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Vitamin B-12 Deficiency in Children Is Associated with Grade Repetition and School Absenteeism, Independent of Folate, Iron, Zinc, or Vitamin A Status Biomarkers[1–3]

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Abstract

Background: Micronutrients are essential to neurocognitive development; yet their role in educational outcomes is unclear.

Objective: We examined the associations of micronutrient status biomarkers with the risk of grade repetition and rates of school absenteeism in a cohort of school children.

Methods: We recruited 3156 children aged 5–12 y from public schools in Bogota, Colombia. Circulating ferritin, hemoglobin, zinc, vitamin A, and vitamin B-12; erythrocyte folate; and mean corpuscular volume (MCV) were measured in blood samples obtained at the beginning of the year. Absenteeism was recorded weekly during the school year, and grade repetition was determined the next year. Risk ratios for grade repetition and rate ratios for absenteeism were estimated by categories of micronutrient status indicators with use of Poisson regression, adjusting for potential confounders.

Results: The risk of grade repetition was 4.9%, and the absenteeism rate was 3.8 d per child-year of observation. Vitamin B-12 deficiency (<148 pmol/L) was associated with an adjusted 2.36-fold greater risk of grade repetition (95% CI: 1.03, 5.41; $P = 0.04$) compared with plasma concentrations ≥148 pmol/L. Other micronutrients were not related to grade repetition. Vitamin B-12 deficiency was also associated with school absenteeism rates. Compared with children with plasma vitamin B-12 concentrations ≥148 pmol/L, vitamin B-12-deficient children had a 1.89-times higher adjusted rate (95% CI: 1.53, 2.34; $P < 0.0001$). Anemia was related to a 72% higher rate (95% CI: 48%, 99%; $P < 0.0001$), whereas every 5-fL difference in MCV was associated with a 7% lower adjusted rate (95% CI: 4%, 10%; $P < 0.0001$).

Conclusions: Vitamin B-12 deficiency was associated with risk of grade repetition and school absenteeism rates in school children from Bogota, Colombia. The effects of correcting vitamin B-12 deficiency on educational outcomes and neurocognitive development of school children need to be determined in intervention studies. *J Nutr* 2015;145:1541–8.

Keywords: vitamin B-12, folate, iron, grade repetition, school absenteeism, school children

Introduction

Grade repetition and school absenteeism are adverse educational outcomes that affect a large number of school-age children worldwide. In 2010, 32.2 million primary school children repeated a school grade. Of them, 70% lived in Latin America and the Caribbean, South and West Asia, or sub-Saharan Africa (1). Grade repetition can lead to poor long-term educational achievements, stigma during adolescence (2), and school dropout (3). School absenteeism is also linked to school dropout (4) and poor economic attainment in adulthood (5).

The nutritional status of children is related to proximal causes of these outcomes, including neurocognitive function (6) and morbidity (7), yet, the link between nutritional exposures and grade repetition or school absenteeism remains poorly understood. Most literature on nutritional correlates of these outcomes has focused on protein-energy malnutrition as reflected in anthropometric status. Stunting is associated with increased risks of low academic performance and grade repetition (8). Obesity is also associated with increased risks of school absenteeism and poor school performance (9, 10). Whether these associations represent causal effects is a matter of debate.

Few studies have examined the impact of micronutrient status on educational outcomes, even though some vitamins and

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minerals are essential to neurocognitive development (6). A substantial body of literature exists on the effects of prenatal micronutrient status and neurocognitive performance and behavior later in life (11), yet, the role of vitamins and minerals during school-age is understudied (12). Iron supplementation to anemic school children has resulted in improved cognitive performance and intelligence scores (13). Vitamin B-12 intake of Kenyan school children was positively related to memory test scores in a prospective study (14), whereas in the cross-sectional National Health and Nutrition Examination Surveys in the United States serum folate but not vitamin B-12 concentrations were related to improved reading test scores in children aged 6–16 y (15). The role of micronutrient status on school absenteeism is similarly uncertain. Micronutrient status of school children is related to infectious morbidity (7), a leading cause of absenteeism (16), but it is unknown whether micronutrients are directly related to absenteeism. Micronutrient deficiencies in school-age children are highly prevalent in many settings. In Bogota, Colombia, 15% of school children have marginal vitamin B-12 status and another 2% are deficient (17); prevalences of vitamin A and iron deficiencies are 14% and 3%, respectively (18). Identifying the effects of micronutrient status during school age on educational outcomes is highly relevant because school-based supplementation policies could be inexpensive, highly cost effective, and logistically feasible.

We conducted a prospective study to investigate the associations of biomarkers of micronutrient status with grade repetition and school absenteeism among children from Bogota, Colombia. We hypothesized that micronutrient status would be inversely related to the incidence of these outcomes.

Methods

Study population. This study was part of the Bogota School Children Cohort, a longitudinal investigation of nutrition and health in school children. Details of the study design were previously published (19). In brief, in February 2006 we randomly sampled classrooms from all 361 public primary schools in Bogota from grades 0 to 5. A total of 3202 children aged 5–12 y from 2981 households were enrolled. Because most children in the public school system were from low- and middle-income families, the study population represented children from these socioeconomic strata.

Information on sociodemographic characteristics was obtained at baseline through parental self-administered questionnaires (82% response). We inquired about parental age and educational level, number of household assets, and food security by using a scale adapted from the Spanish-language version of the USDA Household Food Security Survey Module (20) and the Community Childhood Hunger Identification project (21). The scale was validated previously in this setting (22). During the next few weeks, trained research assistants visited the schools and obtained fasting blood samples and anthropometric measures from the children. Height was measured without shoes to the nearest 1 mm with wall-mounted portable Seca 202 stadiometers, and weight was measured in light clothing to the nearest 0.1 kg on Tanita HS301 solar-powered electronic scales according to standardized protocols (23). Mother's height and weight were measured in 33% and self-reported otherwise.

During the academic year after enrollment, parents or caregivers prospectively filled records of the days children were absent from school by using a diary that was distributed and returned on a weekly basis. One year after the enrollment, we collected information on grade repetition. According to the academic system prevalent in Bogota at the time, a child was not promoted to the next grade when she or he failed 3 subjects, 2 of which were math and language. We used public school listings of the 2007 academic year provided by the Secretary of Education to ascertain the school year's outcome. If children appeared in the same grade in 2007 as they were in 2006, they were considered to have failed. We found 2589 children in these listings. For children who had left the public school system, we asked parents about the outcome of the 2006 grade as part of the follow-up survey conducted in 2007 and obtained information in 245. Information was unavailable for 11% of cohort participants.

Written informed consent from the parents or primary caregivers of all children was obtained before enrollment. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School. The Institutional Review Board at the University of Michigan approved the use of data from the study.

Laboratory methods. Phlebotomists obtained a sample of venous blood from 88% of participating children after an overnight fast. Samples were collected in EDTA-coated tubes and transported the same day, on ice and protected from sunlight, to the National Institute of Health in Bogota, where biochemical analyses were performed according to methods described previously (24). Hemoglobin concentrations were quantified with a hemiglobincyanide method, whereas mean corpuscular volume (MCV) was determined as part of a complete blood count. Plasma retinol was measured with HPLC on a Waters 600 System. Serum zinc concentrations were determined in a separate aliquot that was collected in a metal-free polypropylene tube without anticoagulant, with the use of an atomic absorption technique (25) on a Shimadzu AA6300 spectrophotometer. Plasma ferritin and vitamin B-12 and erythrocyte folate were quantified with competitive chemiluminescent immunoassays in an ADVIA Centaur analyzer (Bayer Diagnostics). Serum C-reactive protein was measured with the use of a turbidimetric immunoassay on an ACS180 analyzer (Bayer Diagnostics).

Data analysis. We included in the analyses 3156 children who had information on grade repetition ($n = 2834$) or school absenteeism ($n = 3084$). Outcomes were risk of grade repetition and rates of school absenteeism. These rates were calculated as the number of days children were absent from school divided by the total number of observed days they should have attended, excluding weekends and holidays. One school year has ~200 d.

To identify potential confounders of the associations between micronutrient status and the outcomes of interest, we first estimated risk of grade repetition and rates of school absenteeism (days per child-years of observation) according to baseline child, maternal, and household characteristics. Children's height-for-age z scores and BMI-for-age z scores were calculated with the use of the WHO reference (26). Maternal BMI was calculated from height and weight as kg/m^2. Covariates were categorized as presented in **Table 1**.

Next, we calculated risk of grade repetition and rates of school absenteeism according to micronutrient status indicators. These analyses were restricted to children with information available on micronutrient biomarkers and either grade repetition ($n = 2587$) or absenteeism ($n = 2774$). MCV, plasma ferritin, serum zinc, and erythrocyte folate were categorized into quartiles of the study population distributions. Plasma retinol was categorized as <10.0 μg/dL (severely vitamin A deficient), 10.0–19.9 μg/dL (deficient), 20.0–29.9 μg/dL (low), or ≥30 μg/dL (adequate) (27), and vitamin B-12 was grouped as <148 pmol/L (deficient), 148–221 pmol/L (marginal deficient), or >221 pmol/L (adequate) (28). Relative risks for grade repetition and rate ratios for school absenteeism with 95% CIs were estimated by categories of micronutrient status predictors with the use of generalized estimating equations with a Poisson distribution and the log-link test. An exchangeable correlation matrix was used in all models to account for the clustering effect of the sampling strategy and for within-family correlations because some siblings were in the sample. Tests of linear trend for ordinal variables were conducted by introducing a continuous predictor that represented ordinal categories into the models. P values for trend were estimated from Wald tests. Adjusted estimates were obtained from multivariable models which included sociodemographic or nutritional indicators that were significantly associated with the outcomes in bivariate analyses or were relevant from a mechanistic viewpoint. Some children had missing information on sociodemographic variables, including father's education and food

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TABLE 1 Grade repetition and school absenteeism according to child, parental, and socioeconomic characteristics among children 5–12 y-old from public schools in Bogota, Colombia

	Grade repetition		Absenteeism		
	n[1]	Risk, %	n[1]	Months of follow-up	Days per child-year[2]
Child's sex					
Female	1440	3.4	1572	3734	3.9
Male	1394	6.5	1512	3431	3.6
P[3]		0.0002			0.34
Child's age, y					
5–6	546	4.2	583	1355	4.2
7–8	884	4.3	980	2280	3.9
9–10	1148	4.8	1238	2895	3.5
11–12	254	9.5	279	632	3.8
P-trend		0.03			0.14
Child's school grade					
0	228	2.2	247	564	4.9
1	304	6.6	324	727	3.7
2	341	5.9	402	921	4.5
3	615	5.0	666	1531	3.5
4	694	5.6	755	1749	3.9
5	652	3.8	690	1672	3.1
P-trend		0.79			0.001
Height-for-age z score[4]					
<-2.0	280	5.7	304	664	4.0
-2.0 to <-1.0	863	6.5	939	2137	3.8
-1.0 to <1.0	1517	3.8	1643	3939	3.6
≥ 1	106	5.7	111	270	3.8
P-trend		0.07			0.41
BMI-for-age z score[4]					
<-2.0	37	5.4	42	104	3.9
-2.0 to <-1.0	301	5.0	318	783	3.6
-1.0 to <1.0	1913	4.6	2084	4781	3.7
1.0 to <2.0	396	6.8	419	1033	4.0
≥ 2.0	116	3.5	130	301	3.5
P-trend		0.65			0.76
Television watching, h/wk					
<10.0	724	4.7	773	1994	3.5
10.0–19.9	581	4.5	617	1596	4.0
20.0–29.9	453	5.7	480	1215	3.7
≥ 30	336	3.3	354	874	3.9
P-trend		0.65			0.45
Time playing outdoors, h/wk					
<1.5	441	3.9	469	1257	4.3
1.5–4.4	513	4.7	555	1422	3.3
4.5–9.9	506	3.6	534	1362	4.1
≥ 10	510	5.5	543	1330	3.9
P-trend		0.38			0.79
Mother's age, y					
20–29	568	4.2	623	1505	3.7
30–34	651	4.6	703	1716	3.7
35–39	573	4.4	596	1563	3.7
≥ 40	609	5.8	640	1677	3.9
P-trend		0.25			0.64
Mother's education					
Incomplete primary (1–4 y)	199	7.5	208	494	3.7
Complete primary (5 y)	458	3.9	500	1246	3.4
Incomplete secondary (6–10 y)	614	5.5	660	1629	3.6
Complete secondary (11 y)	969	4.0	1021	2649	3.9
University (≥ 12 y)	166	4.2	177	453	4.5
P-trend		0.20			0.11

(Continued)

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TABLE 1 *Continued*

	Grade repetition		Absenteeism		
	n[1]	Risk, %	n[1]	Months of follow-up	Days per child-year[2]
Father's education					
Incomplete primary (1–4 y)	173	7.5	186	462	3.3
Complete primary (5 y)	474	6.1	506	1244	3.3
Incomplete secondary (6–10 y)	509	5.1	538	1364	3.9
Complete secondary (11 y)	836	3.7	891	2261	3.9
University (≥12 y)	172	1.7	181	481	4.7
P-trend		0.0009			0.02
Mother's height, cm					
<154.0	568	5.6	613	1550	3.7
154.0–157.9	606	5.0	651	1615	3.6
158.0–161.9	550	5.1	576	1483	3.7
≥162.0	602	3.2	640	1594	4.2
P-trend		0.05			0.26
Mother's BMI, kg/m^2					
<18.5	78	3.9	89	213	5.6
18.5–24.9	1449	4.6	1537	3900	3.8
25.0–29.9	583	4.6	622	1555	3.7
≥30.0	163	4.9	176	453	3.7
P-trend		0.76			0.20
Household's assets[5]					
0–1	201	8.5	223	504	3.7
2	299	5.0	329	772	4.0
3	389	3.9	415	1052	4.1
4	481	3.7	509	1287	3.7
5	535	4.3	568	1471	3.6
6	523	4.6	553	1453	3.7
P-trend		0.21			0.39
Food insecurity					
Secure	551	3.6	587	1522	3.5
Insecure, no hunger	1105	3.8	1172	3011	3.4
Insecure, moderate hunger	433	6.5	466	1156	4.8
Insecure, severe hunger	271	5.9	294	672	3.8
P-trend		0.03			0.02
Absenteeism, % absent days/child					
0	1541	5.3			
>0–5.0	278	1.8			
>5–10.0	334	3.6			
>10–20.0	312	3.2			
>20.0	297	8.4			
P-trend		0.53			

[1] Totals may be <2834 (grade repetition) or <3084 (absenteeism) because of missing values.
[2] Rates are estimated as the number of days absent from school of the total school days observed. An academic year is 40 wk of 5 school days each, for a total of 200 school days.
[3] Wald test from Poisson regression models.
[4] According to the WHO growth reference for children and adolescents (26).
[5] Sum of household assets from a list that included bicycle, refrigerator, blender, television, stereo, and washing machine.

insecurity. Values for these missing covariates were estimated with multiple imputation by using a Markov Chain Monte Carlo method (29) before their inclusion in the models. Child's sex and age, parental education, food insecurity, socioeconomic status indicators, concentrations of all micronutrient status biomarkers, and the index outcome were included in the imputation procedures. Ten cycles of imputation were completed to estimate values for the missing covariates. Results of the imputation strategy were essentially unchanged in sensitivity analyses that included changing the order of imputed variables and adding 2-factor interactions.

In supplemental analyses, we estimated relative risks for grade repetition and rate ratios for absenteeism in relation to nutrient biomarker concentrations as continuous variables with use of restricted cubic splines (30), to account for potential nonlinearity of the associations. Each of these Poisson models included as predictors spline terms for the nutrient in addition to other adjustment covariates, with an exchangeable correlation matrix. Relative risks and rate ratios were estimated in reference to the mean biomarker concentration in the population. Multiple imputation methods were used to estimate values for missing covariates, as described in the previous paragraph.

Finally, because studies in other age groups have found negative interactions between vitamin B-12 and folate on neurocognitive function (31, 32), we a priori decided to estimate risks of grade repetition and rates of school absenteeism by categories of both vitamin B-12 and erythrocyte folate concentrations simultaneously. *P* values for interaction were obtained with the use of Wald tests. All analyses were

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performed with the use of the Statistical Analyses System version 9.3 (SAS Institute Inc.).

Results

Mean age of children at recruitment was 8.7 y; 51.1% were girls. Overall, the risk of grade repetition was 4.9%. Male sex, age, and food insecurity were positively associated with grade repetition (Table 1). Conversely, father's education and mothers' height were inversely related to grade repetition. The overall absenteeism rate was 3.8 d per child-year over 218,065 child-days of observation. Absenteeism was inversely related to school grade and positively associated with father's education and food insecurity (Table 1).

In bivariate analyses, the risk of grade repetition was inversely related to vitamin B-12 status (**Table 2**). Compared with children with plasma vitamin B-12 ≥148 pmol/L, vitamin B-12–deficient children had a 3.4 times higher risk (95% CI: 1.6, 7.4; $P = 0.002$) of grade repetition. Absenteeism was inversely related to hemoglobin, MCV, and plasma vitamin B-12 concentrations (Table 2).

In multivariable analyses, grade repetition was related to vitamin B-12 deficiency. After adjusting for child's sex and age and father's education, the risk of grade repetition was 2.36

TABLE 2 Grade repetition and school absenteeism according to micronutrient status indicators among children 5–12 y-old from public schools in Bogota, Colombia[1]

	Grade repetition		Absenteeism		
	n	Risk, %	*n*	Months of follow-up	Days per child-year[2]
Hemoglobin,[3] g/dL					
<12.7	94	5.3	102	251	5.6
≥12.7	2490	4.8	2668	6597	3.7
P[4]		0.80			0.02
Mean corpuscular volume, fL					
Q1 (median = 81)	582	5.7	635	1510	4.2
Q2 (median = 84)	689	4.8	739	1837	3.8
Q3 (median = 87)	658	4.4	705	1798	3.8
Q4 (median = 93)	553	4.9	581	1391	3.1
P-trend		0.50			0.02
Ferritin, μg/L					
Q1 (median = 21.5)	643	4.2	686	1672	3.8
Q2 (median = 32.1)	642	3.4	690	1655	3.8
Q3 (median = 43.8)	645	5.6	689	1746	4.1
Q4 (median = 65.1)	639	6.0	688	1736	3.3
P-trend[5]		0.06			0.39
Serum zinc, μg/dL					
Q1 (median = 15.5)	640	4.8	688	1709	3.9
Q2 (median = 18.6)	641	4.7	689	1698	3.7
Q3 (median = 21.9)	641	4.4	686	1649	3.4
Q4 (median = 29.7)	641	5.5	687	1743	4.0
P-trend		0.73			0.89
Plasma retinol, μg/dL					
<10	33	9.1	34	93	3.8
10–19.9	320	4.1	339	853	3.9
20–29.9	1084	4.7	1170	2884	3.7
≥30	1146	4.9	1225	3017	3.8
P-trend[5]		0.92			0.90
Erythrocyte folate, nmol/L					
Q1 (median = 620)	623	5.6	667	1660	3.7
Q2 (median = 761)	623	4.2	669	1635	3.8
Q3 (median = 893)	622	4.3	668	1675	3.8
Q4 (median = 1101)	625	5.3	667	1610	3.5
P-trend		0.84			0.77
Plasma vitamin B-12, pmol/L					
<148	37	16.2	43	81	7.1
148–221	383	5.7	401	926	3.6
>221	2074	4.5	2231	5591	3.8
P-trend		0.14			0.33

[1] Q, quartile.
[2] Rates are estimated as the number of days absent from school out of the total school days observed. An academic year is 40 wk of 5 school days each, for a total of 200 school days.
[3] Categorized according to the altitude-adjusted cutoff for anemia.
[4] Wald test from Poisson regression models.
[5] The models included log serum C-reactive protein concentration as an adjustment variable.

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times higher (95% CI: 1.03, 5.41; $P = 0.04$) for children with vitamin B-12 deficiency than for children with plasma vitamin B-12 ≥148 pmol/L (**Table 3**). In analyses of vitamin B-12 as a continuous predictor, the risk increased exponentially as vitamin B-12 values decreased under the population mean (**Supplemental Figure 1**A). Absenteeism was inversely associated with MCV and hemoglobin and vitamin B-12 concentrations (Table 3). Vitamin B-12–deficient children had a 1.89 times higher adjusted rate of absenteeism (95% CI: 1.53, 2.34; $P < 0.0001$) than children with plasma vitamin B-12 ≥148 pmol/L. When vitamin B-12 was considered as a continuous predictor, absenteeism rates increased linearly at vitamin B-12 values <240 pmol/L (Supplemental Figure 1B). Anemia was related to a 72% increased absenteeism rate (95% CI: 48%, 99%; $P < 0.0001$), whereas every 5-fL difference in MCV (~1 SD) was associated with a 7% decreased rate (95% CI: 4%, 10%; $P < 0.0001$). Introducing additional micronutrient biomarkers into the models as predictors did not change the results; therefore, they were excluded for parsimony.

The interactions between vitamin B-12 and erythrocyte folate concentrations on the risk of grade repetition or the rates of absenteeism were not statistically significant ($P = 0.98$ and $P = 0.65$, respectively; data available on request).

Discussion

In this prospective study, we examined the relations between micronutrient status biomarkers and educational outcomes in a representative sample of low- and middle-income children from Bogota, Colombia. Vitamin B-12 deficiency was strongly related to increased risk of grade repetition and school absenteeism. These associations were independent of other micronutrient status indicators and sociodemographic characteristics. Hemoglobin and MCV were inversely related to school absenteeism, whereas food insecurity with hunger was positively associated with it.

Although little is known on the impact of micronutrient status on educational outcomes, a number of studies have examined their role on endpoints closely related to academic performance, including neurocognitive development and behavior. Methyl-donor nutrients, including vitamin B-12 and folate, are involved in the development and normal functioning of the central nervous system through various mechanisms, including production of neurotransmitters, synaptic plasticity, and dendritic arborization and myelination. Some (14, 33, 34) but not all (15, 35, 36) observational studies have found positive relations between vitamin B-12 status and cognitive or behavioral test scores in children. For example, vitamin B-12 intake was positively associated with memory in a prospective study of Kenyan school children (14), and vitamin B-12 serostatus was directly related to intelligence, reasoning, and memory in Guatemalan school-age children (33). These constructs are predictive of academic performance (37, 38), and a vitamin B-12 effect on them could mediate the strong association we found with grade repetition. Another mechanism could be related to school absenteeism. In our study, vitamin B-12–deficient children had twice the rate of absenteeism than did marginally deficient or nondeficient children. Absenteeism can be the result of infectious morbidities, and vitamin B-12 plays important roles on immunologic pathways (39) that may be necessary to control common respiratory and gastrointestinal infections during school years. Nevertheless, vitamin B-12 was not associated with morbidity in these children (7), and absenteeism did not seem related to grade repetition in this population.

An alternative path to explain an effect of vitamin B-12 deficiency on school absenteeism is through behavior and attitudes toward school. Emerging evidence from observational studies in adults has linked low vitamin B-12 intake with depression and mood disorders (40, 41). In a recent intervention study among depressed adults with marginally deficient vitamin B-12 serostatus (140–200 pmol/L), weekly administration of 1 mg of intramuscular vitamin B-12 as co-adjuvant of antidepressive therapy resulted in greater improvements in a depression score scale after 6 wk compared with the administration of antidepressants only (42). Early studies had also suggested an antidepressant role for S-adenosylmethionine (43, 44), a methyl donor whose synthesis depends on vitamin B-12. It might be conceivable that vitamin B-12 deficiency could affect motivation to attend school through depression, anxiety, or other related behavioral alterations. However, for children of school age the evidence on the potential role of vitamin B-12 on these disorders is limited to 3 cross-sectional surveys that evaluated the associations of vitamin B-12 from diet and outcomes including withdrawal/depression or aggressive/delinquent behavior (45) and the presence of depressive symptoms (46, 47). Although none of these studies found a link between vitamin B-12 status and these disorders, reverse causation bias, confounding, or measurement error could explain the findings.

TABLE 3 Multivariable-adjusted RRs of grade repetition and IRRs of school absenteeism among children from public schools in Bogota, Colombia[1]

	Grade repetition, RR (95% CI) (n = 2587)	School absenteeism, IRR (95% CI) (n = 2774)
Sex, male vs. female	1.76 (1.21, 2.58)	0.91 (0.86, 0.98)
Child's age, vs. 5–6 y		
7–8	1.00 (0.57, 1.75)	0.98 (0.89, 1.07)
9–10	1.10 (0.65, 1.86)	0.89 (0.81, 0.98)
11–12	2.00 (1.08, 3.69)	0.97 (0.85, 1.10)
Father's education, per year	0.93 (0.88, 0.99)	1.02 (1.01, 1.03)
Vitamin B-12, <148 pmol/L vs. ≥148 pmol/L	2.36 (1.03, 5.41)	1.89 (1.53, 2.34)
Food insecurity, hunger vs. no hunger/secure		1.28 (1.18, 1.40)
Hemoglobin, <12.7 g/dL		1.72 (1.48, 1.99)
Mean corpuscular volume, per 1 SD (5 fL)		0.93 (0.90, 0.96)

[1] RRs and IRRs were computed from Poisson regression models by using an exchangeable correlation matrix to account for within-family correlations. Each model included as predictors all variables for which estimates are presented. Multiple imputed values were used for missing data on sociodemographic variables. IRR, incidence rate ratio; RR, risk ratio.

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Longitudinal investigations of behavioral and mood disorders of children in relation to vitamin B-12 status by using valid biomarkers of intake are warranted. We had previously reported that a school snack program resulted in improved plasma vitamin B-12 concentrations and reduced absenteeism (24). It is plausible that the program effect on absenteeism may have been mediated in part through improved vitamin B-12 status.

Although many studies have reported positive relations of folate status with neurocognitive development of children (36, 48–53), we did not find any associations with the educational outcomes examined. Several explanations to this discrepancy are possible. First, erythrocyte folate concentrations in our population were high, possibly owing to a flour fortification policy in place since the late 1990s. Second, in many of the prior studies that reported positive associations, folate status was measured in the mothers during pregnancy; its effects on child's cognition may be specific to the prenatal period. Third, there could be noncausal explanations to the findings from previous observational studies, which would be consistent with the lack of effect reported in randomized intervention studies (54, 55).

Iron status indicators were not significantly related to grade repetition. This may be because the prevalence of iron deficiency in the cohort was low, ~3% (18). A recent meta-analyses of iron supplementation trials in school-age children found that positive effects on global cognitive performance and intelligence were restricted to children who were anemic (13). Of note, hemoglobin and MCV were inversely related to school absenteeism. We have recently reported that hemoglobin concentrations in this cohort were inversely associated with ear infections and doctor visits (7), both of which may result in absenteeism.

Our study had several strengths. We used a prospective study design with a large and representative sample of school-age children. Thus, we were able to minimize reverse causation bias. We used biomarkers of intake for several micronutrients as the main exposures, which prevents recall bias. Outcome misclassification was also reduced through prospective data collection because we did not rely on memory to obtain information on grade repetition or school absenteeism. We carefully accounted for missing data in multivariable analyses with the use of multiple imputation techniques. There are also some limitations. Noncausal explanations of the findings are possible because of the observational nature of the study. Vitamin B-12 status in this population is strongly related to socioeconomic conditions (17) and parental education (56). Although we adjusted for these variables in the analysis, it is not possible to completely rule out residual confounding. Second, the use of plasma vitamin B-12 as a proxy of vitamin B-12 status is a limitation in that this measurement represents both vitamin B-12 intake and stores and not necessarily the vitamin available to tissues. Thus, low values may not capture developing deficiencies; instead, they might be indicative of long-term low intake or absorption abnormalities, whereas concentrations of vitamin B-12 metabolites, including homocysteine, methylmalonic acid, or holotranscobalamin, may be more sensitive indicators of deficiency. Financial constraints prevented us from measuring additional biomarkers in this large cohort. Third, outcome measures were unavailable for a group of cohort participants, and selection bias is possible if the lack of outcome data were related to exposure status. Last, generalizability is limited to low- and middle-income children because those from higher socioeconomic strata were not included.

In conclusion, vitamin B-12 deficiency was strongly associated with risk of grade repetition and school absenteeism. Hemoglobin and MCV were inversely related to school absenteeism. Whether correction of vitamin B-12 deficiency through supplementation, food fortification, or inclusion of animal food sources in school feeding programs results in improved educational outcomes among school-age children warrants investigation in randomized intervention studies.

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EV designed the research; MM-P and CM conducted the research; M-CD and EV analyzed the data; M-CD and EV wrote the paper and have primary responsibility for final content. All authors read and approved the final manuscript.

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