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Iron Deficiency, Anemia, and Low Vitamin B-12 Serostatus in Middle Childhood Are Associated with Behavior Problems in Adolescent Boys: Results from the Bogotá School Children Cohort

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Abstract

Background: Iron deficiency (ID) in infancy is related to subsequent behavior problems. The effects of micronutrient status in middle childhood are uncertain.

Objective: The aim of the study was to examine the associations of micronutrient status biomarkers in middle childhood with externalizing and internalizing behavior problems in adolescence.

Methods: We assessed whether ID (ferritin <15 µg/L), anemia (hemoglobin <12.7 g/dL), or blood concentrations of zinc, vitamins A and B-12, and folate at ages 5–12 y were associated with externalizing or internalizing behavior problems in adolescence in 1042 schoolchildren from Bogotá, Colombia. Behavior problems were assessed with the Youth Self-Report questionnaire after a median 6.2 y of follow-up. Mean problem score differences with 95% CIs were estimated between categories of micronutrient status biomarkers with the use of multivariable linear regression.

Results: Mean ± SD externalizing and internalizing problems scores were 52.6 ± 9.6 and 53.8 ± 9.9, respectively. Among boys, middle-childhood ID, anemia, and low plasma vitamin B-12 were associated with 5.9 (95% CI: 1.0, 10.7), 6.6 (95% CI: 1.9, 11.3), and 2.7 (95% CI: 0.4, 4.9) units higher mean externalizing problems scores in adolescence, respectively—after adjustment for baseline age, time spent watching television or playing video games, mother's height, and socioeconomic status. Also in boys, ID was related to an adjusted 6.4 (95% CI: 1.2, 11.6) units higher mean internalizing problems score. There were no associations among girls. Other micronutrient status biomarkers were not associated with behavior problems.

Conclusions: ID, anemia, and low vitamin B-12 in middle childhood are related to behavior problems in adolescent boys. This study was registered at clinicaltrials.gov as NCT03297970. *J Nutr* 2018;148:760–770.

Keywords: iron deficiency, anemia, vitamin B-12, externalizing behavior problems, internalizing behavior problems, middle childhood, adolescence

Introduction

Mental health problems affect 10–20% of children and adolescents worldwide (1) and are associated with adverse health outcomes in the short and long term (2). Among these problems, externalizing and internalizing behavior disorders, including conduct, attention-deficit hyperactivity, depressive, and anxiety disorders, pose a particularly hefty burden accounting for >100 million disability-adjusted life years globally (3). These disorders are at the extreme end of a spectrum of more subtle, yet also highly relevant, behavior problems that are predictive of impaired mental (4) and physical (5) health in adulthood.

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Abbreviations used: ADM, Assessment Data Manager; ID, iron deficiency; SES, socioeconomic status; YSR, youth self-report.

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Nutrition plays an important role in the development of behavior from infancy through adolescence (6), but the effects of individual nutrients throughout the life cycle are not well characterized. Most research has focused on micronutrient status in infancy. For example, iron deficiency (ID) in infancy is associated with lower positive affect in infancy (7) and middle childhood (8), externalizing (9–11) and internalizing (9, 10, 12) behavior problems in adolescence, and lower self-rated emotional health in young adulthood (13). Nevertheless, the effects of exposure to iron or other micronutrient deficiencies later in childhood have not been studied in prospective investigations, to our knowledge. Structural changes in areas of the brain that may be important in the development of behavior problems, including the basal ganglia, hippocampus, amygdala, and prefrontal cortex, occur throughout childhood (14). Rodent experiments indicate that exposure to gestational, perinatal, and post-weaning iron deficiency (15–17), post-weaning zinc (18) or vitamin A (19) deficiencies, low gestational vitamin B-12 (20), or gestational folate deficiency (21) can disrupt the normal development of these regions, but data in humans are scant.

The objective of this study was to investigate the associations between micronutrient status in middle childhood and externalizing and internalizing behavior problems in adolescence in a cohort of schoolchildren from Bogotá, Colombia. We hypothesized that low concentrations of micronutrient status biomarkers (ferritin, hemoglobin, zinc, vitamins A and B-12, and folate) in middle childhood would be related to increased externalizing and internalizing problems in adolescence.

Methods

Study design and population. We conducted a prospective study in the context of the Bogotá School Children Cohort, a longitudinal investigation of nutrition and health in Bogotá, Colombia. Details on the cohort design have been previously reported (22). Briefly, in February 2006, we recruited 3202 randomly selected children aged 5–12 y from primary public schools. A majority of children in the public school system in Bogotá are from low- and middle-income socioeconomic backgrounds. Therefore, our sample pertains to these groups.

Baseline information. At the time of enrollment we collected information on child, parental, and household characteristics with the use of a survey that was sent to the children's homes. The questionnaire inquired about children's background and habits, including the time usually spent watching television and playing video games or playing outdoors. The survey also included questions on parental age, marital status, and education level and on maternal parity, height, and weight. Household characteristics involved the local government's socioeconomic status (SES) classification and the level of food insecurity according to a validated version of the USDA Household Food Security Survey module (23).

During the weeks after enrollment, trained research assistants scheduled data and sample collection school visits, after contacting the primary caregivers requesting that the child fast overnight before the visit day. At these visits, height was measured without shoes to the nearest 1 mm with the use of a wall-mounted portable Seca 202 stadiometer (Seca, Hanover, MD) and weight was measured in light clothing to the nearest 0.1 kg with the use of Tanita H5301 electronic scales (Tanita, Arlington Heights, IL). Height and weight were also measured among the children's mothers who were present at schools (37%). At the same visits, the research assistants obtained fasting blood samples through antecubital venipuncture in 88% of the children. Twelve percent of children were unwilling to provide a blood sample. One aliquot was collected in an EDTA-coated tube and a second one in a metal-free polypropylene tube without anticoagulant for separation of serum. The samples were protected from sunlight and transported in refrigerated coolers on the day of collection to the Colombian National Institute of Health, where they were processed and cryostored for future analyses.

Follow-up. Between 2011 and 2015 we conducted an in-person follow-up assessment in a random sample of approximately one-third of cohort members (n = 1139). Adolescents were assessed at school, or at home if absent from school. At this assessment, we ascertained child behavior with the use of the Spanish-language version of the Youth Self-Report (YSR) questionnaire (24), a widely used method to assess behavioral and emotional problems in adolescents. The YSR is a self-administered questionnaire consisting of 112 statements addressing behaviors or feelings that children rate as false, sometimes true, or very or often true. From responses to these questions, an Assessment Data Manager (ADM) software (25) calculates continuous scores for 8 behavior problem subscales: aggressive behavior, rule breaking behavior, anxious or depressed, withdrawn or depressed, somatic complaints, attention problems, social problems, and thought problems. The sum of the aggressive and rule breaking behavior subscale scores constitutes the total externalizing problems score, whereas the sum of anxious or depressed, withdrawn or depressed, and somatic complaints scores comprises the total internalizing problems score (26). The ADM software standardizes the scores by age and sex to a reference population derived from data collected periodically in US national surveys (25). The YSR has been validated for use in adolescents ages 11–18 y from English-speaking populations (27), has high reliability (24), and is generalizable to Spanish-speaking populations (28). It has been utilized in studies of Chilean (12), Costa Rican (9), and Puerto Rican (24) adolescents. A general questionnaire was also administered to primary caregivers at the follow-up visit to update information on mother's marital status, education, parity, and BMI, and household food security and SES.

The parents or primary caregivers of all children gave written informed consent prior to enrollment into the study and before the follow-up assessment. Children gave written assent to participate. 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. All analyses took place at the Colombian National Institute of Health. Plasma ferritin concentration was measured with the use of a competitive chemiluminescent immunoassay in an ADVIA Centaur analyzer (BayerDiagnostics, Tarrytown, NY). Serum C-reactive protein (CRP) concentration was measured with the use of a turbidimetric immunoassay on an ACS180 analyzer (Bayer Diagnostics, Tarrytown, NY). Hemoglobin concentrations were determined by the hemiglobincyanide method. Serum zinc concentrations were determined with the use of an atomic absorption technique (29) on a Shimadzu AA6300 spectrophotometer. Plasma retinol was measured with HPLC on a Waters 600 System. Plasma vitamin B-12 and erythrocyte folate were also quantified with the use of a competitive chemiluminescent immunoassay in an ADVIA Centaur analyzer.

Data analysis. The YSR was completed by 1097 of the 1139 cohort members who participated in the follow-up assessment; 13 forms could not be processed by the ADM software due to an excess of missing values. Forty-two children who were aged <11 y or >18 y were excluded from the analysis, because the YSR was developed and validated for use in 11–18-y-olds (30); thus, the final sample consisted of 1042 children. The sample size had been calculated to provide >85% statistical power to detect differences in mean behavior scores >10% between extreme quartiles of exposure, assuming a type I error of 5% and mean ± SD scores of 50.0 ± 10.0 in the unexposed. Power would be >95% to detect linear trends in multivariable analyses with as many as 10 covariates. Compared with cohort participants who were not included in the analysis, children in the analytic sample spent more time watching television or playing video games, had better-educated mothers, were of higher SES, and the boys had lower prevalence of anemia at baseline (**Table 1**). They did not differ with regard to the distributions of other micronutrient status biomarkers.

TABLE 1 Sociodemographic characteristics in middle childhood among children included vs. not included in the analysis[1]

Characteristic	Boys: Included ($n = 458$)	Boys: Not included ($n = 1109$)	Girls: Included ($n = 584$)	Girls: Not included ($n = 1051$)
Child's age at baseline, y	8.4 ± 1.6	8.8 ± 1.9	8.5 ± 1.7	8.9 ± 1.8
Height-for-age *z* score[2] at baseline	−0.77 ± 0.94	−0.82 ± 0.95	−0.76 ± 0.97	−0.75 ± 1.05
BMI-for-age *z* score[2] at baseline	0.22 ± 1.09	0.20 ± 1.05	0.07 ± 0.96	0.09 ± 0.92
Time spent watching television or playing video games, h/wk	21.8 ± 17.9	18.0 ± 14.9	20.8 ± 17.6	18.4 ± 14.1
Time playing outdoors, h/wk	8.4 ± 9.1	8.5 ± 10.1	6.7 ± 8.9	7.4 ± 9.8
Mother's education, y	9.2 ± 3.2	8.5 ± 3.3	8.8 ± 3.3	8.5 ± 3.5
Mother's parity	2.7 ± 1.1	2.7 ± 1.1	2.7 ± 1.1	2.8 ± 1.1
Mother's height, cm	157.6 ± 6.5	157.8 ± 6.3	157.7 ± 6.3	157.9 ± 6.5
Mother's BMI, kg/m^2	24.1 ± 3.8	24.2 ± 3.9	24.1 ± 3.6	24.0 ± 3.7
Food insecure, %	74.0	75.1	76.8	76.4
Socioeconomic status, %				
1	5.9	6.9	5.7	6.8
2	29.9	34.6	30.8	33.0
3	56.3	51.4	56.9	53.0
4	7.9	7.0	6.7	7.3
Plasma ferritin, μg/L	41.9 ± 22.9	41.1 ± 23.5	43.4 ± 24.1	42.8 ± 22.7
Iron deficiency,[3] %	3.4	3.2	3.0	3.3
Hemoglobin, g/dL	14.5 ± 1.3	14.5 ± 1.2	14.6 ± 1.1	14.5 ± 1.1
Anemia,[4] %	2.4	4.8	3.5	3.1
Serum zinc, μmol/L	21.7 ± 6.7	21.4 ± 6.3	21.3 ± 6.4	21.4 ± 6.1
Vitamin A, μg/dL	29.3 ± 10.2	29.9 ± 9.8	29.4 ± 9.8	29.9 ± 10.0
Plasma vitamin B-12, pmol/L	322 ± 104	317 ± 103	339 ± 105	333 ± 110
Erythrocyte folate, nmol/L	861 ± 223	875 ± 294	822 ± 227	857 ± 239

[1]Values are means ± SDs unless noted otherwise.
[2]According to the WHO growth reference for children and adolescents (34).
[3]Plasma ferritin concentration <15 μg/L. 47 children with C-reactive protein >10 mg/L were excluded from the analysis.
[4]Hemoglobin <12.7 g/dL.

The primary outcomes of interest were the continuous distributions of total externalizing and internalizing problems scores. Secondary endpoints were the subscales of these composite scores: aggressive and rule breaking behavior for externalizing problems and anxious or depressed, withdrawn or depressed, and somatic complaints for internalizing problems. In supplemental analyses we considered the attention, social, and thought problems subscales.

We considered as exposures biomarkers for micronutrients that are relevant to neurobehavioral development. These included iron, zinc, vitamin A, vitamin B-12, and folate. ID was defined as plasma ferritin <15 μg/L (31). Thirteen children with serum CRP >10 mg/L were excluded from the analysis of ID. Anemia was defined as hemoglobin <12.7 g/dL after adjustment for altitude (32). Vitamin A status was categorized as deficient (plasma retinol <20 μg/dL), low (20 to <30 μg/dL), or adequate (≥30 μg/dL) (33). Serum zinc, plasma vitamin B-12, and erythrocyte folate were categorized into sex-specific quartiles since the prevalence of these micronutrient deficiencies according to conventional cutoffs was low (<2%).

Covariates included sociodemographic, anthropometric, and health-related characteristics measured at baseline. Children's height- and BMI-for-age *z* scores were calculated according to the WHO growth reference for children and adolescents (34). Maternal BMI was calculated as kg/m^2 from objectively measured height and weight in 37% of mothers and from self-reported data in the rest. Correlations between objectively measured and reported values were 0.79 ($P < 0.0001$) for height and 0.81 ($P < 0.0001$) for BMI. Covariates were categorized as presented in **Table 2**.

All analyses were conducted separately by sex. First, to identify independent predictors of the outcomes, we compared the distributions of total externalizing and internalizing problems scores across categories of baseline characteristics using means ± SDs. Next, we examined the distributions of these outcomes by concentrations of micronutrient status indicators. For ordinal exposures in which concentrations have a hierarchical relation with each other, we conducted tests for linear trend by fitting linear regression models with the behavior problems scale as the outcome and a variable representing ordinal categories of each predictor as a continuous covariate. This is a conventional method to examine linearity of associations for nutritional exposures that are categorized into quantiles (35). For ID and anemia, we used the χ^2 score statistic. In all models, an independent correlation matrix was used to account for clustering by sibship, since there were 107 siblings in the sample. Empirical estimates of the variance were specified to overcome potential deviations from the multivariate normality assumption. We estimated mean adjusted differences and 95% CIs for total externalizing or internalizing problems scores between categories of sociodemographic and nutritional predictors with the use of multivariable linear regression. In each model, adjustment variables included independent predictors of the outcome. Child's age at baseline was included in all final models as it was considered important from a mechanistic viewpoint. Other covariates were retained in the final model when they remained statistically significant ($P < 0.05$). We examined the associations of micronutrient status biomarkers with scores on each subscale following an analogous approach.

To further understand if changes in sociodemographic factors from middle childhood through adolescence that could represent underlying changes in micronutrient status were related to the outcomes, we examined differences in total externalizing and internalizing problems by changes in maternal marital status, education, parity, and BMI, and household food security and SES.

All analyses were performed with the use of the Statistical Analyses System version 9.4 (SAS Institute Inc., Cary, NC).

Results

Mean ± SD age at enrollment was 8.5 ± 1.6 y; 56.1% of children were girls. Prevalence of ID, anemia, and vitamin A

TABLE 2 Sociodemographic characteristics in middle childhood and total externalizing problems score at 11–18 y in the Bogotá School Children Cohort

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	n	Mean ± SD
Overall	458	51.9 ± 9.6	584	53.1 ± 9.6
Child's age at baseline, y				
5–6	100	50.3 ± 11.7	122	49.5 ± 10.7
7–8	168	51.9 ± 8.7	216	52.8 ± 9.8
9–10	176	53.0 ± 9.0	221	54.9 ± 8.2
11–12	14	50.4 ± 9.2	25	56.8 ± 8.1
P-trend[2]		0.10		<0.0001
Child's age at assessment, y				
<12	41	50.1 ± 12.0	47	47.3 ± 10.7
12–13	103	48.7 ± 10.2	137	50.1 ± 10.6
14–15	195	53.0 ± 8.8	248	54.4 ± 8.8
>15	119	53.4 ± 8.8	152	55.3 ± 8.1
P-trend		0.001		<0.0001
Height-for-age *z* score[3] at baseline				
<−2.0	40	51.3 ± 11.0	58	52.1 ± 9.2
−2.0 to <−1.0	145	51.9 ± 9.0	186	53.1 ± 9.4
−1.0 to <0.0	166	52.4 ± 10.1	210	52.6 ± 9.7
≥0.0	92	51.7 ± 9.2	122	54.3 ± 10.1
P-trend		0.83		0.22
BMI-for-age *z* score[3] at baseline				
<−1.0	58	50.9 ± 8.8	76	51.5 ± 9.4
−1.0 to <0.0	137	52.3 ± 9.3	200	52.9 ± 9.7
0.0 to <1.0	152	52.6 ± 10.6	202	53.7 ± 9.5
≥1.0	95	51.1 ± 9.2	97	53.2 ± 9.9
P-trend		0.99		0.19
Time spent watching television or playing video games, h/wk				
<10	124	50.5 ± 9.6	155	52.2 ± 9.1
10 to <20	108	52.4 ± 9.9	171	52.6 ± 10.1
20 to <30	110	50.9 ± 9.0	125	53.2 ± 9.5
≥30	104	54.2 ± 9.6	116	54.9 ± 9.4
P-trend		0.02		0.02
Time playing outdoors, h/wk				
<1.5	53	53.7 ± 10.8	109	53.4 ± 9.8
1.5 to <4.5	82	50.1 ± 9.4	113	53.4 ± 9.7
4.5 to <10	97	50.6 ± 8.4	94	52.1 ± 9.9
≥10	96	52.5 ± 10.1	86	52.7 ± 9.4
P-trend		0.94		0.44
Mother's education, y				
Incomplete primary, 1–4	24	53.7 ± 9.2	34	52.7 ± 8.2
Complete primary, 5	76	51.1 ± 10.3	109	54.4 ± 9.0
Incomplete secondary, 6–10	114	52.0 ± 9.0	135	52.5 ± 9.5
Complete secondary, 11	190	51.8 ± 10.1	242	53.1 ± 10.1
University, >11	42	51.9 ± 8.2	41	51.8 ± 9.4
P-trend		0.89		0.31
Mother's parity				
1	47	50.7 ± 11.2	69	52.8 ± 9.7
2	182	51.4 ± 9.7	198	52.5 ± 9.7
3	132	52.2 ± 9.6	184	53.4 ± 9.8
4	45	51.4 ± 5.6	66	53.2 ± 9.4
≥5	40	54.8 ± 10.4	50	54.1 ± 8.2
P-trend		0.08		0.26

(*Continued*)

TABLE 2 *Continued*

Characteristic	Boys		Girls	
	n[1]	Mean ± SD	n	Mean ± SD
Mother's height quartile (median, cm)				
1 (150)	113	50.7 ± 9.6	140	54.4 ± 10.4
2 (155)	121	52.5 ± 9.3	160	53.1 ± 9.7
3 (160)	101	52.0 ± 9.8	126	53.4 ± 9.3
4 (165)	113	52.3 ± 9.8	144	51.5 ± 8.8
P-trend		0.31		0.02
Mother's BMI, kg/m^2				
<18.5	19	54.4 ± 11.0	16	50.6 ± 7.4
18.5 to <25.0	261	52.2 ± 9.5	364	52.9 ± 9.9
25.0 to <30.0	135	50.8 ± 9.4	146	53.3 ± 8.9
≥30.0	30	52.9 ± 10.8	39	55.1 ± 9.5
P-trend		0.36		0.12
Food insecurity in the household				
None	119	50.6 ± 9.4	135	53.8 ± 9.5
Insecure—no hunger	222	52.5 ± 9.5	283	52.6 ± 9.9
Insecure—moderate hunger	78	51.8 ± 10.4	98	54.1 ± 8.9
Insecure—severe hunger	38	52.9 ± 9.1	66	52.0 ± 9.4
P-trend		0.21		0.53
Socioeconomic status				
1 (lowest)	27	52.1 ± 11.8	33	47.6 ± 10.0
2	137	53.0 ± 9.0	180	54.5 ± 9.1
3	258	51.4 ± 9.7	332	53.0 ± 9.7
4	36	51.1 ± 9.7	39	51.4 ± 8.9
P-trend		0.24		0.83

[1] Sums may be less than the total due to missing values in covariates.
[2] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous covariate. Empirical estimates of the variance were used in all models.
[3] According to the WHO growth reference for children and adolescents (34).

deficiency was 3.2%, 3.0%, and 14.8%, respectively. None of the anemic children had ID, 32.1% of the anemic children had vitamin A deficiency, and 14.3% of the nonanemic children had vitamin A deficiency. Among children with and without ID, the prevalence of vitamin A deficiency was 13.8% and 14.9%, respectively. Mean ± SD age at the time of follow-up assessment was 14.7 ± 1.7 y. Children were followed for a median of 6.2 y.

Total externalizing problems. Mean ± SD total externalizing problems scores were 51.9 ± 9.6 in boys and 53.1 ± 9.6 in girls. In bivariate analysis, age at follow-up assessment and time spent watching television or playing video games at baseline were positively associated with total externalizing problems scores in boys and girls (Table 2). In girls, baseline age was positively related to total externalizing problems scores, whereas maternal height and low SES were inversely associated with this outcome (Table 2). ID, anemia, and low vitamin B-12 concentrations were each related to higher total externalizing problems scores among boys (**Table 3**). In girls, anemia was related to lower total externalizing problems scores.

In multivariable analysis, ID, anemia, and low vitamin B-12 serostatus were positively associated with total externalizing problems scores in boys after adjustment for age at baseline, time spent watching television or playing video games, maternal height, and SES. Boys with ID had 5.9 units (95% CI: 1.0, 10.7 units) higher mean total externalizing problems scores

TABLE 3 Micronutrient status in middle childhood and total externalizing problems score at 11–18 y in the Bogotá School Children Cohort

	Boys		Girls	
Micronutrient status indicator	n[1]	Mean ± SD	n	Mean ± SD
Iron deficiency[2]				
Yes	14	57.3 ± 8.2	15	52.1 ± 9.9
No	400	51.9 ± 9.7	489	53.1 ± 9.6
P[3]		0.01		0.71
Anemia[4]				
Yes	10	57.5 ± 6.8	18	48.1 ± 9.2
No	413	51.9 ± 9.7	498	53.4 ± 9.6
P		0.008		0.01
Serum zinc quartile (median boys/girls, μmol/L)				
1 (15.3/15.1)	106	51.7 ± 10.2	127	53.6 ± 10.0
2 (18.6/18.2)	104	51.9 ± 9.4	128	53.8 ± 9.1
3 (22.1/22.3)	104	51.8 ± 10.1	127	52.1 ± 10.2
4 (30.7/29.6)	107	53.0 ± 9.0	128	53.1 ± 9.2
P-trend[5]		0.37		0.40
Vitamin A, μg/dL				
<20	63	53.5 ± 10.6	76	52.7 ± 10.4
20–29.9	188	51.9 ± 9.3	211	52.1 ± 10.0
≥30	173	51.7 ± 9.6	228	54.2 ± 8.8
P-trend		0.31		0.07
Plasma vitamin B-12 quartile (median boys/girls, pmol/L)				
1 (204/218)	105	54.2 ± 9.4	123	53.5 ± 10.0
2 (278/303)	100	51.2 ± 10.0	122	53.6 ± 9.9
3 (345/363)	104	52.0 ± 9.8	123	52.4 ± 9.1
4 (450/452)	103	51.4 ± 9.2	124	53.4 ± 9.2
P-trend		0.06		0.68
Erythrocyte folate quartile (median boys/girls, nmol/L)				
1 (633/573)	100	51.4 ± 8.3	123	53.5 ± 9.5
2 (759/735)	102	51.5 ± 10.6	124	52.1 ± 10.0
3 (898/874)	101	53.0 ± 10.0	124	53.4 ± 9.8
4 (1122/1062)	101	51.9 ± 9.5	124	53.8 ± 9.4
P-trend		0.46		0.60

[1] Sums may be less than the total due to missing values in covariates.
[2] Plasma ferritin concentration <15 μg/L. 13 children with C-reactive protein >10 mg/L were excluded from the analysis.
[3] From linear regression with externalizing problems score as the continuous outcome and the nutrient biomarker as the categorical predictor. Empirical estimates of the variance were used in all models.
[4] Hemoglobin <12.7 g/dL.
[5] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

compared with iron-sufficient boys (**Table 4**). Compared with nonanemic boys, boys with anemia had an adjusted 6.6 units (95% CI: 1.9, 11.3 units) higher total externalizing problems score. Boys with plasma vitamin B-12 in the lowest quartile had adjusted mean total externalizing problems scores 2.7 units (95% CI: 0.4, 4.9) higher than did boys with higher concentrations. Anemia was not associated with total externalizing problems in girls after adjustment for these covariates.

Externalizing problems subscales. The distributions of aggressive and rule breaking behavior subscale scores varied significantly by sociodemographic characteristics and micronutrient status biomarkers (**Supplemental Table 1**). In multivariable analysis among boys, vitamin B-12 concentrations in the lowest quartile were related to a 2.3 units higher mean aggressive behavior score (95% CI: 0.4, 4.3 units) compared with higher concentrations (**Supplemental Figure 1**). Among girls, anemia was associated with a 2.5 units lower mean rule breaking behavior score (95% CI: −3.7, −1.3 units) (Supplemental Figure 1).

Total internalizing problems. In boys and girls, mean total internalizing problems scores were 53.4 ± 9.7 and 54.1 ± 10.1, respectively. In bivariate analysis among girls, baseline age, BMI-for-age *z* score, and maternal BMI were positively related to total internalizing problems scores, whereas maternal education, severe food insecurity, and low SES were inversely related to this outcome (**Table 5**). ID among boys and vitamin A status

TABLE 4 Adjusted mean differences and 95% CIs in total externalizing problems score at 11–18 y according to iron deficiency, anemia, and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort[1]

	Boys	Girls
	Difference (95% CI)	Difference (95% CI)
Iron deficiency,[2] yes vs. no	5.9 (1.0, 10.7)	0.0 (−4.4, 4.4)
Anemia,[3] yes vs. no	6.6 (1.9, 11.3)	−3.3 (−7.7, 1.1)
Plasma vitamin B-12, quartile 1 vs. >1	2.7 (0.4, 4.9)	1.0 (−0.8, 2.9)
Child's age at baseline, per 1 y	0.7 (0.0, 1.4)	1.4 (0.9, 1.9)
Time spent watching television/playing video games, ≥30 h/wk vs. <30 h/wk	3.7 (1.0, 6.4)	1.8 (−0.3, 3.9)
Mother's height quartile (median, cm)		
1 (150)	−1.2 (−4.0, 1.6)	2.8 (0.4, 5.2)
2 (155)	1.0 (−1.8, 3.7)	1.1 (−1.1, 3.3)
3 (160)	−0.4 (−3.5, 2.6)	1.0 (−1.3, 3.3)
4 (165)	Reference	Reference
P-trend[4]	0.64	0.03
Socioeconomic status, 1 (lowest) vs. >1	−1.3 (−6.8, 4.2)	−5.5 (−9.8, −1.1)

[1]Adjusted mean difference and 95% CI from a linear regression model with total externalizing problems score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.
[2]Plasma ferritin concentration <15 μg/L. 13 children with C-reactive protein >10 mg/L were excluded from the analysis.
[3]Hemoglobin <12.7 g/dL.
[4]Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

among girls were positively associated with total internalizing problems scores (Table 6).

In multivariable analysis, ID was related to total internalizing problems scores in boys. After adjustment for child's age and BMI-for-age *z* score, maternal education, and household food insecurity and SES, boys with ID had 6.4 units (95% CI: 1.2, 11.6 units) higher mean total internalizing problems scores than did iron-sufficient boys (Table 7). Vitamin A status was not significantly associated with total internalizing problems scores in girls after adjustment for these covariates.

Internalizing problems subscales. The distributions of anxious or depressed, withdrawn or depressed, and somatic complaints subscale scores varied by sociodemographic characteristics and micronutrient status biomarkers (**Supplemental Table 2**). In multivariable analyses among boys, anxious and depressed scores were positively related to ID and anemia (**Supplemental Figure 2**). Among girls, ID was inversely related to withdrawn and depressed scores (Supplemental Figure 2). Somatic complaints scores were positively associated with ID, anemia, and vitamin B-12 concentrations in the lowest quartile among boys (Supplemental Figure 2).

Attention, social, and thought problems subscales. None of the nutritional status biomarkers examined were associated with attention or social problems scores. Thought problems scores among boys were positively related to ID and anemia (**Supplemental Figure 3**).

Changes in sociodemographic factors. Single motherhood, maternal education level, parity, and BMI, and the percentage of households with food security were higher at the follow-up assessment during adolescence than at recruitment in middle childhood, whereas the percentage of households in the lowest socioeconomic strata did not change (**Supplemental Table 3**). None of the changes in sociodemographic characteristics from middle childhood to adolescence was related to total externalizing or internalizing problems scores among boys or girls (**Supplemental Table 4**).

Discussion

In this longitudinal study of low- and middle-income Colombian schoolchildren, ID, anemia, and low plasma vitamin B-12 in middle childhood were associated with increased total externalizing behavior problems scores in adolescence among boys. In addition, boys with ID in middle childhood had higher total internalizing problems scores in adolescence than did iron-sufficient boys. These associations were independent of other micronutrient status indicators and child, parental, and household characteristics. After adjustment for potential confounding variables, there were no statistically significant associations between the micronutrients examined and total externalizing or internalizing problems scores in girls.

The nutritional causes of behavior problems in adolescence are poorly understood. Previous longitudinal studies primarily focused on the effects of early-life iron status on cognitive and behavioral development. ID in infancy was related to total externalizing (9) and internalizing problems (9, 10) during early adolescence in Costa Rica. In Chile, iron deficiency anemia in infancy was associated with more rule breaking behavior, an externalizing problem, at 15 y of age (11), whereas ID was related to total internalizing problems at age 10 y (12). However, the potential effect of exposure to ID during middle childhood on subsequent behavior problems was not investigated. The results of our study may not be comparable with those from previous investigations, because the mechanisms operating in infancy could differ from those in middle childhood. The mechanisms through which ID in infancy may result in behavior problems during adolescence could involve developmental alterations in myelination throughout the brain (16) as well as diminished hippocampal oligodendrocyte function and dendritic arborization (15), according to rodent studies. Further, ID is associated

TABLE 5 Sociodemographic characteristics in middle childhood and total internalizing problems score at 11–18 y in the Bogotá School Children Cohort

	Boys		Girls	
Characteristic	*n*[1]	Mean ± SD	*n*	Mean ± SD
Overall	458	53.4 ± 9.7	584	54.1 ± 10.1
Child's age at baseline, y				
5–6	100	53.2 ± 11.0	122	51.6 ± 10.9
7–8	168	53.4 ± 9.7	216	54.2 ± 10.3
9–10	176	53.8 ± 8.8	221	54.9 ± 9.1
11–12	14	49.0 ± 8.4	25	58.9 ± 10.1
P-trend[2]		0.88		0.0005
Child's age at assessment, y				
<12	41	54.0 ± 11.7	47	51.4 ± 10.7
12–13	103	51.2 ± 9.8	137	50.9 ± 10.5
14–15	195	53.8 ± 9.5	248	55.3 ± 9.4
>15	119	54.5 ± 9.0	152	56.0 ± 9.8
P-trend		0.12		<0.0001
Height-for-age *z* score[3] at baseline				
<−2.0	40	52.4 ± 10.5	58	52.8 ± 9.6
−2.0 to <−1.0	145	53.4 ± 9.8	186	53.5 ± 10.1
−1.0 to <0.0	166	53.1 ± 9.9	210	54.7 ± 10.3
≥0.0	92	54.6 ± 8.7	122	54.8 ± 10.1
P-trend		0.28		0.10
BMI-for-age *z* score[3] at baseline				
<−1.0	58	53.0 ± 9.9	76	51.5 ± 9.1
−1.0 to <0.0	137	53.9 ± 9.4	200	53.8 ± 10.6
0.0 to <1.0	152	53.0 ± 10.2	202	55.4 ± 9.5
≥1.0	95	53.5 ± 9.2	97	54.4 ± 10.9
P-trend		0.95		0.02
Time spent watching television or playing video games, h/wk				
<10	124	54.3 ± 8.9	155	53.5 ± 8.9
10 to <20	108	52.2 ± 9.7	171	53.7 ± 10.7
20 to <30	110	53.3 ± 10.6	125	53.7 ± 10.7
≥30	104	53.9 ± 9.4	116	55.5 ± 9.5
P-trend		0.94		0.12
Time playing outdoors, h/wk				
<1.5	53	53.1 ± 9.4	109	55.7 ± 9.7
1.5 to <4.5	82	53.3 ± 10.6	113	53.0 ± 10.4
4.5 to <10	97	54.0 ± 8.4	94	53.5 ± 10.0
≥10	96	52.3 ± 9.9	86	53.3 ± 10.3
P-trend		0.64		0.14
Mother's education, y				
Incomplete primary, 1–4	24	55.4 ± 9.2	34	55.8 ± 10.3
Complete primary, 5	76	52.6 ± 9.3	109	55.1 ± 9.9
Incomplete secondary, 6–10	114	53.7 ± 9.1	135	54.5 ± 9.9
Complete secondary, 11	190	53.5 ± 10.5	242	53.7 ± 10.4
University, >11	42	53.1 ± 8.5	41	51.9 ± 8.4
P-trend		0.78		0.04
Mother's parity				
1	47	54.5 ± 10.7	69	55.2 ± 10.3
2	182	53.3 ± 9.6	198	53.5 ± 10.4
3	132	53.7 ± 9.9	184	54.4 ± 9.9
4	45	51.9 ± 8.0	66	55.3 ± 9.9
≥5	40	53.8 ± 10.1	50	53.4 ± 9.3
P-trend		0.59		0.99
Mother's height quartile (median, cm)				
1 (150)	113	53.4 ± 9.7	140	55.1 ± 11.3
2 (155)	121	54.4 ± 10.1	160	53.4 ± 10.4
3 (160)	101	53.2 ± 9.1	126	54.9 ± 9.1
4 (165)	113	53.0 ± 9.8	144	53.0 ± 9.3
P-trend		0.54		0.20
Mother's BMI, kg/m^2				
<18.5	19	52.9 ± 12.9	16	51.3 ± 6.5
18.5 to <25.0	261	53.5 ± 10.0	364	53.8 ± 10.5
25.0 to <30.0	135	53.4 ± 8.9	146	54.1 ± 9.4
≥30.0	30	54.4 ± 8.4	39	57.4 ± 9.7
P-trend		0.68		0.03
Food insecurity in the household				
None	119	52.4 ± 9.8	135	55.3 ± 10.5
Insecure—no hunger	222	53.7 ± 9.5	283	54.0 ± 10.3
Insecure—moderate hunger	78	54.1 ± 10.4	98	54.9 ± 8.8
Insecure—severe hunger	38	52.9 ± 8.9	66	51.5 ± 9.9
P-trend		0.45		0.04
Socioeconomic status				
1 (lowest)	27	55.1 ± 11.1	33	50.7 ± 9.1
2	137	54.2 ± 10.7	180	54.4 ± 10.0
3	258	52.9 ± 9.1	332	54.2 ± 10.3
4	36	52.7 ± 8.7	39	55.4 ± 8.7
P-trend		0.16		0.15

(*Continued*)

TABLE 5 *Continued*

[1] Sums may be less than the total due to missing values in covariates.
[2] Test for linear trend when a variable representing ordinal categories of the characteristic was introduced into a linear regression model as a continuous covariate. Empirical estimates of the variance were used in all models.
[3] According to the WHO growth reference for children and adolescents (34).

with lower D1 and D2 receptor densities (17), elevated concentrations of extracellular dopamine (36), and lower dopamine transporter density (37) in the basal ganglia. Dopamine is essential in the regulation of emotion, reward, motivation, and motor control; thus, dopaminergic dysfunction may be associated with behavior problems. In addition, ID may alter the metabolism of serotonin, norepinephrine, and γ-aminobutyric acid, which could relate to emotional or behavioral development (38). Whether the same mechanisms could explain the effects of exposure to ID in middle childhood is speculative. Iron gradually concentrates in the basal ganglia (38); activity in this region during a cognitive task in children and adolescents aged 6–20 y is associated with future working memory capacity, a marker of cognitive and behavioral development (39). It is also possible that ID measured in middle childhood was already present in infancy. In this case, our findings could represent the cumulative effect of ID on behavioral development. Of note, ID in middle childhood was associated with externalizing and internalizing problems in boys only. Some rodent studies suggest that males may be more sensitive to the effects of ID than females (17, 40); however, previous epidemiologic studies did not examine sex-specific associations.

Anemia in middle childhood was also associated with higher total externalizing problems scores in adolescence among boys. In this population, anemia was not due to ID and was generally uncorrelated with biomarkers of micronutrients that are relevant for hemoglobin metabolism, including zinc, folate, and vitamins B-12 and A (22). Other causes of anemia, such as

TABLE 6 Micronutrient status in middle childhood and total internalizing problems score at 11–18 y in the Bogotá School Children Cohort

	Boys		Girls	
Micronutrient status indicator	*n*[1]	Mean ± SD	*n*	Mean ± SD
Iron deficiency[2]				
Yes	14	60.1 ± 9.3	15	53.6 ± 7.9
No	400	53.3 ± 9.6	489	54.3 ± 10.1
P[3]		0.005		0.74
Anemia[4]				
Yes	10	58.0 ± 11.6	18	52.1 ± 10.2
No	413	53.3 ± 9.7	498	54.4 ± 10.0
P		0.18		0.32
Serum zinc quartile (median boys/girls, μmol/L)				
1 (15.3/15.1)	106	54.1 ± 8.7	127	54.8 ± 10.1
2 (18.6/18.2)	104	52.0 ± 10.1	128	54.4 ± 9.5
3 (22.1/22.3)	104	53.9 ± 10.3	127	54.3 ± 10.9
4 (30.7/29.6)	107	53.9 ± 9.6	128	53.7 ± 9.7
P-trend[5]		0.72		0.41
Vitamin A, μg/dL				
<20	63	55.3 ± 10.0	76	53.3 ± 9.7
20–29.9	188	53.3 ± 9.9	211	53.6 ± 11.0
≥30	173	52.9 ± 9.4	228	55.3 ± 9.2
P-trend		0.14		0.05
Plasma vitamin B-12 quartile (median boys/girls, pmol/L)				
1 (204/218)	105	54.3 ± 10.4	123	54.4 ± 9.5
2 (278/303)	100	52.3 ± 9.3	122	55.0 ± 10.1
3 (345/363)	104	53.5 ± 9.8	123	52.9 ± 11.2
4 (450/452)	103	53.8 ± 9.5	124	55.1 ± 8.8
P-trend		0.95		0.98
Erythrocyte folate quartile (median boys/girls, nmol/L)				
1 (633/573)	100	52.2 ± 9.2	123	54.3 ± 10.7
2 (759/735)	102	53.8 ± 9.9	124	53.4 ± 9.6
3 (898/874)	101	53.9 ± 9.5	124	55.2 ± 10.1
4 (1122/1062)	101	54.0 ± 10.0	124	54.6 ± 9.6
P-trend		0.20		0.54

[1] Sums may be less than the total due to missing values in covariates.
[2] Plasma ferritin concentration <15 μg/L. 13 children with C-reactive protein >10 mg/L were excluded from the analysis.
[3] From linear regression with internalizing problems score as the continuous outcome and the nutrient biomarker as the categorical predictor. Empirical estimates of the variance were used in all models.
[4] Hemoglobin <12.7 g/dL.
[5] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

parasitic infections, sickle cell disease, and thalassemia, are relatively infrequent in children from Bogotá. Thus, the nature of the association between anemia and externalizing behavior problems is uncertain. It could reflect low intake of nutrients we did not measure, such as riboflavin or vitamin C.

Among boys, low plasma vitamin B-12 was associated with higher externalizing problems scores, possibly due to increased aggressive behavior. Growing evidence suggests that vitamin B-12 status is associated with cognition in childhood (41). However, evidence related to potential effects on behavior is limited. Three cross-sectional studies examined the association between vitamin B-12 intake and externalizing and internalizing problems (42) or depressive symptoms (43, 44) in adolescence. None found an association, which could be due to lack of variability in the exposure, reverse causation bias, measurement error, or confounding. The mechanisms to explain a potential effect of vitamin B-12 on behavior could be related to its role in the metabolism of *S*-adenosylmethionine, a methyl donor involved in the synthesis of dopamine, serotonin, and norepinephrine. *S*-adenosylmethionine may improve mood among adults with depressive disorders (45), especially in males.

Although the micronutrient status biomarkers studied were not related to the primary outcomes in girls, anemia and ID were related to decreased scores in the rule breaking behavior and withdrawn or depressed subscales, respectively. In rodent models, ID among females was associated with higher serotonin transporter density in several brain regions whereas among males ID was related to lower serotonin transporter density (40). This may help explain why anemia would be associated

TABLE 7 Adjusted mean differences and 95% CIs in total internalizing problems score at 11–18 y according to iron deficiency and sociodemographic characteristics in middle childhood in the Bogotá School Children Cohort[1]

	Boys	Girls
	Difference (95% CI)	Difference (95% CI)
Iron deficiency,[2] yes vs. no	6.4 (1.2, 11.6)	0.9 (−2.6, 4.4)
Child's age at baseline, per 1 y	0.1 (−0.6, 0.7)	1.1 (0.5, 1.7)
BMI-for-age *z* score[3] at baseline, <-1.0 vs. ≥-1.0	−0.7 (−3.8, 2.4)	−3.7 (−6.0, −1.4)
Mother's education, y		
Incomplete primary, 1–4	Reference	Reference
Complete primary, 5	−2.3 (−6.7, 2.2)	−1.9 (−5.8, 2.0)
Incomplete secondary, 6–10	−1.5 (−5.9, 2.8)	−1.9 (−5.7, 1.9)
Complete secondary, 11	−1.8 (−6.1, 2.5)	−3.7 (−7.4, 0.0)
University, >11	−2.3 (−7.2, 2.7)	−6.3 (−10.9, −1.6)
P-trend[4]	0.70	0.005
Food insecurity, severe hunger vs. no severe hunger	−1.2 (−4.2, 1.8)	−3.3 (−6.0, −0.6)
Socioeconomic status, 1 (lowest) vs. >1	1.9 (−3.2, 7.0)	−3.6 (−7.2, −0.1)

[1] Adjusted mean difference and 95% CI from a linear regression model with total internalizing problems score as the continuous outcome. Predictors included all variables presented. Empirical variances were specified.
[2] Plasma ferritin concentration <15 μg/L. 13 children with C-reactive protein >10 mg/L were excluded from the analysis.
[3] According to the WHO growth reference for children and adolescents (34).
[4] Test for linear trend when a variable representing ordinal categories of the predictor was introduced into the model as a continuous covariate.

with higher behavior problems in boys but lower behavior problems in girls.

Family-level SES indicators, including mother's height, mother's education, and household food security, were inversely related to internalizing problems among girls. Low maternal education level and household food insecurity may impact parental mental and physical health (46, 47), whereas mother's height is a marker of intergenerational SES (48). Parental wellbeing and SES have previously been associated with the development of behavior problems (49). On the other hand, household SES, as measured through a neighborhood-level indicator, was positively associated with externalizing and internalizing problems in girls. The positive association with neighborhood SES is contrary to results from studies in the US (50) and the Netherlands (51). In Colombia, neighborhoods with low SES may have higher social cohesion which can modify the association between neighborhood SES and behavior problems (52). Changes in sociodemographic factors that could represent underlying changes in micronutrient status from middle childhood to adolescence were unrelated to the outcomes of interest. This suggests that a potential effect of micronutrients in middle childhood might be independent of these factors.

This study has several strengths. First, its longitudinal nature minimizes the potential for reverse causation bias. Prospective collection of outcome information reduces misclassification. We used objectively measured biomarkers of micronutrient status as the exposures, which precludes recall bias. The YSR questionnaire is valid in populations similar to ours (28). Finally, we controlled for many potential confounders of the association between micronutrient status and behavior problems.

There are limitations as well. First, we did not have a baseline measurement of behavior problems in middle childhood. If behavior problems in adolescence were already present at the time of exposure assessment, reverse causation cannot be disregarded as an explanation of some of these results. Second, we lacked an assessment of micronutrient status during adolescence. If micronutrient status in middle childhood was correlated with that during adolescence, then the results may reflect exposure in adolescence rather than exposure in middle childhood. Third, some of the biomarkers we used may result in misclassification of micronutrient status. For example, plasma vitamin B-12 represents both intake and stores of the vitamin, not necessarily the vitamin available in tissues. Since plasma vitamin B-12 concentrations are maintained while depletion occurs in the tissues, low plasma vitamin B-12 may not capture tissue deficiencies. Further, low concentrations may reflect long-term marginal intake or absorption abnormalities rather than deficiency (53). Concentrations of vitamin B-12 metabolites, methylmalonic acid, or homocysteine may be more sensitive to capturing deficiency than plasma vitamin B-12 concentrations alone (53), but we did not have the means to quantify these analytes. Fourth, boys included in the analysis were less likely to be anemic than those who were not included. If the anemic children who were not included had less total externalizing problems than those included, we may have overestimated the association of anemia with externalizing behavior problems. Fifth, not all children had an available measurement for all micronutrients examined. Sixth, unmeasured independent predictors of behavior problems that may be associated with micronutrient status indicators, such as blood lead concentrations, history of child behavior problems, history of parental mental health conditions, or exposure to violence, could have resulted in residual confounding. Another limitation is that we did not have objectively measured anthropometric data in all mothers. Measurement error could have obscured the associations of these covariates with the outcomes. The prevalences of ID and anemia are low, which limits the public health significance of our findings since improvement of these conditions would only benefit a few children. Finally, our results might not be generalizable to other populations, especially high-income children. Nevertheless, in a recent national nutrition survey, prevalences of ID, anemia, and vitamin B-12 deficiency among children of the highest socioeconomic strata were comparable to those of children in our study (54).

In conclusion, ID in middle childhood was strongly related to both externalizing and internalizing behavior problems in male adolescents. Anemia and low vitamin B-12 serostatus in boys were each related to increased externalizing behavior problems. Intervention studies are warranted to test whether improving the status of these micronutrients in middle childhood enhances behavioral development throughout adolescence.

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