

## Undernutrition, fatty acid and micronutrient status in relation to cognitive performance in Indian school children: a cross-sectional study

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While undernutrition and anaemia have previously been linked to poor development of children, relatively little is known about the role of B-vitamins and fatty acids on cognition. The present study aims to explore the associations between indicators of body size, fatty acid and micronutrient status on cognitive performance in 598 Indian school children aged 6–10 years. Baseline data of a clinical study were used to assess these associations by analyses of variance adjusting for age, sex, school, maternal education and cognitive tester. The Kaufman Assessment Battery for Children II was used to measure four cognitive domains, including fluid reasoning, short-term memory, retrieval ability and cognitive speediness. Scores were combined into an overall measure, named mental processing index (MPI). Body size indicators and Hb concentrations were significantly positively related to cognitive domains and MPI, such that increases of 1 SD in height-for-age and weight-for-age *z*-scores would each translate into a 0.09 SD increase in MPI,  $P=0.0006$  and  $0.002$ , respectively. A 10 g/l increase in Hb concentrations would translate into a 0.08 SD increase in MPI,  $P=0.0008$ . Log-transformed vitamin B<sub>12</sub> concentrations were significantly inversely associated with short-term memory, retrieval ability and MPI ( $\beta$  (95% CI) =  $-0.124$  ( $-0.224$ ,  $-0.023$ ),  $P=0.02$ ). Other indicators of Fe, iodine, folate and fatty acid status were not significantly related to cognition. Our findings for body size, fatty acids and micronutrients were in agreement with previous observational studies. The inverse association of vitamin B<sub>12</sub> with mental development was unexpected and needed further study.

### Undernutrition: Fatty acids: Micronutrients: Cognition: Children

Low intakes of energy, protein and other nutrients, together with high infection rates and poor socio-economic status may lead to linear growth retardation and impaired child development<sup>(1)</sup>. Cross-sectional studies have linked stunting (short stature for age) and low weight-for-age to poor development and school achievement in infants and children<sup>(2)</sup>. The detrimental effects of undernutrition early in life (<2 years of age) on intellectual development seem irreversible and remain apparent during childhood and adolescence<sup>(3,4)</sup>.

The *n*-3 fatty acid DHA and the *n*-6 fatty acid arachidonic acid are important structural components of the human central nervous system<sup>(5)</sup> and play a role in brain functioning through their involvement in aspects of neuron function and of neurotransmitter synthesis<sup>(6)</sup>. These two fatty acids can be synthesised by the human body from the  $\alpha$ -linolenic acid (ALA) and linoleic acid. However, dietary intake of the *n*-3 fatty acid ALA in children is considered to be low<sup>(7,8)</sup>, which possibly limits adequate cognitive functioning. In fact, high fish intake during pregnancy has been associated with better cognitive development of infants<sup>(9–11)</sup> and maternal and infant DHA supplementation may benefit visual, motor

and mental development of infants and young children<sup>(12–18)</sup>. For healthy children >2 years of age such evidence is currently limited<sup>(19)</sup>.

Among the micronutrients, Fe and iodine interventions have been shown to improve intelligence scores of children<sup>(20,21)</sup>. Fe is needed for the formation of Hb for adequate oxygen transport in the human body. In the brain, Fe is required for myelination and neurotransmitter synthesis<sup>(22)</sup>. Iodine is an important component of the thyroid hormones, thyroxine and tri-iodothyronine, which plays a major role in the growth and development, function and maintenance of the central and peripheral nervous system<sup>(23)</sup>. For the B-vitamins, however, little research has been conducted to investigate whether these vitamins are of influence on mental development in children. Vitamin B<sub>12</sub> (cobalamin) deficiency has been associated with lower scores on cognitive tests in Guatemalan<sup>(24)</sup> and Dutch<sup>(25)</sup> children. Folate is important for closure of the neural tube during fetal development<sup>(26)</sup>, but no studies have investigated the role of folate on cognitive functioning in children after birth. In the brain, folate is required for neurotransmitter production and myelination<sup>(27,28)</sup>. Because of the

**Abbreviations:** ALA,  $\alpha$ -linolenic acid; HAZ, height-for-age *z*-scores; MPI, mental processing index; sTfR, soluble transferrin receptor; WAZ, weight-for-age *z*-scores.

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interactions with folate metabolism, vitamin B<sub>12</sub> is indirectly involved in neurotransmitter synthesis. Furthermore, the vitamin B<sub>12</sub> cofactors adenosylcobalamin and methylcobalamin are involved in myelination of the spinal cord and the brain<sup>(29)</sup>.

The primary objective of the present study is to investigate the associations between indicators of body size, fatty acid status, and Fe, iodine and B-vitamin status on overall cognitive performance in 598 Indian school-age children. Secondary, we will explore the relationships of the nutritional parameters with specific cognitive domains known to be sensitive to differences in nutritional status in children. We hypothesise that the indicators of body size, fatty acid and micronutrient status (Fe, iodine, folate and vitamin B<sub>12</sub>) will be positively related to overall cognitive performance and specific cognitive domains.

### Experimental methods

The Children's Health And Mental Performance Influenced by Optimal Nutrition study was designed to investigate the efficacy of foods fortified with *n*-3 fatty acids and micronutrients on improving intellectual performance and growth in Indian school children<sup>(30)</sup>. The baseline data of the present study, collected in the period between November 2005 and February 2006, were used to assess the associations between height-for-age (HAZ) and weight-for-age *z*-scores (WAZ), Hb concentration and indicators of *n*-3 and *n*-6 fatty acid, Fe, iodine, folate and vitamin B<sub>12</sub> status and cognitive performance. These nutritional parameters were selected based on their possible relationship with children's mental development.

### Subjects

Two primary schools serving children from a poor socio-economic background in Bangalore city, India were selected for participation in the study. Almost all children living in the surrounding communities attended these schools, where they were taught in the local Kannada language. Before study start, parents or caretakers of all children aged 6–10 years, attending grades 2–5 of these schools were invited for a meeting during which the study procedures were explained to them. Informed, written consent from the parents and verbal assent from their children was obtained from 645 parent–child pairs. Children were included in the study if they were: (1) apparently healthy, without any chronic illness and physical/mental handicaps; (2) not severely anaemic (Hb < 80 g/l); (3) not severely undernourished (< –3 SD for WAZ and HAZ-scores of National Health Centre for Statistics/WHO standards<sup>(31)</sup>); (4) not intending to use micronutrient supplements during the study; (5) planning to reside in the study area during the next 12 months. Children who were frequently absent from school (>40 d during 6 months before start of the study) and children who took micronutrient supplements in the period of 3 months before the study start were excluded. A total of 598 children were enrolled in the study. Details on the enrolment, including a flow chart of children recruited in the study have been published elsewhere<sup>(30)</sup>.

### Socio-demographic information

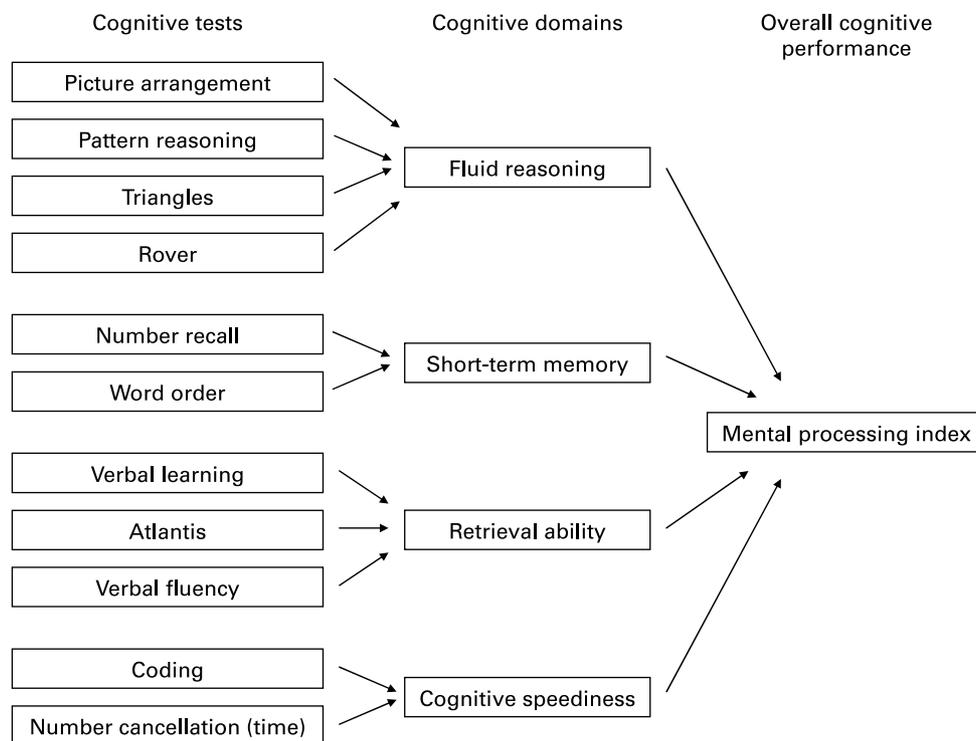
Socio-demographic information on household composition, parental education, income and use of fortified foods was collected by a structured questionnaire that was administered to the mother or primary caretaker of the subjects. The age of the children was verified by the school records.

### Cognitive performance

Cognitive performance was evaluated using age-appropriate, validated psychometric tests that were administered by seven masters-level psychologists in Kannada language. The psychologists were trained extensively during 3 weeks before the study to ensure standardisation in the test administration and scoring procedures. The cognitive test battery was administered to each child on a single day over three sessions of which two took place in the morning and one in the afternoon. Care was taken to ensure all the children had breakfast before testing began in the morning since omitting breakfast is known to impair cognitive performance<sup>(32)</sup>. The cognitive test battery consisted of eleven subtests, including six core tests of the Kaufman Assessment Battery for Children, second edition for children 3–18 years (pattern reasoning, triangles, rover, number recall, word order, atlantis)<sup>(33)</sup>, two tests from Wechsler Intelligence Scale for Children-Revised and Wechsler Intelligence Scale for Children-4 (picture arrangement, coding) and three additional tests from Rey Auditory Verbal Learning Test (auditory-verbal learning test), NEPSY (neuropsychological assessment tool, verbal fluency) and number cancellation, which was specifically designed for the study. The eleven subtests covered four cognitive domains as specified in Carroll's model as described in the Kaufman Assessment Battery for Children, second edition manual<sup>(33)</sup>, including fluid reasoning, short-term memory, retrieval ability and cognitive speediness (Fig. 1). These domains were chosen because they have shown to be influenced by previous nutritional interventions<sup>(34)</sup>. The test battery underwent an extensive adaptation process to ensure its suitability in the local cultural context<sup>(35)</sup>. For each subtest, a sum score was calculated and converted into a standardised *z*-score. The domain score was composed by taking the average of standardised *z*-scores for the tests constituting a domain. The average of the domain scores named the mental processing index (MPI) was a composite measure of overall cognitive performance based on the Kaufman Assessment Battery for Children, second edition manual<sup>(33)</sup>. Our model of clustering of individual sum scores to form a composite score in four separate cognitive domains showed good validity assessed by structural equation modelling techniques<sup>(36)</sup>.

### Anthropometry

Anthropometric measurements were conducted in duplicate according to standard techniques<sup>(37)</sup> by trained research assistants. Height was recorded to the nearest 0.1 cm using a locally made stadiometer (BioRad, Chennai, India) that was fixed to a wall. Body weight was recorded to the nearest 0.1 kg using a digital weighing scale (Breuer, Germany). During the measurements, children wore their school



**Fig. 1.** Clustering of cognitive tests in domain scores. Fluid reasoning involves basic processes of reasoning and other mental activities that depend only minimally on learning and acculturation; short-term memory is an ability that requires apprehending and holding information in immediate awareness briefly and then using that information within a few seconds; retrieval ability comprises the capacity to store information in long-term memory and to retrieve that information fluently and efficiently; cognitive speediness measures the ability of rapid cognitive processing of information involving attention.

uniform and no shoes, caps or hats. HAZ and WAZ were computed by data on height, weight, age and sex using the National Health Centre for Statistics/WHO growth reference data<sup>(31)</sup>. Children with HAZ and WAZ  $< -2$  SD of this reference median were classified as stunted and underweight, respectively. We did not include weight-for-height z-scores, because National Health Centre for Statistics/WHO reference data were lacking for children  $> 10$  years of age, which concerned 57 children aged 10–11 years in the present study.

#### Biochemical indicators

A whole blood sample (10 ml) was collected in the morning from non-fasted children by venepuncture in an EDTA vacutainer. A spot urine sample was also collected in a sterile plastic container, and the samples were transported to the laboratory on ice. Care was taken to limit the exposure of the samples to light. Hb concentrations were determined within 4 h of collection using an AcT Diff2 Counter (Beckman Coulter Inc., Fullerton, CA, USA). One aliquot of whole blood for erythrocyte folate estimation was immediately treated with freshly prepared 1% ascorbic acid. The remaining blood was immediately centrifuged (3000 rpm, 10 min, 4°C), and the plasma was stored in 2 ml eppendorf tubes at  $-80^{\circ}\text{C}$  until analysis. One millilitre of erythrocytes was washed with 5 ml saline containing EDTA (1 litre normal saline + 0.00324 g disodium EDTA), flushed under nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis for fatty acid content. Serum ferritin was measured by an enzyme immunoassay

(Access<sup>®</sup> 2 Beckman Coulter autoanalyser, Brea, CA, USA)<sup>(37)</sup> against an external 3-level control material (WHO Standard 80/578; Ramco Laboratories Inc., Houston, TX, USA). Serum soluble transferrin receptor (sTfR) was measured by using an enzyme immunoassay (Ramco Laboratories Inc.) with two-level control materials provided by the manufacturer. C-reactive protein was analysed by a turbidimetric method (Roche Hitachi 902, Indianapolis, IN, USA)<sup>(38)</sup>. Plasma vitamin B<sub>12</sub> and red blood cell folate were analysed using a chemiluminescence system (ACS:180, Bayer Diagnostics, Tarrytown, NY, USA)<sup>(39,40)</sup>. Fatty acid content of erythrocyte membrane phospholipids was analysed using GC with a flame ionization detector (Varian 3800, Palo Alto, CA, USA). The procedure involved the extraction of total lipids, isolation of phospholipid fraction by TLC and transmethylation of phospholipids<sup>(41–43)</sup>. The fatty acid methyl esters were separated by chain length and degree of saturation by injection onto a 50 m  $\times$  0.2 mm capillary column (Varian, Palo Alto, CA, USA) with nitrogen as the carrier gas. Urinary iodine was measured using the Sandell–Kolthoff reaction as modified by Pino *et al.*<sup>(44)</sup>. Satisfactory agreement in urinary iodine was obtained on urine samples at four different concentrations measured and the Ensuring the Quality of Urinary Iodine Procedures, Centers for Disease Control and Prevention (Atlanta, GA, USA). The following criteria were used to define micronutrient deficiencies: anaemia: Hb  $< 115$  g/l<sup>(45)</sup>; Fe deficiency: serum ferritin  $< 15$  mg/l and/or sTfR  $> 7.6$  mg/l<sup>(46)</sup>; folate deficiency: erythrocyte folate  $< 305$  nmol/l<sup>(47)</sup>; vitamin B<sub>12</sub> deficiency: plasma vitamin B<sub>12</sub>  $< 148$  pmol/l<sup>(48)</sup>; iodine deficiency: urinary iodine  $< 100$   $\mu\text{g/l}$ <sup>(49)</sup>.

### Statistical analyses

Values for serum ferritin concentrations from the subjects with elevated C-reactive protein (>10 mg/l) were excluded from statistical analyses. Body Fe stores were calculated from serum ferritin and sTfR concentrations using the formula by Cook *et al.*<sup>(50)</sup>. Differences in mean cognitive outcomes between boys and girls, schools and different levels of education of the mother were assessed by *t* tests. Distributions of parameters of fatty acid status, serum ferritin and sTfR, erythrocyte folate, plasma vitamin B<sub>12</sub> and urinary iodine were normalised by natural logarithm (ln) transformation before analysis. Associations between the nutritional parameters and the cognitive scores were analysed using ANOVA (SAS General Linear Modelling procedure) taking into account age, sex, school, maternal education level and assessor of cognitive tests as covariates. All available data were analysed, missing values were not replaced. All analyses were performed using Statistical Analysis Software version 9.1 statistical software package (SAS Institute Inc., Cary, NC, USA).

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethics committees at St John's National Academy of Health Sciences, Bangalore, India and Wageningen University, The Netherlands. Written informed consent was obtained from the parents of all subjects and verbal assent from all the subjects. Verbal consent was witnessed and formally recorded.

### Results

Five hundred and ninety eight children completed the baseline measurements on cognitive performance, anthropometry and Hb concentrations. Data on biochemical indicators of

micronutrient and fatty acid status were available for at least 529 and 541 children, respectively. The socio-demographic characteristics and nutritional status of the subjects are presented in Table 1. Mean age of the children was 8.7 (SD 1.2) years and 49% of them were boys. Nearly, half of the mothers were uneducated and median family income was 2700 Indian rupees per month, which is close to the poverty line of US\$2 per d. Twenty-two percent of the children were stunted and 30% were underweight. The prevalence of anaemia was 9%, while that of Fe, folate, vitamin B<sub>12</sub> and iodine deficiencies were 31, 17, 23 and 47%, respectively.

### Associations of covariates with cognitive performance

Age was significantly positively related with all cognitive outcomes ( $\beta = 0.31$  (95% CI 0.27, 0.35),  $P < 0.0001$  for MPI). Mean cognitive scores for boys and girls are presented in Table 2. Scores on the domains of retrieval ability and cognitive speediness and MPI were significantly lower in boys compared with girls ( $P < 0.001$ ). These findings did not change when scores were corrected for age (data not shown). There was a significant difference in performance on short-term memory and retrieval ability between the two schools (data not shown). Children of mothers with <6 years of education had significantly lower MPI scores compared with children of mothers with  $\geq 6$  years of education (means were  $-0.05$  (SD 0.66) v.  $0.07$  (SD 0.63),  $P = 0.03$ , respectively).

### Associations of nutritional parameters with cognitive performance

Table 3 provides an overview of the associations between the nutritional parameters and the indicators of cognitive performance. Scatter plots of the correlations between the MPI and

**Table 1.** Characteristics of the study population  
(Mean values and standard deviations; median and percentile values)

Characteristic	n	Mean	SD	Median	Percentiles	
					25th	75th
<b>Socio-demographic parameters</b>						
Sex (% males)	598	49				
Age (years)	598	8.7	1.2			
Family income (Indian rupees/month)	598			2700	2000	3800
Uneducated mothers (%)	571	45.5				
<b>Anthropometric measures</b>						
Height (m)	598	122.9	7.8			
Weight (kg)	598	21.4	3.8			
<b>Erythrocyte phospholipid fatty acid status (% of total fatty acids (wt/wt))</b>						
Linoleic acid	541			14.12	13.07	15.21
Arachidonic acid	541			14.61	12.75	16.18
$\alpha$ -Linolenic acid	541			0.21	0.17	0.25
EPA	541			0.16	0.13	0.20
DHA	541			3.21	2.70	3.65
<b>Micronutrient status</b>						
Blood Hb (g/l)	598	127.8	9.9			
Serum ferritin ( $\mu$ g/l)	535			23.6	14.9	34.2
Serum soluble transferrin receptors (mg/l)	538			5.7	4.9	6.8
Total body Fe stores (mg/kg)	535	3.22	3.20			
Erythrocyte folate (nmol/l)	529			515	371	745
Plasma vitamin B <sub>12</sub> (pmol/l)	533			197	151	266
Urinary iodine ( $\mu$ g/l)	542			108	66	200

**Table 2.** Cognitive domain scores for boys and girls\*  
(Mean values and standard deviations)

	Boys (n 293)		Girls (n 305)	
	Mean	SD	Mean	SD
Mental processing index	-0.09	0.64†	0.09	0.64
Fluid reasoning	0.01	0.81	-0.01	0.77
Short-term memory	-0.06	0.92	0.05	0.88
Retrieval ability	-0.12	0.76†	0.11	0.75
Cognitive speediness	-0.21	0.88†	0.20	0.83

\* Domain scores are expressed in z-scores.  
† Scores between boys and girls were significantly different, t test ( $P < 0.001$ ).

HAZ, WAZ, Hb and vitamin B<sub>12</sub> concentrations are shown in Fig. 2. HAZ scores were significantly positively related to all cognitive domains and MPI. WAZ were significantly positively associated with all cognitive parameters, except cognitive speediness. The associations of HAZ and WAZ would in theory mean that an increase of 1 SD in HAZ and WAZ would correspond with 0.09 SD increase in MPI,  $P = 0.0006$  and  $P = 0.002$ , respectively.

No significant relationships were detected between linoleic acid, arachidonic acid, EPA and DHA and any of the cognitive parameters. ALA was significantly inversely related to the MPI, but no significant associations were observed with the separate cognitive domains.

Hb concentrations were significantly positively related to all cognitive domains and MPI. Our findings suggest that an increase of 10 g/l in Hb concentration would translate into a 0.08 SD increase in MPI,  $P = 0.0008$ . There was a significant inverse association between sTfR concentrations and retrieval ability. Other indicators of Fe status were not significantly related to cognitive performance. Similarly, there were no significant associations between urinary iodine concentrations and cognitive parameters. In contrast, significantly inverse relationships were found between erythrocyte folate concentrations and fluid reasoning ( $\beta = -0.10$  (95% CI -0.19, -0.01) and short-term memory (-0.11 (95% CI -0.23, 0.02)). However, when vitamin B<sub>12</sub> status was added to the model, these inverse associations were not significant anymore for fluid reasoning (-0.07 (95% CI -0.17, 0.02) and for short-term memory (-0.08 (95% CI -0.21, 0.05)). Vitamin B<sub>12</sub> concentrations were significantly inverse related to short-term memory and retrieval ability and the MPI. These associations remained significant after further adjusting for Hb and folate status and HAZ ( $\beta$  (95% CI) were -0.19 (95% CI -0.36, -0.03) for short-term memory; -0.20 (95% CI -0.33, -0.08) for retrieval ability; -0.12 (95% CI -0.22, -0.02),  $P = 0.02$  for MPI).

**Discussion**

The present study shows that indicators of body size, HAZ and WAZ and Hb concentrations were significantly positively related to various cognitive domain scores and MPI, while plasma vitamin B<sub>12</sub> concentrations were significantly inversely associated with short-term memory and retrieval ability and MPI. Other indicators of Fe, folate, iodine and fatty acid status were not significantly related to cognitive performance.

**Table 3.** Overview of associations of nutritional factors with cognitive performance\*  
( $\beta$  coefficients and 95% confidence intervals)

	n	Mental processing index			Fluid reasoning			Short-term memory			Retrieval ability			Cognitive speediness		
		$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	$\beta$	95% CI	
Height-for-age z-score	570	0.085	0.037, 0.133	0.114	0.056, 0.172	0.079	-0.002, 0.160	0.061	0.000, 0.123	0.086	0.019, 0.152					
Weight-for-age z-score	570	0.091	0.033, 0.150	0.123	0.054, 0.193	0.115	0.018, 0.213	0.077	0.003, 0.152	0.049	-0.032, 0.130					
Linoleic acid††	513	0.007	-0.251, 0.265	0.200	-0.112, 0.511	-0.053	-0.484, 0.378	0.017	-0.304, 0.337	-0.133	-0.488, 0.222					
Arachidonic acid††	513	-0.053	-0.211, 0.105	0.047	-0.143, 0.237	-0.039	-0.267, 0.260	-0.067	-0.263, 0.129	-0.187	-0.343, 0.453					
$\alpha$ -Linolenic acid††	513	-0.097	-0.195, 0.000	-0.079	-0.196, 0.046	-0.124	-0.286, 0.038	-0.075	-0.196, 0.046	-0.112	-0.245, 0.021					
EPA††	513	-0.006	-0.122, 0.110	0.041	-0.099, 0.018	0.026	-0.168, 0.219	-0.087	-0.230, 0.057	-0.004	-0.164, 0.155					
DHA††	513	-0.061	-0.204, 0.083	-0.032	-0.204, 0.140	-0.010	-0.250, 0.230	-0.038	-0.216, 0.140	-0.164	-0.360, 0.032					
Blood Hb	570	0.007	0.003, 0.012	0.005	0.000, 0.010	0.009	0.002, 0.016	0.008	0.002, 0.013	0.008	-0.002, 0.014					
Serum ferritin†	507	0.031	-0.035, 0.096	0.020	-0.059, 0.099	0.040	-0.068, 0.149	0.034	-0.047, 0.115	0.028	-0.062, 0.118					
Serum soluble transferrin receptor†	510	-0.096	-0.258, 0.065	-0.106	-0.300, 0.088	-0.027	-0.241, 0.295	-0.295	-0.494, -0.097	-0.010	-0.232, 0.212					
Total body Fe stores	507	0.006	-0.009, 0.020	0.006	-0.011, 0.023	0.003	-0.021, 0.027	0.011	-0.007, 0.029	0.003	-0.017, 0.022					
Erythrocyte folate†	501	-0.038	-0.112, 0.036	-0.101	-0.191, -0.011	-0.108	-0.230, 0.015	0.053	-0.038, 0.144	0.003	-0.099, 0.105					
Plasma vitamin B <sub>12</sub> †	497	-0.124	-0.224, -0.023	-0.029	-0.151, 0.094	-0.212	-0.360, -0.044	-0.195	-0.319, -0.071	-0.058	-0.195, 0.079					
Urinary iodine†	515	-0.007	-0.057, 0.043	0.026	-0.035, 0.086	-0.044	-0.127, 0.039	-0.009	-0.054, 0.072	-0.020	-0.089, 0.049					

\* Using a model adjusted for age, sex, school, maternal education and assessor of cognitive tests. The  $R^2$  of the models with the different nutritional indicators ranged from 0.34 to 0.37 for MPI, 0.37 to 0.39 for fluid reasoning, 0.08 to 0.10 for short-term memory, 0.25 to 0.27 for retrieval ability and 0.34 to 0.36 for cognitive speediness.  
† Variables were normalised by natural logarithm transformation.  
‡ Fatty acids were measured in the erythrocyte membranes in the phospholipid fraction.

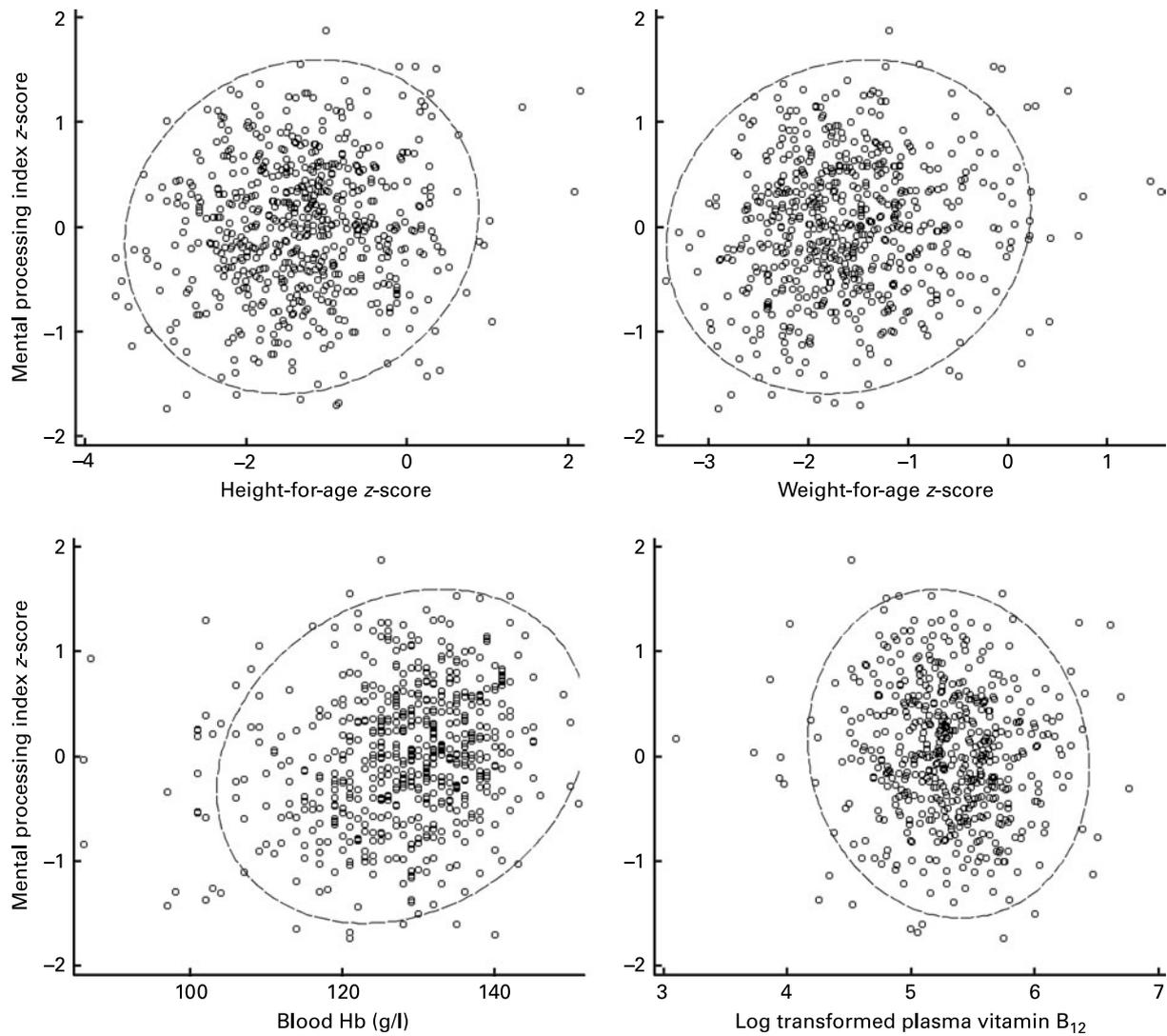


Fig. 2. Scatter plots of correlations between the mental processing index and height-for-age z-score ( $r$  0.10,  $P$ =0.012), weight-for-age z-score ( $r$  0.11,  $P$ =0.007), Hb concentration ( $r$  0.21,  $P$ <0.0001) and log-transformed vitamin B<sub>12</sub> ( $r$  0.09,  $P$ =0.046).

Strengths of this cross-sectional study were the availability of biochemical parameters of micronutrient and fatty acid status in a relatively large sample of >500 children from a low socio-economic background. The sample is representative for school children aged 6–10 years from poor socio-economic classes in Bangalore city and the surrounding peri-urban areas, based on a similar prevalence of anaemia measured and similar average heights and weights in our studies and other studies conducted in children in Bangalore<sup>(51)</sup>. The cognitive test battery was thoroughly adapted to local language and culture and showed good internal and external validity, which is essential to detect any associations between cognitive functioning and nutritional status<sup>(52)</sup>. In addition, we chose to assess the cognitive abilities that have been shown to be influenced by nutritional interventions before<sup>(53)</sup>.

A limitation of a cross-sectional study design is the inability for causal inference. Furthermore, the high number of comparisons made between nutritional and cognitive variables may have yielded false-positive findings (type I error).

However, we tried to limit the number of comparisons by the use of composite scores for the cognitive tests. In addition, we aimed to look for patterns among our findings, such as the consistent positive association of HAZ with all cognitive parameters. Another limitation of the study was the finding that our overall model explained only 10–40% of the variation in cognitive parameters. Genetic variation and environmental factors such as socio-emotional stimulation at home may account for this unexplained variation. In additional analyses we explored whether the interactions of age and sex with the nutritional indicators could explain any variation in cognitive test scores, but the results of these analyses did not yield further insights.

In agreement with our findings, lower HAZ, reflecting longer term undernutrition, has previously been associated with poorer cognitive performance in younger (1–3 years)<sup>(54–56)</sup> and school children<sup>(57–59)</sup>. Moreover, intervention studies have demonstrated that protein–energy supplementation in young children benefits cognitive development on the longer term<sup>(4,60,61)</sup> and therefore an

adequate intake of energy and protein is required for optimal development.

Erythrocyte fatty acid status was unrelated to cognitive performance, which is in line with findings from a cohort study in children aged 7 years<sup>(62)</sup>. Possibly, the range in fatty acid status among the children was too narrow to determine effects on cognition. It may also be that erythrocyte or plasma fatty acid status does not resemble brain fatty acid status at school age when most brain growth has been completed. A study in human subjects estimated that DHA requirements of the brain are rather low and the authors suggested that the liver may synthesise sufficient amounts of DHA to maintain brain DHA concentrations, provided that dietary intake of the precursor ALA is adequate<sup>(63)</sup>. Moreover, animal studies indicated that synthesis of DHA in the liver is enhanced and the turnover of DHA in the brain is reduced when diets were low in ALA and free of DHA<sup>(63)</sup>. Thus, intake and erythrocyte concentrations of *n*-3 fatty acids may not be related to brain function. Besides, there is some evidence that children with attention-deficit hyperactivity disorders have lower plasma-erythrocyte DHA and higher linoleic and arachidonic acid concentrations than control children<sup>(64–67)</sup>, which could be attributed to differences in fatty acid metabolism<sup>(68)</sup>. Therefore, more research is needed to investigate whether specific subgroups of children may be sensitive to fatty acid interventions and whether fatty acids may predominantly influence certain aspects of behaviour, such as attention.

For Hb, we showed a very small but significant positive relationship with mental performance. However, for the other parameters of Fe status, no such relationships could be detected. Possibly, this relationship becomes only apparent when Fe deficiency has caused anaemia, which was the case in only 6% of the present study population. This has also been reported in a review of literature of observational studies showing that (Fe deficient) anaemic children have poorer cognitive development and school performance than non-anaemic children, and it was concluded that it is unclear whether Fe deficiency without anaemia impairs mental performance<sup>(69)</sup>. In contrast, Fe supplementation has been shown to improve mental performance in children >2 years of age in (Fe deficient) anaemic as well as in non-anaemic children<sup>(20,21)</sup>, indicating that extra Fe may also be beneficial for development of non-anaemic children. The higher cognitive scores with increasing Hb concentrations found in the present study, suggest that the Hb level for optimal mental performance may be higher than the current definition of anaemia (<115 g/l).

Against our expectations, both folate and vitamin B<sub>12</sub> were inversely associated with some of the cognitive domain scores. For folate these inverse relationships disappeared after controlling for vitamin B<sub>12</sub> status, while for vitamin B<sub>12</sub> the inverse associations with memory remained significant even after controlling for folate, Hb and height-for-age. Our findings are in contrast with two earlier observational studies indicating that children with lower plasma vitamin B<sub>12</sub> concentrations had poorer cognitive test scores<sup>(24,25)</sup> and could be due to chance. In elderly, however, eight studies did not show significant associations between plasma vitamin B<sub>12</sub> and cognitive test performance<sup>(70)</sup> and one study showed an inverse relationship<sup>(71)</sup>. Our finding and the observations in elderly contradict to the overt clinical signs of vitamin

B<sub>12</sub> deficiency of neurological damage. Therefore, it has been questioned whether plasma vitamin B<sub>12</sub> is a suitable indicator to study effects on cognition<sup>(70,72)</sup>. It is of interest to investigate whether higher plasma homocysteine concentrations are related to poorer mental performance in children, as has been observed in elderly<sup>(70,71)</sup>. In both children and adults, plasma homocysteine concentrations are increasing when folate and vitamin B<sub>12</sub> intake are low<sup>(73)</sup> and elevated homocysteine may impair cognitive functioning through neurotoxic and vasotoxic effects<sup>(74)</sup>. Also other indicators of vitamin B<sub>12</sub> status, such as holotranscobalamin and methylmalonic acid may be worth evaluating in future research<sup>(72)</sup>.

In addition, we could speculate on other confounding factors that influence the relationship between higher plasma vitamin B<sub>12</sub> concentrations and poorer cognitive performance. Possibly, consumption of animal products infected with pathogens or vegetables contaminated with vitamin B<sub>12</sub>-producing bacteria from manure may improve vitamin B<sub>12</sub> status<sup>(75,76)</sup> and simultaneously increase the risk of disease, resulting in poor school attendance and impaired cognition. However, no literature is available to support this hypothesis.

Despite the evidence in literature that iodine deficiency is detrimental to cognitive development<sup>(77)</sup> and that iodine supplementation improves cognitive functioning in children<sup>(78)</sup>, we failed to detect any association between urinary iodine concentrations and cognition, which may be due to day-to-day within subject variation in iodine excretion in urine<sup>(49)</sup>.

In conclusion, findings of the present study are in agreement with other observational studies showing that undernutrition (lower HAZ and WAZ) and lower Hb concentrations adversely influence cognitive performance in school-age children, while serum ferritin and sTfR concentrations, and indicators of iodine, folate and fatty acid status were unrelated and an inverse association was found for vitamin B<sub>12</sub> and memory. Future research is needed to elucidate the role of B-vitamins and homocysteine in cognitive development of children and to investigate whether fatty acid status at school age may be of influence on specific cognitive functions not measured in the present study, such as attention.

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