The Journal of Nutrition
Nutritional Epidemiology

Vitamin A Deficiency Is Associated with Gastrointestinal and Respiratory Morbidity in School-Age Children[1–3]

Kathryn A. Thornton,[4] Mercedes Mora-Plazas,[5] Constanza Marín,[5] and Eduardo Villamor[6]*

[4]Department of Epidemiology and Biostatistics, Michigan State University, East Lansing, MI; [5]Foundation for Research in Nutrition and Health (FINUSAD), Bogotá, Colombia; and [6]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, MI

Abstract

Infection is an important cause of morbidity throughout childhood. Poor micronutrient status is a risk factor for infection-related morbidity in young children, but it is not clear whether these associations persist during school-age years. We examined the relation between blood concentrations of micronutrient status biomarkers and risk of gastrointestinal and respiratory morbidity in a prospective study of 2774 children aged 5–12 y from public schools in Bogotá, Colombia. Retinol, zinc, ferritin, mean corpuscular volume, hemoglobin, erythrocyte folate, and vitamin B-12 concentrations were measured in blood at enrollment into the cohort. Children were followed for 1 academic year for incidence of morbidity, including diarrhea with vomiting, cough with fever, earache or ear discharge with fever, and doctor visits. Compared with adequate vitamin A status (≥30.0 μg/dL), vitamin A deficiency (<10.0 μg/dL) was associated with increased risk of diarrhea with vomiting [unadjusted incidence rate ratio (IRR): 2.17; 95% CI: 0.95, 4.96; *P*-trend = 0.03] and cough with fever (unadjusted IRR: 2.36; 95% CI: 1.30, 4.31; *P*-trend = 0.05). After adjustment for several sociodemographic characteristics and hemoglobin concentrations, every 10 μg/dL plasma retinol was associated with 18% fewer days of diarrhea with vomiting ($P < 0.001$), 10% fewer days of cough with fever ($P < 0.001$), and 6% fewer doctor visits ($P = 0.01$). Every 1 g/dL of hemoglobin was related to 17% fewer days with ear infection symptoms ($P < 0.001$) and 5% fewer doctor visits ($P = 0.009$) after controlling for sociodemographic factors and retinol concentrations. Zinc, ferritin, mean corpuscular volume, erythrocyte folate, and vitamin B-12 status were not associated with morbidity or doctor visits. Vitamin A and hemoglobin concentrations were inversely related to rates of morbidity in school-age children. Whether vitamin A supplementation reduces the risk or severity of infection in children over 5 y of age needs to be determined. J. Nutr. 144: 496–503, 2014.

Introduction

Infection is a major cause of morbidity throughout childhood (1). Among school-age children, respiratory and gastrointestinal infections account for increased school absenteeism and parental absenteeism from work (2), as well as a considerable proportion of physician visits (3). Despite their heavy burden, environmental determinants of the risk of infection, including nutritional status, remain poorly understood in this age group.

In children aged ≤5 y, poor micronutrient status has been associated with risk of infection-related morbidity (4). Vitamin A supplementation reduces mortality and measles morbidity, although its effects on diarrhea and non-measles respiratory infection remain equivocal (5,6). Also in this age group, zinc supplementation prevents diarrhea, is recommended as an adjuvant treatment of acute diarrhea (7), and reduces the risk of lower respiratory tract infection (8). The role of iron has been a subject of concern; iron deficiency is associated with impaired cellular immunity, yet supplementation may result in adverse health outcomes related to infections with pathogens that require iron for growth (9). A protective effect of iron supplementation against diarrhea, respiratory infection, or fever may be limited to anemic children (10).

In contrast, evidence on the role of specific micronutrients in morbidity experienced by children aged >5 y is scant. Some trials have addressed the question in this age group, although most used multiple micronutrient interventions (11–16). Because micronutrients were administered together, it is uncertain from these studies which particular nutrients may have had an effect on morbidity. Only 1 of these studies was conducted in Latin America, and the effects of micronutrients may vary in different settings according to the underlying nutritional status of the population and the specific epidemiologic pattern of agents causing infectious morbidity. Sentinel surveillance suggests that the proportional contribution of various microbes to gastrointestinal and

[1] The Bogotá School Children Cohort Study is currently sponsored by the ASISA Research Fund at the University of Michigan. K.A.T. is supported by NIH training grant T32HD046377.
[2] Author disclosures: K. A. Thornton, M. Mora-Plazas, C. Marín, and E. Villamor, no conflicts of interest.
[3] Supplemental Table 1 is available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: villamor@umich.edu.

Manuscript received September 25, 2013. Initial review completed November 4, 2013. Revision accepted January 13, 2014.
First published online February 5, 2014; doi:10.3945/jn.113.185876.

respiratory illnesses differs between preschool and school-age children (17–19). In addition, susceptibility to infection and maturation of the immune system vary with age (20). Thus, findings on the relation between micronutrient status and infection in infants and preschool children may not necessarily be generalizable to older children.

We conducted a prospective study to investigate the association between blood concentrations of micronutrient status biomarkers and morbidity among school children in Bogotá, Colombia. We hypothesized that micronutrient concentrations would be inversely related to incidence of gastrointestinal and respiratory symptoms, as well as the number of doctor visits.

Participants and Methods

Study Population. This study was conducted in the context of the Bogotá School Children Cohort, an ongoing longitudinal investigation of health and nutrition in school children. Details on the study design have been reported previously (21). In summary, in February 2006, we recruited a representative group of 3202 children aged 5–12 y from public primary schools of Bogotá through random sampling. These children came from a total of 2981 households. The sample represents children from low- and middle-income families in Bogotá because the public school system enrolls a majority of children within these socioeconomic strata (22). We obtained information on sociodemographic characteristics and health habits of the children and their families through a caregiver self-administered questionnaire at the time of enrollment (82% response). Trained research assistants who visited the schools in the following weeks made anthropometric measurements and collected blood samples from the children. The assistants measured height to the nearest 1 mm with wall-mounted Seca 202 stadiometers and weight to the nearest 0.1 kg with Tanita HS301 electronic scales using standardized protocols (23).

Throughout the academic year after enrollment into the cohort, parents or primary caregivers kept daily records of the incidence of morbidity episodes using a pictorial diary that was distributed and returned on a weekly basis. The diaries had drawings that depicted children with symptoms including vomiting, diarrhea, fever, cough, and earache/discharge. Caregivers were asked to check each day the child had these conditions or visited a doctor. A doctor visit included any visit to a physician or hospital. We received 50,153 diaries in total, with a median of 17 diaries per child. Diaries are often used to record participants' symptoms in studies of gastrointestinal and respiratory illness, including randomized controlled trials (RCTs)[7] in which illness is defined according to clinical symptoms (24–26). The use of symptom diaries has been validated in various settings (27,28), and previous studies indicate that pictorial diaries validly capture incidence of morbidity in developing countries (29,30).

The parents or primary caregivers of all children provided written informed consent before enrollment into the study. The study protocol was approved by the Ethics Committee of the National University of Colombia Medical School. The Health Sciences and Behavioral Sciences Institutional Review Board at the University of Michigan approved the use of data from the study.

Laboratory Methods. Fasting blood samples were obtained by venipuncture in 2816 (88%) children at baseline on the day of recruitment. The samples were collected in EDTA tubes and transported on ice, protected from sunlight, to the National Institute of Health (Bogotá, Colombia), where biochemical analyses were performed according to methods described previously (31). We performed a complete blood count and determined mean corpuscular volume (MCV). Hemoglobin concentrations were determined with the hemiglobincyanide method. Plasma retinol was measured with HPLC on a Waters 600 System. Plasma ferritin and vitamin B-12, as well as erythrocyte folate, were quantified using competitive chemiluminescent immunoassays in an ADVIA Centaur analyzer (Bayer Diagnostics). In a separate aliquot that was collected in a metal-free polypropylene BD Diagnostics tube without anticoagulant, serum zinc concentrations were determined using an atomic absorption technique (32) on a Shimadzu AA6300 spectrophotometer. Serum C-reactive protein (CRP) was measured with a turbidimetric immunoassay on an ACS180 analyzer (Bayer Diagnostics). All analytes were measured in duplicate. Internal quality assurance at the National Institute of Health laboratory involved daily calibration of instruments. External quality assurance of this laboratory was performed by the Centers for Disease Control (Atlanta, GA).

Data Analyses. The final sample consisted of 2774 children after excluding 42 participants without morbidity diaries. Outcomes were rates of gastrointestinal and respiratory morbidity, including diarrhea with vomiting, cough with fever, and earache or ear discharge with fever, as well as rates of doctor visits. Clinically diagnosed episodes of gastrointestinal illness have been associated with reports of diarrhea and vomiting combined (33–35). Cough with fever had a positive predictive value of 83% for laboratory-confirmed influenza infection among children aged 5–12 y (36), and this case definition has been used to monitor influenza-like illness in Latin America (37). Cough with fever is also reported in school-age children experiencing the common cold due to a variety of viral and bacterial infections (26). Although the diagnosis of acute otitis media requires clinical examination, symptoms including moderate to severe ear pain with fever are indicators of severe illness (38) and ear drainage is often related to bacterial infection (39). Rates were calculated as the number of days with each combination of symptoms divided by the total number of days the child was under observation. Rates of doctor visits were similarly calculated.

To identify potential confounders of the associations between micronutrient status and morbidity, we first estimated incidence rates of morbidity and doctor visits according to baseline child and maternal characteristics. Children's height-for-age Z-scores and BMI-for-age Z-scores were estimated using the WHO reference (40). Maternal BMI was calculated with measured height and weight in 34% of mothers and with self-reported data in the remainder. Food insecurity in the household was evaluated using a scale adapted from the Spanish-language version of the USDA Household Food Security Survey Module (41) and the Community Childhood Hunger Identification project (42) that was validated previously in this setting (43). When 3 of 5 child-specific questions were answered affirmatively, child food insecurity in the household was considered present.

We then calculated incidence rates of morbidity and doctor visits according to micronutrient status indicators. Zinc, ferritin, MCV, hemoglobin, and erythrocyte folate were categorized into sex- and age-specific quartiles of the study population distributions; vitamin A was categorized as <10.0 (severely deficient), 10.0–19.9 (deficient), 20.0–29.9 (low), or ≥30.0 μg/dL (adequate) (44), and vitamin B-12 was grouped as >221 (adequate) or ≤221 pmol/L (low) (45). We estimated incidence rate ratios (IRRs) and 95% CIs for morbidity and doctor visits by categories of micronutrient status predictors with the use of generalized estimating equations with the Poisson distribution. An exchangeable correlation structure was specified to account for within-child and within-family correlations of outcome measures. Tests of linear trend were conducted for ordinal characteristics by introducing a variable representing the ordinal categories as a continuous predictor into the models. Because ferritin and retinol concentrations can be altered in the presence of inflammation, models with these micronutrients also included a variable for ln-transformed CRP (46). Finally, adjusted IRRs were estimated from multivariable models for each morbidity event and doctor visits. These models included as predictors sociodemographic and micronutrient status indicators that were significantly associated with the outcomes in the previous analyses. Some children had missing information on sociodemographic variables, including maternal education (12%), parity (12%), and child food insecurity (13%). Values for these missing covariates were estimated with multiple imputation using a Markov Chain Monte Carlo method before their inclusion in the models (47). For each outcome, child sex, age, maternal education, parity, household child food insecurity, vitamin A and hemoglobin concentrations, and the index outcomes were included in the imputation procedure. Ten cycles of imputation were completed to estimate values for the missing covariates. All analyses were conducted with Statistical Analysis System software (version 9.3; SAS Institute).

[7] Abbreviations used: CRP, C-reactive protein; IRR, incidence rate ratio; MCV, mean corpuscular volume; RCT, randomized controlled trials.

TABLE 1 Morbidity and doctor visits according to sociodemographic characteristics in 2774 school children from Bogotá, Colombia[1]

			Diarrhea with vomiting		Cough with fever		Earache/discharge with fever		Doctor visits	
	n	Months of follow-up	Days of morbidity	Rate per child-year	Days of morbidity	Rate per child-year	Days of morbidity	Rate per child-year	Days of visits	Rate per child-year
Child characteristics										
Sex										
Girls	1373	5716	468	0.98	1534	3.22	397	0.83	981	2.06
Boys	1401	5387	364	0.81	1137	2.53	224	0.50	953	2.12
P				0.21		0.03		0.02		0.81
Age (y)										
5–6	547	2117	195	1.11	601	3.41	135	0.77	453	2.57
7–8	858	3496	358	1.23	938	3.22	219	0.75	680	2.33
9–10	1092	4491	223	0.60	929	2.48	215	0.57	648	1.73
11–12	264	987	56	0.68	202	2.46	52	0.63	153	1.86
P				<0.001		0.01		0.28		0.01
Height-for-age Z-score[2]										
<−2.0	268	1019	91	1.07	283	3.33	107	1.26	190	2.24
−2.0 to <−1.0	842	3327	227	0.82	764	2.76	143	0.52	547	1.97
−1.0 to <1.0	1476	6097	457	0.90	1458	2.87	325	0.64	1087	2.14
≥1.0	98	405	43	1.27	101	2.99	33	0.98	78	2.31
P				0.86		0.73		0.42		0.84
BMI-for-age Z-score[2]										
<−2.0	36	162	10	0.74	45	3.34	15	1.11	28	2.08
−2.0 to <−1.0	298	1236	98	0.95	302	2.93	55	0.53	193	1.87
−1.0 to <1.0	1851	7358	509	0.83	1740	2.84	379	0.62	1279	2.09
1.0 to <2.0	385	1618	153	1.13	411	3.05	146	1.08	281	2.08
≥2.0	111	460	45	1.17	105	2.74	13	0.34	118	3.08
P				0.18		0.95		0.52		0.17
Maternal characteristics										
Education										
Incomplete primary	197	774	72	1.12	263	4.08	110	1.71	231	3.58
Complete primary	478	1975	170	1.03	510	3.10	124	0.75	288	1.75
Incomplete secondary	629	2597	169	0.78	655	3.03	124	0.57	401	1.85
Complete secondary	978	4226	315	0.89	922	2.62	179	0.51	767	2.18
University	167	716	46	0.77	168	2.82	34	0.57	162	2.72
P				0.31		0.05		0.01		0.82
Parity										
1	290	1324	123	1.11	291	2.64	64	0.58	277	2.51
2	879	3722	238	0.77	828	2.67	172	0.55	629	2.03
3	726	3019	177	0.70	599	2.38	150	0.60	483	1.92
≥4	534	2138	202	1.13	748	4.20	145	0.81	418	2.35
P				0.69		0.02		0.24		0.90
Height (cm)										
Q1, median of 150	586	2465	159	0.77	636	3.10	104	0.51	417	2.03
Q2, median of 155	620	2562	181	0.85	647	3.03	169	0.79	439	2.06
Q3, median of 160	548	2359	148	0.75	512	2.60	109	0.55	457	2.32
Q4, median of 165	612	2542	238	1.12	620	2.93	148	0.70	446	2.11
P				0.13		0.54		0.53		0.73
BMI (kg/m^2)										
<18.5	84	340	57	2.01	114	4.02	38	1.34	85	3.00
18.5–24.9	1463	6198	440	0.85	1506	2.92	357	0.69	1115	2.16
25.0–29.9	595	2476	162	0.79	544	2.64	88	0.43	404	1.96
≥30.0	170	726	48	0.79	213	3.52	33	0.55	146	2.41
P				0.20		0.82		0.11		0.73
Household characteristics										
Child food insecurity										
No	2014	8595	608	0.85	1870	2.61	465	0.65	1569	2.19
Yes	390	1511	150	1.19	562	4.46	92	0.73	210	1.67
P				0.10		<0.001		0.63		0.06

(Continued)

TABLE 1 *Continued*

			Diarrhea with vomiting		Cough with fever		Earache/discharge with fever		Doctor visits	
	n	Months of follow-up	Days of morbidity	Rate per child-year	Days of morbidity	Rate per child-year	Days of morbidity	Rate per child-year	Days of visits	Rate per child-year
Average number of people sleeping in same room										
1	93	402	34	1.02	50	1.49	12	0.36	57	1.70
2	1173	5095	303	0.71	1140	2.68	212	0.50	896	2.11
3	707	2897	268	1.11	772	3.20	229	0.95	518	2.15
≥4	449	1803	159	1.06	525	3.49	108	0.72	309	2.06
P				0.05		0.008		0.01		0.87
Number of assets[3]										
0–1	211	790	81	1.23	293	4.45	74	1.12	135	2.05
2	316	1233	77	0.75	319	3.10	71	0.69	237	2.31
3	401	1677	136	0.97	455	3.26	94	0.67	280	2.00
4	481	2037	149	0.88	457	2.69	74	0.44	358	2.11
5	535	2328	166	0.86	556	2.87	170	0.88	428	2.21
6	533	2327	175	0.90	455	2.35	91	0.47	400	2.06
P				0.61		0.01		0.21		0.94

[1] For ordinal predictors, *P* value was a test of linear trend when a variable that represented the categories of the predictor was introduced as continuous into a generalized estimating equation model with a Poisson distribution. For dichotomous predictors, *P* value is from the Wald test obtained with a generalized estimating equation model with robust standard errors. An exchangeable correlation matrix was used in all models to account for within-child and within-family correlations of days of morbidity. Q, quartile.
[2] According to the WHO reference (40).
[3] Sum of household assets from a list that included bicycle, refrigerator, blender, television, stereo, and washing machine.

Results

The mean age of children at enrollment was 8.7 ± 1.8 y; 50.5% were boys. Plasma vitamin A concentration was 29.7 ± 9.9 μg/dL; 12.3% of children were vitamin A deficient (10.0–19.9 μg/dL), and an additional 1.2% were severely deficient (<10.0 μg/dL). Mean concentrations of zinc, ferritin, MCV, hemoglobin, erythrocyte folate, and vitamin B-12 were 140.2 ± 41.6 μg/dL, 42.2 ± 23.3 μg/L, 86.0 ± 5.3 fL, 14.5 ± 1.2 g/dL, 858 ± 257 nmol/L, and 327 ± 106 pmol/L, respectively; 16.6% of children had low vitamin B-12 status (≤221 pmol/L). Micronutrient concentrations varied with sex and age at recruitment (**Supplemental Table 1**). CRP was elevated (>10 mg/L) in 1.6% of children at baseline.

Children were observed for a median 126 d per child (IQR: 77, 168) and contributed 337,937 total days of observation. Girls experienced more days of cough with fever and earache/discharge with fever than boys (**Table 1**). Child age was inversely associated with rates of diarrhea with vomiting, cough with fever, and doctor visits. Maternal parity was positively related to rates of cough with fever, whereas education was inversely related to cough with fever and earache/discharge with fever. Children living in households with child food insecurity had more days of cough with fever compared with those who were not (IRR: 1.71; 95% CI: 1.30, 2.25) but also had fewer doctor visits (IRR: 0.76; 95% CI: 0.57, 1.02).

Vitamin A concentrations were inversely related to diarrhea with vomiting (*P*-trend = 0.03) and cough with fever (*P*-trend = 0.05) (**Table 2**). Severely vitamin A–deficient children experienced more than twice as many days of gastrointestinal symptoms as those with adequate retinol concentrations (≥30 μg/dL) (IRR: 2.17; 95% CI: 0.95, 4.96). Children with severe vitamin A deficiency also had 2.4 times as many days of cough with fever compared with those with adequate vitamin A concentrations (IRR: 2.36; 95% CI: 1.30, 4.31). After adjustment for sex, age, maternal education and parity, child food insecurity in the household, and hemoglobin, every 10 μg/dL plasma retinol was related to 18% fewer days of diarrhea with vomiting ($P < 0.001$), 10% fewer days of cough with fever ($P < 0.001$), 7% fewer days of ear symptoms ($P = 0.06$), and 6% fewer doctor visits ($P = 0.01$) (**Table 3**). These results were robust to sensitivity analyses in which the models were refitted after excluding 170 children who had symptoms during the first week of follow-up. These sensitivity analyses were conducted to assess whether the findings were affected by potential reverse causation bias related to concurrent infection at the time of micronutrient status assessment.

Hemoglobin concentrations were inversely associated with earache or ear discharge with fever (*P*-trend = 0.008). Children with hemoglobin concentrations in the lower 2 sex- and age-adjusted quartiles experienced twice as many days of earache/discharge with fever compared with those with concentrations in quartile 4 (quartile 1 vs. quartile 4, IRR: 1.98, 95% CI: 1.09, 3.62; quartile 2 vs. quartile 4, IRR: 2.10, 95% CI: 1.18, 3.73). After adjustment for sex, age, maternal education and parity, child food insecurity in the household, and vitamin A concentrations, every 1 g/dL of hemoglobin was related to 17% fewer days with ear infection symptoms ($P < 0.001$) and 5% fewer doctor visits ($P = 0.009$) (Table 3). Zinc, ferritin, MCV, erythrocyte folate, and vitamin B-12 concentrations were not associated with morbidity or doctor visits. We conducted sensitivity analyses of multiple imputations for missing data in the multivariable models by refitting these models among children with complete information on all covariates. These analyses yielded essentially the same results as those presented using imputed values for sociodemographic variables.

Discussion

In this large longitudinal study of school children, baseline vitamin A deficiency was related to increased rates of diarrhea with vomiting and cough with fever. The inverse association of retinol concentration with these morbidities was independent of other micronutrient status indicators and sociodemographic characteristics.

Most evidence regarding the effect of vitamin A on child morbidity is from studies conducted primarily in infants and preschool children (5), which reported inconsistent results of vitamin A

TABLE 2 Morbidity and doctor visits according to micronutrient concentrations in Colombian school-age children[1]

			Diarrhea with vomiting		Cough with fever		Earache/discharge with fever		Doctor visits	
	n	Months of follow-up	Days of morbidity	Rate per year	Days of morbidity	Rate per year	Days of morbidity	Rate per year	Days of visits	Rate per year
Vitamin A in plasma (μg/dL)										
<10.0	34	149	21	1.69	77	6.19	10	0.80	30	2.41
10.0–19.9	339	1370	137	1.20	339	2.97	98	0.86	262	2.30
20.0–29.9	1170	4660	354	0.91	1179	3.04	264	0.68	887	2.28
≥30.0	1225	4911	319	0.78	1073	2.62	249	0.61	755	1.85
P				0.03		0.05		0.26		0.11
Zinc in serum (μg/dL)										
Q1[2]	684	2789	244	1.05	696	3.00	178	0.77	534	2.30
Q2	684	2711	168	0.74	688	3.05	125	0.55	398	1.76
Q3	685	2667	152	0.68	557	2.51	134	0.60	409	1.84
Q4	684	2833	265	1.12	718	3.04	182	0.77	585	2.48
P				0.80		0.79		0.92		0.66
Ferritin in plasma (μg/L)										
Q1	685	2706	172	0.76	585	2.59	149	0.66	497	2.20
Q2	685	2681	172	0.77	659	2.95	112	0.50	437	1.96
Q3	686	2836	274	1.16	780	3.30	173	0.73	512	2.17
Q4	685	2793	213	0.92	640	2.75	187	0.80	479	2.06
P				0.17		0.43		0.32		0.94
Mean corpuscular volume (fL)										
Q1	658	2554	201	0.94	720	3.38	182	0.86	531	2.49
Q2	662	2723	154	0.68	487	2.15	77	0.34	353	1.56
Q3	666	2721	224	0.99	639	2.82	195	0.86	566	2.50
Q4	662	2584	211	0.98	649	3.01	139	0.65	381	1.77
P				0.51		0.80		0.92		0.40
Hemoglobin (g/dL)										
Q1	697	2749	200	0.87	724	3.16	193	0.84	387	1.69
Q2	677	2742	250	1.09	693	3.03	204	0.89	674	2.95
Q3	687	2733	176	0.77	524	2.30	123	0.54	423	1.86
Q4	696	2854	206	0.87	729	3.07	101	0.42	449	1.89
P				0.56		0.50		0.008		0.58
Erythrocyte folate (nmol/L)										
Q1	663	2638	163	0.74	723	3.29	184	0.84	426	1.94
Q2	665	2654	248	1.12	666	3.01	131	0.59	501	2.27
Q3	666	2742	176	0.77	561	2.46	144	0.63	435	1.90
Q4	665	2615	212	0.97	618	2.84	145	0.67	484	2.22
P				0.57		0.19		0.52		0.65
Vitamin B-12 in plasma (pmol/L)										
≤221.0	444	1624	94	0.69	383	2.83	86	0.64	296	2.19
>221.0	2231	9064	709	0.94	2196	2.91	517	0.68	1583	2.10
P				0.19		0.87		0.80		0.83

[1] For ordinal predictors, *P* value was a test of linear trend when a variable that represented the categories of the predictor was introduced as continuous into a generalized estimating equation model with a Poisson distribution. For dichotomous predictors, *P* value is from the Wald test obtained with a generalized estimating equation model with robust standard errors. An exchangeable correlation matrix was used in all models to account for within-child and within-family correlation of days of morbidity. All models estimating *P*-trend with vitamin A or ferritin were adjusted for ln of C-reactive protein. Q, quartile.
[2] All quartiles are sex and age adjusted.

supplementation on diarrhea (48–50) and respiratory illness (48,51–53). Some RCTs examined the effect of foods fortified with micronutrients including vitamin A on morbidity in school-age children (11–14). A micronutrient-fortified powder administered to Thai school children reduced the incidence of diarrhea and respiratory-related symptoms (12). In contrast, supplementation to Indian school-age children had no effect on reported morbidity incidence (13). It is not possible to determine a causal role for vitamin A from these studies because other micronutrients were included in the interventions, and the nutrients were delivered in different forms.

Our results suggest that vitamin A may play a role in the incidence of gastrointestinal infections during the school-age years. A range of viral, bacterial, or parasitic agents cause diarrheal disease in children of this age (17). Norovirus is a common cause of diarrhea with vomiting in school-age children (35), likely producing these symptoms through pathogenic effects on the intestinal epithelial barrier (54). The role of vitamin A deficiency might be explained through its adverse effects on components of gastrointestinal mucosal immunity, including mucin gene expression and secretory IgA response (55–57).

In contrast with studies of young children in which vitamin A had no protective effects on respiratory infection, we found an inverse relation between plasma retinol concentrations and incidence of respiratory morbidity among children aged ≥5 y. Vitamin A may have different effects on respiratory illness throughout childhood if the microorganisms causing infection in children

TABLE 3 IRRs (95% CIs) of morbidity and doctor visits according to sociodemographic characteristics and micronutrient concentrations in Colombian school children[1]

	Diarrhea with vomiting	Cough with fever	Earache/discharge with fever	Doctor visits
Male sex	0.82 (0.72, 0.94)	0.79 (0.73, 0.86)	0.59 (0.50, 0.70)	1.02 (0.93, 1.12)
Age, per y	0.88 (0.85, 0.92)	0.92 (0.90, 0.94)	0.94 (0.89, 0.98)	0.93 (0.91, 0.95)
Maternal education, per y	0.98 (0.95, 1.00)	0.98 (0.96, 0.99)	0.90 (0.88, 0.93)	0.98 (0.97, 1.00)
Parity, per child	1.07 (0.98, 1.16)	1.14 (1.08, 1.19)	1.08 (0.97, 1.21)	1.02 (0.97, 1.07)
Child food insecurity	1.27 (1.04, 1.56)	1.49 (1.34, 1.66)	0.88 (0.65, 1.21)	0.74 (0.63, 0.88)
Vitamin A, per 10.0 μg/dL	0.82 (0.77, 0.89)	0.90 (0.87, 0.94)	0.93 (0.85, 1.00)	0.94 (0.90, 0.99)
Hemoglobin, per g/dL	1.01 (0.95, 1.08)	1.00 (0.97, 1.04)	0.83 (0.78, 0.89)	0.95 (0.92, 0.99)

[1] Multiple imputed values were used for missing data on sociodemographic variables; $n = 2764$. Values for missing micronutrient data were not imputed; therefore, these models exclude 10 children with missing measures of vitamin A or hemoglobin status at baseline. IRR estimated with generalized estimating equation models with a Poisson distribution, using an exchangeable correlation matrix to account for within-child and within-family correlations of days of morbidity. IRR, incidence rate ratio.

vary by age. In studies of children hospitalized with community-acquired pneumonia, the proportion of identifiable infections attributable to bacterial agents was greatest in children aged ≥5 y, whereas that attributable to viral pathogens was highest in infants (58,59). Vitamin A deficiency may increase the risk or severity of bacterial rather than viral infections because deficiency favors a proinflammatory T helper 1 cell–mediated response and impairs a T helper 2 response that is necessary to control infections by extracellular microorganisms, including bacteria (60). Furthermore, experimental evidence demonstrates that retinoic acid enhances both primary and recall antibody responses to antigens through effects on T and B cells (61). Our findings could also have noncausal explanations. Plasma retinol concentrations typically decrease during infections as part of the acute-phase response (62). If children experienced a heavy burden of infection at the time of vitamin A assessments, the inverse association observed could represent an effect of infection on retinol rather than the opposite. Nevertheless, adjustment for CRP and sensitivity analyses excluding children with morbidity during the first week of follow-up did not change the results, providing some evidence against this potential explanation.

In our study, hemoglobin concentrations were inversely associated with rates of earache or ear discharge with fever, whereas the iron status indicators ferritin and MCV were not. Hemoglobin is commonly used as an indicator of iron-deficiency anemia; however, in non-anemic populations, it may not be a useful marker of iron status. The altitude-adjusted prevalence of anemia (hemoglobin < 12.7 g/dL) and low ferritin in this cohort were low, at 3.7 and 3.3%, respectively (31). Thus, the inverse association between hemoglobin and ear symptoms observed in our study could be attributable to hemoglobin itself and not necessarily to iron. Hemoglobin is known primarily for transporting oxygen and carbon dioxide in erythrocytes, but hemoglobin expression may occur in non-erythroid cells, including alveolar cells and macrophages (63,64). These cells are involved in innate immunity, and experimental work suggests that non-erythrocyte hemoglobin expression may be related to protection against oxidative stress (65) or regulation of NO signaling (66). Few investigations have evaluated the relation between hemoglobin and ear infection in children. In a recent case-control study of Yemeni children aged 0.6–15 y, neither hemoglobin nor ferritin concentrations were associated with chronic suppurative otitis media (67). The majority of ear symptoms reported in our cohort were likely due to acute infections, which are typically caused by bacteria, including *Streptococcus pneumonia*, nontypeable *Haemophilus influenzae*, and *Moraxella catarrhalis* (68). Chronic suppurative otitis media is associated with a broader range of pathogens than acute otitis media (69), and the discrepant findings may be related to etiologic differences in disease or reverse causation bias in the Yemen study of prevalent rather than incident cases.

We did not find associations of other micronutrients, including ferritin, MCV, zinc, folate, or vitamin B-12, with gastrointestinal or respiratory symptoms in our cohort. Iron supplementation in school-age children has been related to increased incidence of diarrhea in an area with mesoendemic malaria (16) and fewer episodes of upper respiratory tract infection (70). Zinc supplementation in 2 RCTs conducted among school children did not reduce rates of parasitic reinfection (71,72). Although folate deficiency is associated with alterations in immune function (73), few epidemiologic studies have considered the effect of folate or vitamin B-12 on morbidity. In a prospective study of Indian children aged 6–30 mo, folate concentrations were inversely associated with both persistent diarrheal episodes and acute lower respiratory tract infection, whereas low vitamin B-12 concentrations were only related to increased risk of acute diarrhea in non-breastfed infants (74,75). One possible explanation for the lack of associations between these micronutrients and morbidity in our study is that their concentrations were relatively high. There were very few children with concentrations below conventional cutoff points for deficiency. For example, only 2 children had erythrocyte folate <305 nmol/L (76), and only 3.3% were iron-deficient according to ferritin concentrations (31). Lack of variability in the exposures at the lower end of their distributions may have prevented us from observing associations with morbidity that are due to deficiency of these nutrients.

Both vitamin A and hemoglobin concentrations were associated with fewer doctor visits after controlling for various sociodemographic characteristics. Because sicker children may have higher consultation rates than those who are healthier, these associations provide internal consistency to the findings on morbidity. Child food insecurity was related to fewer doctor visits, but also to increased rates of cough with fever. This observation may reflect an inability of poor families to consult a physician when a child is ill.

Although the micronutrient blood concentrations examined in this study are generally considered adequate biomarkers of intake (77), the effect of diet on morbidity is unknown. Among participants in this cohort, provision of a school snack that supplemented 30% and 50% of the children's daily requirements of energy and iron, respectively, was associated with significantly fewer days of cough with fever, diarrhea and vomiting, and doctor visits over a short 3-mo period (21). We did not find a relation between iron status indicators and morbidity over a longer time span; thus, the previously reported snack effect might be mediated through other nutrients we did not measure. Additional examination of the effect of diet on child morbidity is warranted.

Our study has many strengths. We determined the micronutrient status of a large and representative sample of school children using valid biomarkers. Prospectively collected information on the outcomes minimizes the potential for reverse causation bias, as well as outcome misclassification bias that may result from differential recall of morbidity events. We carefully controlled in the analysis for important potential confounders of the associations of micronutrients with morbidity. Although information on some of the possible confounding sociodemographic variables was missing in a small proportion of children, sensitivity analyses indicated that the results were not biased by using imputed values for missing data. Nonetheless, given the observational nature of the study, residual confounding by unmeasured characteristics cannot be completely ruled out as a noncausal explanation for the findings. Another limitation is that we were unable to confirm the infectious etiology of the morbidities evaluated in our study. The validity of pictorial diaries to capture pediatric morbidity in this setting is unknown, and the results might be biased if reporting of the outcomes was differential with respect to the children's micronutrient status. Finally, we were unable to differentiate whether the associations observed represent the potential effect of micronutrients on the incidence or the duration of disease.

In conclusion, vitamin A concentrations were inversely related to rates of gastrointestinal and respiratory morbidity, and hemoglobin concentrations were inversely associated with symptoms of ear infection in a large cohort of apparently healthy school children. These results suggest that both vitamin A and hemoglobin may be important in maintaining immune function or limiting severity of infection in children older than 5 y of age. Intervention studies are warranted to confirm that improving vitamin A status reduces the risk of morbidity in school-age children.

Acknowledgments

E.V. designed the research; M.M.-P. and C.M. conducted the research; K.A.T. analyzed the data; and K.A.T. and E.V. wrote the paper and had primary responsibility for final content. All authors read and approved the final manuscript.

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