and A5).

As part of the data screening, distributions of the scores on the cognitive tests were checked for normality, and floor effects on the RAVLT tests in Indonesia were examined. Four

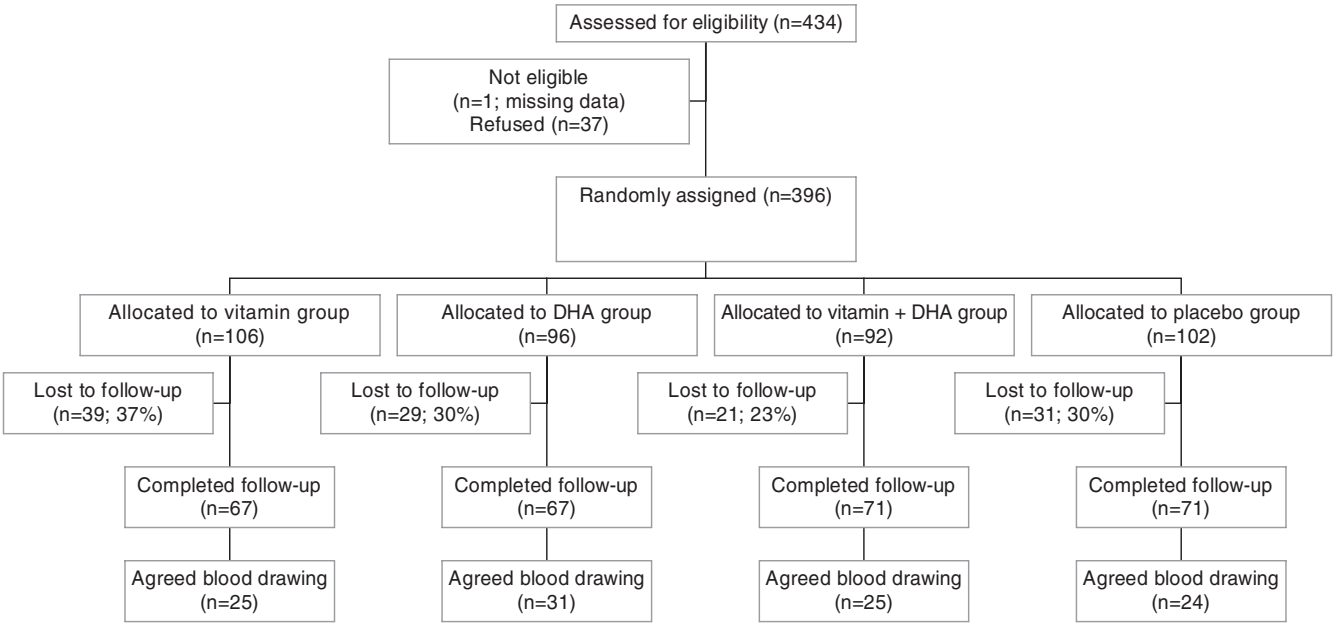

FIGURE 1. Trial profile: Australia.

psychologists who were blinded to the treatment assignment independently reviewed all individual scores on this test and identified 69 children in Indonesia with scores that were below the lowest cutoff. These unexpectedly low scores may have been due in part to a lack of understanding of the test instructions by either the children or the interviewers. Because RAVLT scores for these children could be considered unreliable and because no differences were observed in baseline characteristics for children with and without reliable RAVLT scores (data not shown), treatment analyses on the RAVLT scores were performed and reported excluding the data for these 69 children. No floor or ceiling effects were observed on the other cognitive tests, and all other analyses were performed on the whole sample. All data were handled and stored according to Good Clinical Practice Guidelines (Internet: http://www.fda.gov/cder/guidance/959fnl.pdf).

Statistical analysis

The 2 countries differed substantially not only in nutritional status but also in other characteristics likely to influence performance on tests, such as experience with and attitude toward testing and type of education. Therefore, data for Indonesia and Australia were analyzed separately. The specific analyses for each type of outcomes are detailed below.

Biochemical outcomes

The main effects of micronutrients and DHA+EPA and the interaction between micronutrients and DHA+EPA on change in biochemical indicators and cognitive test outcomes were analyzed by using analysis of covariance, including sex and age and cohort in Australia as covariates and evaluating interactions with sex and age. Sex and age were selected a priori to be included as covariates in the model because there is evidence from the literature that sex and age might interact with nutritional interventions for their effect on cognitive performance (20, 26). In addition, in Australia, cohort of recruitment (2003 or 2004) was introduced as an additional covariate in the models, because baseline characteristics (age and some of the cognitive test scores) of children recruited in the 2003 and 2004 cohorts differed significantly. In Indonesia, C-reactive protein concentrations were included as covariates for the analyses of serum ferritin and serum zinc to control for unusually high concentrations due to the acute phase response during infections.

Cognitive outcomes

Cognitive measures included a series of multiple psychological tests thought to be related to a few specific cognitive domains (such as memory, attention, and fluid intelligence), consistent with the Carroll model (27). The selection of these tests has been described in detail elsewhere (19), and more details on the tests are described in Table 2. Evaluating multiple outcomes may increase the possibility of observing false-positive effects due to chance. For these reasons, we evaluated the effect of the intervention on clusters of the individual cognitive tests by means of a factor analysis using oblique rotation (28). This procedure also provided an opportunity to compare cognitive constructs at baseline in Indonesia and Australia. Similarity between the factor structure in the 2 countries was assessed by means of a proportionality coefficient (28). In both countries, 3 factors emerged, as will be described in detail elsewhere (C Wilson, C Transler, H van der Knaap, et al, unpublished observations, 2007). In Australia, the first factor was dominated by coding, design fluency, vocabulary, digits backwards, mathematical reasoning, and block design and can be interpreted as representing general or fluid intelligence. Factor 2 was dominated by RAVLT-A3, RAVLT-slope and RAVLT delayed recall and was interpreted as representing verbal learning and memory. Factor 3 was dominated by the visual attention test and therefore reflected attention. In Indonesia, factor 1 was dominated by design fluency and block design and can be interpreted as representing fluid intelligence. Factor 2 was dominated by RAVLT-A3, RAVLT-slope, delayed recall, and vocabulary and was interpreted as representing verbal learning and memory, and factor 3 was dominated by visual attention, coding, digits backwards, and mathematical reasoning

TABLE 3
Baseline demographic and biochemical characteristics in Australia and Indonesia[1]

	Australia				Indonesia			
	Micronutrients ($n = 67$)	DHA+EPA ($n = 67$)	Micronutrients+ DHA+ EPA ($n = 71$)	Placebo ($n = 71$)	Micronutrients ($n = 92$)	DHA+EPA ($n = 94$)	Micronutrients + DHA + EPA ($n = 94$)	Placebo ($n = 88$)
Age (y)	8.5 ± 1.0	8.8 ± 1.0	8.8 ± 0.9	8.5 ± 1.0	8.2 ± 0.9	8.1 ± 1.1	8.2 ± 1.1	8.1 ± 1.1
Female [*n* (%)]	31 (42)	30 (45)	28 (44)	28 (39)	40 (43)	49 (52)	45 (48)	44 (50)
Height (cm)	135 ± 8	137 ± 8	137 ± 7	135 ± 7	120 ± 7	119 ± 7	119 ± 7	119 ± 7
Weight (kg)	32.0 ± 8.4[a,b]	33.4 ± 8.3[a]	34.0 ± 8.4[a]	30.0 ± 5.5[b]	20.4 ± 3.2	20.6 ± 3.4	20.4 ± 3.2	20.0 ± 3.4
BMI (kg/m^2)	17.3 ± 2.9[a,b]	17.5 ± 2.9[a]	17.9 ± 3.0[a]	16.1 ± 1.9[b]	14.1 ± 1.1	14.3 ± 1.1	14.2 ± 0.9	14.1 ± 1.0
MUAC (cm)	NA	NA	NA	NA	16.6 ± 1.3	16.8 ± 1.4	16.8 ± 1.3	16.6 ± 1.4
Height-for-age *z* score	0.8 ± 1.0	0.8 ± 0.8	0.8 ± 0.9	0.7 ± 1.0	−1.4 ± 1.0	−1.4 ± 1.0	−1.4 ± 1.0	−1.5 ± 0.8
Weight-for-age *z* score	0.6 ± 1.1[a,b]	0.7 ± 0.9[a,b]	0.8 ± 1.0[a]	0.3 ± 1.0[b]	−1.7 ± 0.7	−1.5 ± 0.8	−1.6 ± 0.7	−1.7 ± 0.7
Weight-for-height *z* score	NA	NA	NA	NA	−1.1 ± 0.8	−0.9 ± 0.7	−1.0 ± 0.7	−1.1 ± 0.7
Highest education in the household (y)	14.0 ± 3.6	15.0 ± 3.6	14.0 ± 3.0	14.0 ± 2.6	8.0 ± 3.4	7.8 ± 3.4	7.9 ± 3.4	8.0 ± 3.2
n for blood analyses	29	34	33	31	89	91	93	84
Anemia[2] [*n* (%)]	0 (0)	0 (0)	3 (9.1)	1 (3.2)	9 (10.1)	10 (11.0)	13 (14.0)	6 (7.1)
Iron deficiency[3] [*n* (%)]	1 (3.5)	2 (5.9)	6 (18.2)	5 (16.1)	13 (14.6)	26 (28.6)	17 (18.3)	25 (29.8)
Iron-deficiency anemia[4] [*n* (%)]	0 (0)	0 (0)	2 (6.1)	0 (0)	3 (3.4)	5 (5.5)	4 (4.3)	2 (2.4)
Zinc deficiency[5] [*n* (%)]	3 (11.1)	5 (14.7)	7 (21.2)	6 (19.4)	14 (15.7)	20 (22.0)	17 (18.3)	18 (21.4)

[1] All values are $\bar{x}$ ± SD unless otherwise indicated. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUAC, midupper arm circumference (measured in Indonesia only); NA, not available in Australia. Means in the same row with different superscript letters are significantly different, $P < 0.05$ (Tukey's test). For the other variables, no significant differences between groups were observed (Tukey's test).

[2] Defined as hemoglobin < 11.5 g/dL.

[3] Defined as serum ferritin < 15.0 μg/L.

[4] Defined as hemoglobin < 11.5 g/dL and serum ferritin < 15.0 μg/L (15).

[5] Defined as serum zinc < 10 μmol/L (22).

and was thought to represent attention and concentration (Fons vd Vijver, personal communication, 2006). For both countries, factor scores were calculated by using the weighed means of the tests loading on that factor. Analysis of covariance adjusted for sex and age in Indonesia, and sex, age, and cohort in Australia, was performed by using the change in factor scores as an outcome and evaluating interactions with sex and age. All analyses were performed by using the SAS (version 9.0) statistical software package (SAS Institute Inc, Cary, NC).

RESULTS

Australia

In Australia, 396 children were randomly allocated to the 4 treatment groups, and a total of 276 children completed the study (**Figure 1**). The 120 children (30%) who dropped out during the study either lost interest ($n = 32$), did not like the taste of the supplement or juices ($n = 23$), could not participate further because of family issues ($n = 15$), had moved ($n = 7$), or withdrew for other specified or unknown reasons ($n = 43$). There were no significant differences between treatment groups in the number of dropouts or in the reasons for dropping out. Children who dropped out during the study did not differ significantly in baseline characteristics from children who completed the study (data not shown). A relatively large number of children or their parents did not consent to blood sampling, and thus the final sample sizes for blood indexes were 25, 31, 25, and 24 in the micronutrients, DHA+EPA, micronutrients+DHA+EPA, and placebo groups, respectively.

Adherence to the feeding regimen (in % of d; $\bar{x}$ ± SD) assessed by returned sachet count was 73 ± 16%, 79 ± 15%, 76 ± 17%, and 75 ± 16% in children in the micronutrients, DHA+EPA, micronutrients+DHA+EPA, and placebo groups, respectively. Adherence assessed by self-reported calendars was 79 ± 18%, 84 ± 12%, 82 ± 17%, and 84 ± 12% in the micronutrients, DHA+EPA, micronutrients+DHA+EPA, and placebo groups, respectively. There were no significant differences in treatment adherence or loss to follow-up between treatment groups.

Baseline demographic and biochemical characteristics of the children in each group are shown in **Table 3**. Children in the placebo group had lower weights, body mass indexes, and weight-for-age *z*-scores than did children in the micronutrients+DHA+EPA and children in the DHA+EPA group. No other baseline differences between groups were observed.

Biochemical indicators

The changes in blood mineral, vitamin, and fatty acid concentrations between baseline and 12 mo for the 4 groups are presented in **Table 4**. The micronutrient treatment improved concentrations of serum ferritin, body iron stores, red blood cell folate, and vitamin B-12. There was also a treatment effect of

TABLE 4
Changes in biochemical indicators for the 4 intervention groups in Australia[1]

	Group				Treatment effect after 12 mo		
	Micronutrients ($n = 25$)	DHA+EPA ($n = 31$)	Micronutrients + DHA + EPA ($n = 25$)	Placebo ($n = 24$)	Micronutrients (95% CI)	DHA+EPA (95% CI)	*P* for interaction
Serum ferritin (μg/L)							
Baseline	31.9 ± 22.4	29.8 ± 10.8	26.8 ± 15.3	23.1 ± 10.3	7.35	1.72	0.38
12 mo	35.7 ± 11.1	31.7 ± 14.9	40.4 ± 25.3	28.5 ± 13.0	(2.25, 12.45)	(−3.42, 6.85)	
Change	8.6 ± 9.5	2.4 ± 10.1	12.9 ± 20.1	4.6 ± 8.8			
Hemoglobin (g/dL)							
Baseline	13.1 ± 0.8	13.1 ± 0.7	12.6 ± 0.7	13.0 ± 0.8	0.10	0.09	0.19
12 mo	13.0 ± 0.7	13.1 ± 0.7	12.8 ± 0.7	13.1 ± 0.7	(−0.17, 0.36)	(−0.18, 0.35)	
Change	−0.2 ± 0.8	−0.0 ± 0.6	0.2 ± 0.7	0.1 ± 0.6			
Red blood cell folate (nmol/L)							
Baseline	439 ± 186	424 ± 205	435 ± 178	442 ± 187	171.14	50.01	
12 mo	534 ± 118	408 ± 161	594 ± 150	395 ± 137	(107.97, 234.31)	(−13.58, 113.60)	0.07
Change	107 ± 190	−16 ± 149	213 ± 128	−19 ± 123			
Vitamin B-12 (pmol/L)							
Baseline	373 ± 104	376 ± 137	381 ± 130	372 ± 153	114.65	8.56	0.04
12 mo	426 ± 119	326 ± 113	494 ± 190	346 ± 131	(80.50, 148.80)	(−25.75, 42.87)	
Change	50 ± 76	−54 ± 61	99 ± 109	−15 ± 99			
Serum zinc (μmol/L)							
Baseline	11.7 ± 1.7	11.3 ± 1.8	11.0 ± 1.8	11.2 ± 1.7	0.05	0.36	0.60
12 mo	12.3 ± 1.7	12.2 ± 1.7	11.4 ± 1.8	11.6 ± 1.6	(−0.64, 0.73)	(−0.33, 1.04)	
Change	0.6 ± 1.2	1.0 ± 1.6	0.9 ± 2.2	0.5 ± 1.7			
Transferrin receptor (mg/L)							
Baseline	3.5 ± 0.6	3.8 ± 0.6	3.6 ± 0.7	3.6 ± 0.5	0.01	0.09	0.72
12 mo	3.4 ± 0.6	3.6 ± 0.9	3.4 ± 0.5	3.2 ± 0.5	(−0.20, 0.22)	(−0.13, 0.30)	
Change	−0.2 ± 0.4	−0.2 ± 0.6	−0.1 ± 0.7	−0.2 ± 0.4			
Body iron stores[2] (mg/kg)							
Baseline	4.36 ± 1.77	4.09 ± 1.43	3.45 ± 2.79	3.27 ± 1.64	0.87	0.06	0.06
12 mo	5.17 ± 1.32	4.34 ± 1.68	5.14 ± 2.28	4.45 ± 1.66	(0.35, 1.39)	(−0.46, 0.59)	
Change	1.10 ± 0.98	0.30 ± 1.05	1.67 ± 1.98	0.79 ± 1.30			
ALA plasma mass (μg/mL)							
Baseline	2.1 ± 0.8	2.0 ± 0.7	2.2 ± 0.8	2.0 ± 0.7	−0.18	0.15	0.28
12 mo	1.9 ± 0.7	2.2 ± 0.7	2.3 ± 1.0	2.2 ± 1.0	(−0.51, 0.16)	(−0.19, 0.49)	
Change	−0.2 ± 0.8	0.2 ± 0.8	0.2 ± 0.7	0.2 ± 1.0			
EPA plasma mass (μg/mL)							
Baseline	7.9 ± 2.8	7.4 ± 2.8	7.7 ± 3.3	7.5 ± 2.6	0.21	2.23	0.58
12 mo	8.2 ± 2.5	10.0 ± 2.9	9.4 ± 4.1	7.0 ± 2.4	(−1.18, 1.60)	(0.82, 3.64)	
Change	0.2 ± 3.3	2.4 ± 3.4	2.1 ± 2.6	−0.3 ± 3.2			
DPA plasma mass (μg/mL)							
Baseline	12.5 ± 2.6	12.0 ± 3.2	12.4 ± 3.3	12.5 ± 3.1	−0.19	−0.19	0.81
12 mo	13.1 ± 1.9	12.9 ± 3.1	12.6 ± 3.9	12.4 ± 2.5	(−1.64, 1.25)	(−1.65, 1.28)	
Change	0.6 ± 3.3	1.0 ± 3.6	0.4 ± 3.0	−0.3 ± 3.6			
DHA plasma mass (μg/mL)							
Baseline	35.2 ± 10.4	31.1 ± 10.6	32.7 ± 10.3	33.2 ± 11.0	−1.21	11.06	0.73
12 mo	36.8 ± 8.5	47.4 ± 12.7	47.4 ± 10.7	38.4 ± 14.0	(−6.07, 3.66)	(6.13, 20.51)	
Change	2.9 ± 9.2	15.8 ± 11.4	14.9 ± 9.9	4.9 ± 13.0			
Total plasma n−3 (μg/mL)							
Baseline	58.8 ± 14.3	53.5 ± 15.2	55.9 ± 15.9	56.2 ± 15.3	−1.49	13.35	0.90
12 mo	60.9 ± 10.3	73.5 ± 17.5	72.7 ± 17.2	60.9 ± 17.7	(−8.55, 5.57)	(6.19, 20.51)	
Change	3.3 ± 14.5	19.5 ± 17.1	17.5 ± 14.4	4.9 ± 17.7			

[1] All values are $\bar{x}$ ± SD. ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Treatment effect estimated by analysis of covariance adjusted for sex, age, and cohort year.

[2] Calculated as log[(1500 × serum transferrin receptor + 350)/(serum ferritin)] −2.8229/0.1207 (24, 25).

TABLE 5
Baseline, 12-mo, and change in factor scores of cognitive tests and main effects of micronutrient and docosahexaenoic acid + eicosapentaenoic acid (DHA+EPA) treatment in Australia[1]

	Group				Mean estimated treatment effect after 12 mo		
	Micronutrients ($n = 66$)	DHA + EPA ($n = 67$)	Micronutrients + DHA + EPA ($n = 69$)	Placebo ($n = 70$)	Micronutrients (95% CI)	DHA + EPA (95% CI)	*P* for interaction
General intelligence							
Baseline	−0.08 ± 1.08[2]	0.19 ± 0.87	−0.01 ± 1.04	−0.08 ± 1.08	−0.03	−0.03	0.63
12 mo	0.88 ± 1.19	1.15 ± 0.93	0.91 ± 1.01	0.88 ± 1.19	(−0.14, 0.08)	(−0.13, 0.08)	
Change	0.96 ± 0.40	0.97 ± 0.46	0.92 ± 0.45	0.96 ± 0.40			
Verbal learning and memory							
Baseline	−0.17 ± 0.95	0.12 ± 1.00	0.03 ± 1.11	0.01 ± 0.94	0.23	−0.04	0.66
12 mo	0.32 ± 0.96	0.30 ± 0.97	0.41 ± 0.99	0.19 ± 0.94	(0.001, 0.46)	(−0.27, 0.19)	
Change	0.48 ± 0.99	0.17 ± 0.95	0.38 ± 0.86	0.19 ± 0.98			
Visual attention							
Baseline	−0.19 ± 0.92	0.08 ± 1.08	0.06 ± 1.03	0.05 ± 0.96	0.15	−0.16	0.18
12 mo	1.13 ± 0.66	1.05 ± 0.75	1.03 ± 0.68	1.02 ± 0.72	(−0.07, 0.37)	(−0.38, 0.07)	
Change	1.32 ± 0.82	0.97 ± 1.00	0.97 ± 0.95	0.98 ± 0.90			

[1] Values reflect factor scores with a mean of 0 and an SD of ± 1; treatment effect was estimated by ANCOVA adjusted for sex, age, and cohort.
[2] $\bar{x}$ ± SD (all such values).

DHA+EPA on change in plasma EPA, plasma DHA, and total plasma n−3 fatty acids.

Cognitive test outcomes

After 12 mo of intervention, there was a treatment effect of the micronutrients on change in factor 2, which represented verbal learning and memory [estimated effect size 0.23 (95% CI: 0.01, 0.46)]. No significant effects were observed on the other factors (**Table 5**). There were no significant interactions between micronutrients and DHA+EPA for change in factor scores. No interactions between treatment and sex or age were observed. In general, similar, albeit not statistically significant, trends were observed at 6 mo (data not shown but available on request).

Indonesia

In Indonesia, 384 children were randomly allocated to the 4 intervention groups, and a total of 368 children completed the study at 12 mo (**Figure 2**). A total of 16 children (4.2%) dropped out during the study because they either had moved from the study area ($n = 13$), did not attend school anymore ($n = 1$), or had been diagnosed with chronic infectious disease (pulmonary tuberculosis; $n = 1$). In addition, after random assignment, one

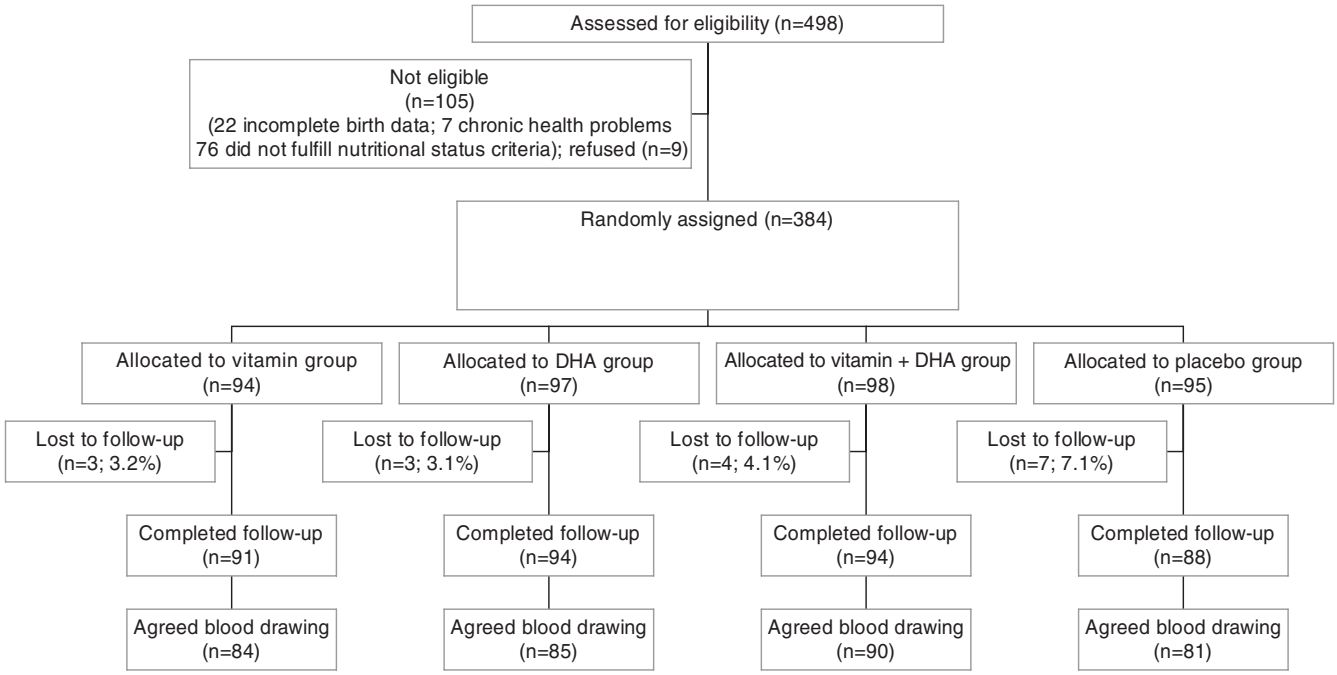

FIGURE 2. Trial profile: Indonesia.

child was registered twice because that child was known by 2 different names. There were 84, 85, 90, and 81 blood samples for final analyses in the micronutrient, DHA+EPA, micronutrients+DHA+EPA, and placebo groups, respectively.

Adherence to the intervention products as recorded during the feeding sessions at schools was 86 ± 5%, 85 ± 9%, 85 ± 6%, and 87 ± 5% for children in the micronutrient, DHA+EPA, micronutrients+DHA+EPA, and placebo groups, respectively. There were no significant differences between groups in adherence to the intervention. Adherence for biscuit consumption (% of d that child consumed the required 3 biscuits) ranged from 80% to 82% in the 4 groups.

Baseline demographic and biochemical characteristics of the children in each group are shown in Table 3. There were no significant differences between treatment groups in baseline characteristics.

Biochemical indicators

Data on biochemical indicators for the 4 intervention groups in Indonesia are shown in **Table 6**. The micronutrient treatment improved concentrations of serum ferritin, hemoglobin, red blood cell folate, and vitamin B-12; decreased serum transferrin receptor concentrations; and improved body iron stores. No significant DHA+EPA effects were observed for vitamin and mineral outcomes after 12 mo of intervention.

The DHA+EPA treatment increased changes in plasma DHA and total plasma n−3 fatty acids. In addition, the micronutrient treatment increased changes in plasma DHA and total n−3 fatty acid concentrations.

Cognitive test outcomes

In Indonesia, no significant treatment effects were observed on any of the factors after 12 mo of intervention (**Table 7**). However, an interaction ($P = 0.03$) was found between micronutrient treatment and sex on change in scores on factor 2 (verbal learning and memory), such that a positive micronutrient treatment effect was observed in girls (0.32; 95% CI: −0.01, 0.64) but not in boys (−0.04; 95% CI: −0.38, 0.29).

DISCUSSION

In Australia, an intervention with a fortified drink containing multiple micronutrients with or without DHA and EPA consumed daily for 1 y improved micronutrient status in school-aged children. In addition, the fortified drink with DHA and EPA with or without micronutrients improved plasma DHA and plasma total n−3 fatty acids. These improvements in micronutrient and fatty-acid status were seen even though children in Australia were ostensibly well nourished at baseline as indicated by the adequate mean micronutrient concentrations. However, the small number of blood samples in the Australian sample might have biased this conclusion.

Fortification with multiple micronutrients with or without DHA improved scores on tests representing verbal learning and memory. No effects were seen on tests measuring general intelligence or attention. Previous studies in 10–13-y-old UK schoolchildren found an improvement in nonverbal reasoning and intelligence after vitamin and mineral supplementation (29, 30).

Even though we reduced the number of outcome variables by means of a factor analysis, we cannot exclude the possibility that our findings are due to chance. However, we believe that our findings may reflect true effects because *1*) the observed effect size was small, but with high precision; *2*) a similar effect was observed in Indonesian girls; and *3*) similar effects on verbal learning and memory have been reported in supplementation trials in nonanemic, iron-deficient, adolescent girls and school-aged children in the United States and Canada (31, 32), and these effects were particularly pronounced in anemic children (8). The improvements observed in our study may therefore have been due in part to a moderately improved iron status as demonstrated by the increased serum ferritin concentrations and body iron stores.

However, our intervention provided a mix of micronutrients and we can only speculate about the possible biological pathways for the observed effect on verbal learning and memory. In addition to the moderately improved iron status, the micronutrient mix improved significantly the concentrations of red blood cell folate and serum vitamin B-12. Evidence from several cross-sectional studies suggests an association between vitamin B-12 and folate and cognitive performance in children (1, 3), but results from intervention studies are lacking. Evidence is emerging, but still conflicting, for a role of zinc on children's motor and cognitive development (4). We did not observe increases in mean serum zinc concentrations in our groups, despite the surprisingly high estimated prevalence of zinc deficiency. Our mix contained only one-half the RDA for zinc to avoid possible interaction with iron (17), which was apparently insufficient to increase the mean group serum zinc concentrations in our population.

There were no treatment effects of DHA+EPA on cognitive factor scores. Our findings are in contrast with findings from recent studies in the United Kingdom, where supplementation with DHA in combination with EPA has been shown to improve scores on reading, spelling, and behavior after 3 mo in schoolchildren with developmental coordination disorders (7). In addition, 5 mo of intervention with a spread fortified with DHA and EPA resulted in improvements on tests of verbal learning and memory in healthy South African schoolchildren (5). The dosages used in those 2 studies were markedly higher (558 mg EPA plus 174 mg DHA and 182 mg DHA+EPA in the United Kingdom and South African study, respectively) than in our study (88 mg DHA, 22 mg EPA). It is possible that our dose was too low for a measurable effect or that our intervention did not contain the right combination of DHA and EPA, even though it was sufficient to increase plasma DHA and plasma total n−3 fatty acid status in these children.

In Indonesia, the intervention improved micronutrient and fatty acid status after 12 mo. There was a (nonsignificant) trend for improved scores on tests representing verbal learning and memory after the micronutrient treatment among girls. Fortification with DHA+EPA did not result in beneficial effects on cognitive scores. We are not sure why this effect was apparent only in girls. Girls in Indonesia were on average slightly younger (8.0 ± 1.0 y) than boys (8.3 ± 1.1 y) and had higher serum vitamin B-12 concentrations (427 ± 213 and 419 ± 166 pmol/L in girls and boys, respectively). No other significant differences in baseline nutritional status between boys and girls were observed. The girls had higher baseline scores on the factor representing attention and concentration (−0.39 ± 1.38 compared with −0.75 ± 1.62 in boys), which agrees with the observation that in this age group girls are often seen to be better motivated and perform better on these tests (26). However, in contrast, girls had lower baseline scores on the verbal learning and memory

TABLE 6
Changes in biochemical indicators for the 4 intervention groups in Indonesia[1]

	Group				Treatment effect after 12 mo		
	Micronutrients ($n = 90$)	DHA + EPA ($n = 85$)	Micronutrients + DHA + EPA ($n = 84$)	Placebo ($n = 81$)	Micronutrients	DHA + EPA	*P* for interaction
Serum ferritin (μg/L)							
Baseline	28.1 ± 23.3[2]	28.5 ± 27.5	30.2 ± 17.2	24.9 ± 13.8	26.09	−4.81	0.28
12 mo	64.8 ± 32.7	33.3 ± 18.2	60.9 ± 26.1	30.2 ± 19.5	(19.47, 32.70)	(−11.42, 1.80)	
Change	37.2 ± 38.5	4.5 ± 26.3	30.9 ± 26.9	5.6 ± 16.2			
Hemoglobin (g/dL)							
Baseline	12.9 ± 1.1	12.9 ± 1.1	12.7 ± 1.1	12.8 ± 1.0	0.28	0.04	0.03
12 mo	13.1 ± 1.0	12.9 ± 1.0	13.1 ± 0.9	13.0 ± 1.0	(0.03, 0.52)	(−0.21, 0.28)	
Change	0.2 ± 0.9	0.0 ± 0.9	0.5 ± 1.2	0.2 ± 0.7			
Red blood cell folate (nmol/L)							
Baseline	591 ± 211	580 ± 217	559 ± 231	596 ± 197	124.51	30.07	0.13
12 mo	607 ± 183	502 ± 170	643 ± 188	514 ± 167	(69.98, 179.34)	(−24.75, 84.89)	
Change	18 ± 224	−88 ± 199	89 ± 250	−73 ± 221			
Vitamin B-12 (pmol/L)							
Baseline	426 ± 178	439 ± 166	433 ± 196	487 ± 225	81.99	−0.93	0.08
12 mo	478 ± 189	403 ± 146	457 ± 156	425 ± 168	(49.61, 114.38)	(−33.31, 31.45)	
Change	63 ± 154	−42 ± 117	22 ± 133	−61 ± 145			
Serum zinc (μmol/L)							
Baseline	11.1 ± 1.9	10.8 ± 1.8	11.0 ± 1.7	11.0 ± 2.1	0.14	0.12	0.58
12 mo	11.1 ± 1.6	10.9 ± 1.6	11.2 ± 1.6	11.1 ± 1.4	(−0.36, 0.64)	(−0.38, 0.62)	
Change	0.0 ± 2.4	0.1 ± 1.8	0.3 ± 2.0	0.2 ± 2.3			
Transferrin receptor (mg/L)							
Baseline	4.5 ± 1.2	4.7 ± 1.7	4.7 ± 1.5	4.6 ± 1.0	−0.46	−0.09	0.77
12 mo	3.8 ± 0.9	4.4 ± 1.5	3.8 ± 0.9	4.3 ± 1.0	(−0.72, −0.20)	(−0.35, 0.17)	
Change	−0.7 ± 1.1	−0.3 ± 0.9	−0.8 ± 1.4	−0.3 ± 0.7			
Body iron stores (mg/kg)[3]							
Baseline	3.03 ± 2.61	2.74 ± 2.81	2.73 ± 3.32	2.35 ± 2.78	2.93	0.21	0.04
12 mo	6.76 ± 1.60	3.79 ± 2.40	6.46 ± 1.82	3.21 ± 2.65	(2.53, 3.33)	(−0.18, 0.61)	
Change	3.83 ± 2.57	1.11 ± 2.01	3.81 ± 3.45	0.84 ± 1.95			
ALA plasma mass (μg/mL)							
Baseline	1.1 ± 0.5	1.1 ± 0.5	1.1 ± 0.5	1.3 ± 0.6	0.13	0.06	0.46
12 mo	1.0 ± 0.4	1.0 ± 0.4	1.1 ± 0.4	1.0 ± 0.5	(−0.01, 0.27)	(−0.09, 0.20)	
Change	−0.1 ± 0.6	−0.1 ± 0.5	−0.1 ± 0.5	−0.2 ± 0.7			
EPA plasma mass (μg/mL)							
Baseline	2.9 ± 2.6	3.2 ± 2.4	3.3 ± 3.6	2.9 ± 1.5	0.25	−0.54	0.61
12 mo	2.9 ± 3.7	2.7 ± 1.6	2.8 ± 1.6	2.6 ± 1.9	(−0.61, 1.20)	(−1.40, 0.33)	
Change	−0.1 ± 4.5	−0.6 ± 2.5	−0.5 ± 3.7	−0.3 ± 2.0			
DPA plasma mass (μg/mL)							
Baseline	6.2 ± 2.2	6.4 ± 2.0	6.2 ± 2.4	6.3 ± 2.0	0.40	−0.18	0.12
12 mo	5.7 ± 1.5	5.7 ± 1.5	6.0 ± 1.6	6.0 ± 2.1	(−0.14, 0.94)	(−0.72, 0.36)	
Change	−0.5 ± 2.3	−0.7 ± 2.1	−0.2 ± 2.3	−0.3 ± 2.1			
DHA plasma mass (μg/mL)							
Baseline	39.2 ± 13.7	41.3 ± 13.9	41.1 ± 14.4	41.3 ± 13.0	4.09	7.06	0.14
12 mo	40.3 ± 11.1	46.4 ± 10.4	51.1 ± 12.6	39.6 ± 13.3	(1.01, 7.17)	(3.98, 10.14)	
Change	0.7 ± 11.8	5.5 ± 13.9	10.3 ± 12.8	−1.7 ± 11.9			
Total plasma n−3 (μg/mL)							
Baseline	50.1 ± 17.1	52.7 ± 17.3	52.3 ± 19.0	52.5 ± 15.6	4.86	6.35	0.22
12 mo	50.6 ± 14.3	56.3 ± 12.4	61.5 ± 15.0	49.9 ± 16.5	(0.85, 8.87)	(2.34, 10.36)	
Change	0.0 ± 16.7	4.0 ± 17.3	9.5 ± 16.9	−2.6 ± 14.5			

[1] DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. Treatment effect estimated by ANCOVA adjusted for sex and age.
[2] $\bar{x}$ ± SD (all such values).
[3] Body iron stores (mg/kg) calculated as log[(1500 × serum transferrin receptor + 350)(serum ferritin)]−2.8229/0.1207 (24, 25).

TABLE 7
Baseline, 12-mo, and change in factor scores of cognitive tests and main effects of micronutrients and docosahexaenoic acid + eicosapentaenoic acid (DHA + EPA) treatment in Indonesia[1]

	Group				Mean estimated treatment effect after 12 mo		
	Micronutrients ($n = 61$)	DHA + EPA ($n = 65$)	Micronutrients + DHA + EPA ($n = 62$)	Placebo ($n = 54$)	Micronutrients	DHA + EPA	*P* for interaction
General intelligence							
Baseline	0.01 ± 0.89[2]	−0.01 ± 0.85	0.10 ± 0.82	−0.11 ± 0.84	−0.12	−0.13	0.32
12 mo	0.37 ± 0.94	0.25 ± 1.08	0.37 ± 1.00	0.50 ± 1.06	(−0.40, 0.15)	(−0.41, 0.14)	
Change	0.42 ± 1.10	0.40 ± 0.95	0.42 ± 0.83	0.68 ± 1.03			
Verbal learning and memory							
Baseline	−0.03 ± 0.99	0.05 ± 1.07	0.02 ± 1.06	−0.07 ± 0.94	0.14	−0.11	0.63
12 mo	0.28 ± 1.07	0.15 ± 1.01	0.21 ± 1.00	0.14 ± 0.88	(−0.10, 0.37)	(−0.35, 0.12)	
Change	0.34 ± 0.99	0.13 ± 0.84	0.34 ± 0.67	0.31 ± 0.90			
Visual attention							
Baseline	−0.25 ± 0.90	0.10 ± 1.06	0.06 ± 0.92	0.08 ± 0.86	0.11	−0.19	0.28
12 mo	1.19 ± 0.95	1.20 ± 1.06	1.11 ± 0.91	1.15 ± 1.06	(−0.10, 0.32)	(−0.40, 0.02)	
Change	1.46 ± 0.76	1.19 ± 0.83	1.16 ± 0.67	1.26 ± 0.78			

[1] Values reflect factor scores with a mean of 0 and an SD of ± 1; treatment effect estimated by ANCOVA adjusted for sex and age.
[2] $\bar{x}$ ± SD (all such values).

tests (−0.07 ± 0.76 compared with 0.11 ± 0.93 in boys), which may indicate that the observed sex effect was partly representing a regression to the mean. Improvements after the micronutrient interventions in tests assessing verbal abilities and short-term memory have been observed in 3 studies in developing countries (9–11), although in one study the effects were restricted to children who were deficient in iron and iodine (10). In contrast, Jinabhai et al (12) provided biscuits fortified with iron and vitamin A and did not observe an effect on cognitive test scores. This study had a duration of 16 wk and used only one subscore of the RAVLT test, which may have been why no improvement in performance was observed.

We hypothesized that marginally nourished children would benefit more from the intervention than would well-nourished children. However, we observed similar effects in both countries, with the effect limited to girls only in Indonesia. These unexpected findings in Indonesia, particularly in the boys, may have been due in part, to differences in academic experience, familiarity with test materials and previous exposure to testing and assessment (33). At baseline, the children in Indonesia were on average 6 mo younger than their Australian counterparts, which may partly, but not completely, explain the lower baseline test scores observed in Indonesia than in Australia. The lower baseline scores in Indonesia, especially for the verbal tests, may in addition raise questions about whether, in the Indonesian study, these tests were sensitive enough and how well the children understood the test instructions. It is commonly observed that children in impoverished conditions perform less well on intelligence tests (34). The observed scoring pattern, especially on the verbal tests, suggests that perhaps more validation and training would have been required in our study in Indonesia (20, 33).

Short-term hunger, possibly as the result of missing breakfast (35), might be an additional reason for the lower test scores observed in Indonesia. In Indonesian infants, Pollitt et al (36) observed responses on mental development only when micronutrients combined with a protein-energy supplementation were provided, and for this reason we combined the intervention in Indonesia with a small protein-energy snack. It is possible that compromised nutrition early in life may have prevented beneficial effects of micronutrient interventions later in life. Despite their relatively adequate mean micronutrient concentrations and a surprisingly low 4% of children with iron-deficiency anemia, 27% of the children in Indonesia were stunted, which suggests a long-term history of marginal intakes.

In summary, an intervention with a micronutrient-fortified drink improved micronutrient status and improved scores on tests assessing verbal memory and learning in school-aged Australian children. A similar effect was observed in Indonesian girls. No significant effects were found on tests measuring abilities related to general intelligence and attention. The addition of DHA+EPA to the fortified drink did not result in beneficial effects on cognitive performance despite the fact that n−3 fatty acid status was improved. Further research is recommended to define the exact role of n−3 fatty acids on the cognitive function of children, with or without micronutrient interventions.

Recently, a group of child developmental scientists emphasized the importance of, among others, nutrition and micronutrient interventions for optimizing the development, including cognitive development, of undernourished children under the age of 5 y (1). In this study, we report that, even in an adequately nourished, school-aged population, improvements in micronutrient status and verbal learning and memory can be achieved by fortification with multiple micronutrients.

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