Titers of antibody to common pathogens: relation to food-based interventions in rural Kenyan schoolchildren[1–4]

Jonathan H Siekmann, Lindsay H Allen, Mitchell R Watnik, Penelope Nestel, Charlotte G Neumann, Yehuda Shoenfeld, James B Peter, Meeta Patnik, Aftab A Ansari, Ross L Coppel, and M Eric Gershwin

ABSTRACT

Background: Undernutrition is widely perceived to affect the development of an effective immune system.

Objective: We used a mini-analysis system to quantitate antibody titers and evaluate the sera of 200 Kenyan schoolchildren for antibodies to *Helicobacter pylori* [isotypes of immunoglobulins A (IgA), G (IgG), and M (IgM)], hepatitis A virus, rotavirus, tetanus toxoid (IgG), and a panel of recombinant malarial antigens ($MSP1_{19}$, MSP2, Ag512, MSP4, and MSP5).

Design: Children participated in a school-based feeding intervention with meat, milk, or nonanimal-source foods or in a nonintervention control group. Microvolumes (200 mL) of sera were analyzed at baseline and after 1 y.

Results: Nearly all children had elevated titers of antibody to *H. pylori*, hepatitis A virus, rotavirus, and malaria at the outset, despite a high prevalence of apparent biochemical micronutrient deficiencies and stunting, but many had titers of tetanus toxoid IgG antibodies below the protective concentration. Children with low hemoglobin had a greater proportion of elevated *H. pylori* IgM antibody titers at baseline, which suggests that current infection with *H. pylori* may be associated with anemia. Compared with the control subjects, only the group eating meat had a significant increase in *H. pylori* IgM antibodies during the intervention ($P = 0.019$). No other group comparisons with the control subjects were statistically significant. The additional finding that the sera of some children showed inadequate tetanus-protective antibodies, despite immunization, suggests that the vaccination program was suboptimal.

Conclusions: A large battery of immune assays can be performed on microvolumes of sera. Furthermore, despite evidence of malnutrition, children do develop significant antibody-mediated responses to common pathogens. *Am J Clin Nutr* 2003;77:242–9.

KEY WORDS Micronutrient deficiency, immune response, supplementation, *Helicobacter pylori*, hepatitis A virus, tetanus toxoid, rotavirus, malaria, schoolchildren, Kenya

INTRODUCTION

Micronutrient deficiencies affect more than 2 billion people globally (1). Although less prevalent in higher-income populations, these deficiencies do occur in such groups, especially among premature infants, infants, children, and the elderly (2–4). Micronutrient deficiencies may impair immune function, increase the risk of opportunistic infections and the severity of disease, and cause suboptimal antibody responses to immunization (5). These relations have been observed in human populations, in laboratory animals deprived of one dietary element, and in the rare patient with a single nutrient deficiency (6).

High antibody titers are expected in well-nourished individuals currently or previously exposed to an infectious pathogen. Antibodies are associated with virus neutralization, complement activation to neutralize pathogens such as bacteria, and hypersensitivity (7). Enzyme-linked immunosorbent assay (ELISA) measurements of antigen-specific circulating antibodies may provide a snapshot of immune status.

In the present study, we evaluated titers of antibody to *Helicobacter pylori* [isotypes of immunoglobulin A (IgA), G (IgG), and M (IgM)], hepatitis A virus (HAV), rotavirus, tetanus toxoid (IgG), and malaria in Kenyan schoolchildren after they were fed a supplementary isocaloric diet containing meat, milk, or nonanimal-source foods for 1 y while at school and in a control group of schoolchildren who received no supplementary food. This is the first such study to compare possible changes in immune function in a low-income country after a nutrient intervention with

[1] From the Departments of Nutrition (JHS and LHA), Statistics (MRW), and Rheumatology/Allergy and Clinical Immunology (MEG), University of California, Davis; the OMNI Research Project, Washington, DC (PN); the School of Public Health, University of California, Los Angeles (CGN); the Autoimmune Diseases Research Unit, Chaim Sheba Medical Center, Tel-Hashomer, Israel (YS); the Specialty Laboratories, Santa Monica, CA (JBP and MP); the Department of Pathology, Emory University School of Medicine, Atlanta (AAA); and the Department of Microbiology, Monash University, Clayton, Australia (RLC).

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[4] Reprints not available. Address correspondence to ME Gershwin, Division of Rheumatology/Allergy and Clinical Immunology, School of Medicine, University of California, Davis, One Shields Avenue, TB 192, Davis, CA 95616. E-mail: megershwin@ucdavis.edu.

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TABLE 1
Estimated energy and micronutrient contents of the 3 *githeri* meals, by school term and percentage of recommended daily intake provided by each meal[1]

	Term 3, 1998	Terms 1 and 2, 1999
Meat		
Serving size (g)	185 (includes 60 g meat)	225 (includes 85 g meat)
Energy		
(kJ)	999 [14]	1215 [17]
(kcal)	239 [14]	291 [17]
Vitamin A (μg RE)[2]	112 [28]	112 [28]
Iron (mg)	2.42 [24]	2.94 [29]
Riboflavin (mg)	0.12 [20]	0.15 [24]
Vitamin B-12 (μg)	0.75 [63]	0.91 [76]
Zinc (mg)	2.38 [48]	2.89 [58]
Milk		
Serving size (g)	100 (+ 200 mL)	100 (+ 250 mL)
Energy		
(kJ)	1007 [14]	1219 [16]
(kcal)	241 [14]	292 [16]
Vitamin A (μg RE)[2]	244 [61]	412 [68]
Iron (mg)	1.52 [15]	1.57 [16]
Riboflavin (mg)	0.44 [73]	0.53 [88]
Vitamin B-12 (μg)	0.96 [80]	1.16 [97]
Zinc (mg)	1.46 [29]	1.66 [33]
Nonanimal-source food		
Githeri		
Serving size (g)	185	230
Energy		
(kJ)	1003 [14]	1299 [18]
(kcal)	240 [14]	311 [18]
Vitamin A (μg RE)[2]	210 [53]	364 [67]
Iron (mg)	3.16 [32]	3.93 [39]
Riboflavin (mg)	0.12 [20]	0.15 [25]
Vitamin B-12 (μg)	0 [0]	0 [0]
Zinc (mg)	1.35 [27]	1.68 [34]

[1] Energy and nutrient values determined by Murphy et al with the use of the WorldFood nutrient database. Recommendations for energy intake assume a 20-kg child and 85 kcal·kg^{-1}·d^{-1} intake, based on graphs of mean energy intake for girls and boys (9). Recommended micronutrient intakes are for children aged 4–8 y from dietary reference intakes (10). Percentage of recommended intake in brackets. RE, retinol equivalents.

[2] Only retinol from the vegetable oil spread (fortified at 70 μg retinol/g) is listed for the energy and meat diets. For the milk diet, the sums of retinol from the vegetable oil spread and retinol from milk are listed. Retinol contribution from the vegetable oil spread was determined by multiplying the grams of the spread per serving by 70 μg/g.

energy and with animal-source foods. Data from the Nutrition Collaborative Research Support Program study in this population group in the 1980s indicated a high prevalence of anemia and stunting and a high probability of the inadequate intake of many micronutrients (8).

The goals of the present study were *1*) to assess the feasibility of simultaneously performing a battery of antibody assays by using small amounts of serum (200 mL) from these children; *2*) to determine whether children with biochemical indicators of micronutrient deficiencies have low titers of antibody to common pathogens and adequate titers of antibody to tetanus toxoid; *3*) to measure the association between diphtheria-pertussis-tetanus (DPT) immunizations and adequate tetanus toxoid response; *4*) to measure the association between antibodies to *H. pylori* (IgA, IgG, and IgM isotypes) and anemia, low plasma ferritin concentrations, and low vitamin B-12 concentrations; and *5*) to determine whether a food-based intervention to reduce micronutrient deficiencies would increase antibody titers.

SUBJECTS AND METHODS

Subjects

The schoolchildren were participants in a long-term feeding intervention. Data were collected at baseline (August 1998) and after one school year (August 1999). The study took place in 3 locations (Kyeni South) of the Embu district in the Eastern Province of Kenya. This is a high-elevation area (2000 m) near the base of Mt Kenya and close to the Equator. The region is characterized by distinct rainy and dry seasons, with mild weather year-round. *Plasmodium falciparum* malaria is endemic.

The staple foods are maize and beans, and dark-green leafy vegetables are frequently consumed. The children in this area typically obtain >75% of their energy intake from maize and beans, 1% from milk (35 g/d), and <1% from meat (11 g/d) (8). A total of 555 children aged 5–14 y participated in the intervention study. This study is based on data from 200 children for whom there were sufficient blood samples in August 1999, with approximately equal numbers of boys and girls. Paired *t* tests revealed that sex, age, and anthropometric characteristics of this subgroup at baseline were not significantly different ($P < 0.05$) from those of the larger population of participating children (data not shown).

Given 3 intervention groups (isocaloric diet based on meat, milk, or nonanimal-source foods) and a control group, a 5% concentration of significance, and no design effect of school, 50 subjects per group would provide sufficient power (≥0.75) to detect a 2-fold increase in antibody titers for all antibodies measured except tetanus toxoid IgG (power = 0.16).

The study was approved by the Human Subject Protection Committee of the University of California, Los Angeles; the Office of the President, University of Nairobi; the Kenyan Ministries of Education and Health; and the Human Subjects Review Committee of the University of California, Davis.

Dietary interventions

The in-school intervention consisted of meat, milk, or fat [(a vitamin A–fortified vegetable oil spread) Kimbo; Unilever Kenya, Nairobi, Kenya] served together with *githeri*, which is a local maize-and-bean stew prepared with tomatoes, iodized salt, the same vegetable oil spread, and chopped, dark leafy greens. The dark-green vegetables used in the *githeri* meals are nearly identical to kale. With consumption estimated (by us) at 20–100 g/d, no materials are present that would be expected to enhance immunity. Twelve schools were randomly assigned to 1 of 4 groups, with roughly equal numbers in each group, resulting in 3 schools per group: group 1, the meat group (*githeri* + ground beef); group 2, the milk group (*githeri* + cow milk); group 3, the nonanimal-source food group (*githeri* + vegetable oil spread); and group 4, the control group (no supplementary food). The children received the supplementary diet as a mid-morning snack in school, 5 d/wk except during holidays. Consumption was observed, and leftovers were weighed and accounted for.

The meat, milk, and nonanimal-source diets are described in **Table 1** in terms of serving size, composition, and the estimated percentage of the recommended daily intake for energy and

micronutrients provided by each meal. During the third school term (September-November) of 1998, the meat diet contained 60 g of minced beef, and the milk diet contained 200 mL of whole cow milk. All 3 diets provided <240 kcal/serving. Beginning in January 1999, the diets were changed to provide 300 kcal/serving, and thus the amount of supplementary food eaten was increased. Specifically, the amount of meat in the meat *githeri* was increased by 15 g, to a total of 85 g, and the portion size was increased from 185 g to 225 g. The amount of milk given to the milk group increased by 50 mL, to a total of 250 mL, but the *githeri* portion was held constant. The portion of *githeri* in the nonanimal-source food group increased from 185 g to 230 g/serving. The amount of vegetable oil spread also increased in the milk and nonanimal-source food groups. These changes increased the energy content of the meat, milk, and nonanimal-source food diets to 291, 292, and 311 kcal/serving, respectively.

The meals contained substantial amounts of some micronutrients: because the vegetable oil spread is fortified and whole milk is a good source of vitamin A, the milk and nonanimal-source foods provided between one-half and two-thirds of the recommended daily intake for vitamin A; the meat meal provided between one-quarter and one-third and the nonanimal-source food meal provided just over one-third of the recommended daily intake for iron; the milk meal provided > 70% of the recommended intake for riboflavin, whereas the other 2 meals provided only 20–25%; both the meat and milk meals provided > 60% of the recommended daily intake for vitamin B-12; and the meat meal provided about one-half the recommended daily intake of zinc, whereas the other 2 meals provided about one-third.

Schoolchildren in the control group did not receive any supplementary food in school. At the conclusion of the intervention, their parents received one milk goat per child as a compensation for the food their children missed.

Sample collection

In August 1998, experienced local physicians collected < 13 mL of venous blood from each study child (baseline samples). Blood samples were separated into aliquots of plasma, serum, and washed red blood cells for the analysis of antibodies and micronutrient status. Hemoglobin was measured on-site with the use of a drop of venous blood (Hemocue, Ängelholm, Sweden). This process was repeated in August 1999, after 1 y of the dietary interventions. DPT data were obtained from health records.

Antibody analyses

Blood samples of 50 children from each study group for whom there were sufficient blood samples in August 1999 were selected. The baseline blood samples from some of these children, however, were insufficient for all the assays (maximum $n = 19$), and those children were excluded from that particular analysis. Except for the assay with recombinant malaria antigens, the number of samples with insufficient volume did not differ significantly among the groups.

H. pylori antibodies (IgA, IgG, and IgM isotypes) were measured by enzyme immunoassay (EIA) using goat antihuman IgG, IgM, and IgA labeled with alkaline phosphatase (EC 3.1.3.1). Strong responders were classified as having a response > 15 EIA units.

Tetanus toxoid (IgG) antibody was measured by ELISA with the use of alkaline phosphatase–labeled goat antihuman immunoglobulin. Spectrophotometric measurement at 405 nm with an EIA microplate reader assessed the amount of specific antibody present. Values < 0.1 IU/mL were considered below the minimum protective threshold concentration.

Total antibodies to HAV were analyzed with an in vitro HAV EIA kit, using monoclonal antibodies to HAV (HAV-Total; Diagnostic Automation Inc, Calabasas, CA). An HAV antibody titer > 20 mIU/mL is indicative of current or past infection and is designated "positive."

Antibodies to rotavirus were measured by the semi-quantitative complement fixation method, with sheep erythrocytes and antierythrocyte serum (hemolysin). The presence of an antibody titer ≥ 8 indicates exposure to rotavirus.

Malaria antibodies to the recombinant proteins $MSP1_{19}$, MSP2, Ag512, MSP4, and MSP5 were measured by ELISA. These antigens were chosen on the basis of previous studies by one of us (RC) that found that the immune response to these epitopes is reflective of malaria exposure. Responders were classified as > 2 SD above the mean of nonexposed control subjects [> 0.53 optical density (OD), determined at UC Davis on banked sera from nonexposed Americans]. Units are OD at 405 nm.

Micronutrient analyses

Plasma ferritin assays were run in duplicate with the use of an immunoradiometric assay (Diagnostic Products Corporation, Los Angeles). Serum iron, zinc, and copper (3 readings of each sample) were measured by inductively coupled plasma emission spectroscopy (11); duplicate samples of plasma vitamin B-12 and folate were measured by radioimmunoassay (ICN Diagnostics, Costa Mesa, CA); plasma retinal was measured by HPLC [adaptation of the method of Catignani and Bieri (12)]; and erythrocyte riboflavin was measured by HPLC (13, 14). The red blood cell riboflavin content was measured as flavin adenine dinucleotide and flavin mononucleotide, indicators of long-term riboflavin status (15). Commercial controls were performed with each batch for plasma ferritin (CV: 9–18%; Chiron Diagnostics, East Walpole, MA), vitamin B-12 (CV: 5%), and folate (CV: 6.5%) (both from ICN Diagnostics). Pooled samples from 14 fasted, healthy Americans were used as controls for serum iron (CV: 3%), zinc (CV: 4%), and copper (CV: 6%), plasma retinol (CV: 9%), and red blood cell riboflavin (CV: 8%).

Data analyses

To meet normality assumptions (16), *H. pylori* IgA, IgG, and IgM and tetanus toxoid IgG were transformed with the use of natural logarithms plus a constant. Rotavirus complement fixation titers were transformed by converting the dilution ratios into whole numbers (eg, < 1:8 = 0, 1:8 = 1, 1:16 = 2). Because the residuals for tetanus toxoid were not normally distributed, a ranking operation was performed to achieve a normal distribution. Malaria OD data were not transformed. HAV variables were binary, and, thus, logistic regression was used. Except for HAV, analysis of covariance using the SAS GLM procedure (SAS software, version 8e; SAS Institute Inc, Cary, NC) was used to measure changes in antibody titers between baseline and 1 y after feeding among the 3 intervention groups and the control group.

In the analysis of covariance models, we used the change between the baseline and the final measure as the outcome variable. This approach allowed us to make conclusions about the effects of the treatments and to eliminate the effects of preexisting differences between groups. Furthermore, the quantitative baseline measurements and the school were used as explanatory variables. Testing for a statistically significant change overall was achieved with the *t* test, when the data could be appropriately transformed.

TABLE 2
Characteristics of subjects and the percentage with micronutrient deficiencies by intervention group and overall at baseline[1]

	Meat	Milk	Nonanimal-source food	Control	Overall
Sample size (*n*)	48	44	47	46	185
Percentage male (%)	56.3	65.9	55.3	54.3	57.8
Age (y)	7.5 ± 1.0[2]	7.3 ± 1.1	6.9 ± 1.2	7.3 ± 1.3	7.2 ± 1.2
SES	96.5 ± 34.3	86.1 ± 33.1	95.9 ± 28.5	101.1 ± 53.3	95.1 ± 38.6
HAZ	−1.50 ± 0.80	−1.36 ± 1.04	−1.18 ± 1.12	−1.04 ± 1.15	−1.27 ± 1.04
WHZ	−0.33 ± 0.82	−0.32 ± 0.63	−0.05 ± 0.70	−0.42 ± 0.84	−0.28 ± 0.76
Hemoglobin (g/L)	117 (101, 124)[3]	114 (104, 126)	112 (94, 127)	119 (107, 126)	116 (101, 126)
<115 g/L (%)	44.7	52.3	53.2	42.2	48.1
Plasma ferritin (μg/L)	50.9 (40.0, 80.0)	24.8 (18.2, 61.4)	56.4 (39.2, 102.4)	40.2 (17.6, 66.8)	47.8 (30.4, 78.0)
<15 μg/L (%)	4.4	20.5	0	14.0	9.6
Serum iron (μmol/L)	7.8 (6.6, 9.4)	9.1 (7.2, 10.9)	8.5 (6.3, 12.0)	8.7 (7.1, 11.5)	8.6 (6.8, 11.0)
<9.0 μmol/L (%)	65.2	46.5	56.5	55.6	55.4
Serum zinc (μmol/L)	9.4 (8.1, 11.4)	9.7 (8.6, 11.5)	9.3 (8.2, 11.1)	10.5 (8.9, 11.8)	9.8 (8.4, 11.4)
<10.7 μmol/L (%)	60.9	69.8	69.6	51.1	62.5
Serum copper (μmol/L)	21.5 (19.6, 23.9)	19.3 (17.5, 22.2)	21.0 (18.4, 23.6)	19.8 (17.2, 22.6)	20.4 (18.0, 23.5)
<11.0 μmol/L (%)	0	0	0	0	0.01
Plasma vitamin B-12 (pmol/L)	128 (95, 195)	152 (109, 246)	192 (158, 262)	215 (159, 307)	175 (117, 252)
<125 pmol/L (%)	48.9	39.5	19.6	11.4	29.9
125–221 pmol/L (%)	31.9	32.6	43.5	43.2	38.0
Plasma folate (nmol/L)	29.9 (23.4, 35.0)	36.1 (27.6, 43.4)	34.8 (27.1, 43.0)	29.0 (24.0, 35.1)	31.4 (25.0, 40.2)
<6.8 nmol/L (%)	0	0	0	0	0
6.8–13.6 nmol/L (%)	2.1	0	0	2.3	1.1
Plasma retinol (μmol/L)	0.41 (0.35, 0.49)	0.48 (0.40, 0.65)	0.52 (0.42, 0.62)	0.50 (0.39, 0.57)	0.47 (0.38, 0.58)
<0.35 μmol/L (%)	25.6	20.0	13.0	20.0	19.8
0.35–0.70 μmol/L (%)	74.4	65.0	80.4	73.3	72.9
RBC riboflavin (μmol/L)	212 (190, 262)	198 (175, 222)	203 (173, 226)	182 (160, 210)	199 (173, 231)
<170 μmol/L (%)	15.2	23.5	24.4	33.3	24.7

[1] SES, socioeconomic status; HAZ, height-for-age *z* score; WHZ, weight-for-height *z* score; RBC, red blood cell. There were no significant differences by group.
[2] $\bar{x}$ ± SD.
[3] Median; quartiles (quartile 1, quartile 3) in parentheses.

A tetanus toxoid antibody titer >0.1 IU/mL is usually an indication of a successful series of vaccinations, which includes an initial dose and 2 booster doses. The association between the number of DPT immunizations (either 1 or 3) and population assessments of weak responses (<0.1 IU/mL) to tetanus toxoid IgG was determined with the use of Pearson's chi-square test.

Because of reports suggesting a causal role of *H. pylori* infection in the anemia of young children (GJ Fuchs, SA Sarker, L Davidsson, unpublished observations, 2001), and because *H. pylori* infection may cause chronic gastritis and reduce gastric acid secretion, vitamin B-12 malabsorption, and subsequent deficiency (17, 18), the association between *H. pylori* IgA, IgG, and IgM antibodies >15 EIA units and the prevalence of low hemoglobin, ferritin, and vitamin B-12 was examined with the use of the SAS CATMOD procedure (SAS Institute Inc). Age, sex, socioeconomic status (SES), height-for-age *z* score (HAZ), and weight-for-height *z* score (WHZ) at baseline were also initially included in the models and dropped from the final model if not significant.

RESULTS

Baseline age, anthropometry, and micronutrient status

The percentage of males and the mean age, SES score, HAZ, and WHZ at baseline by intervention group and overall are shown in **Table 2**. There were no significant differences between the 4 groups in these variables.

The biochemical indicators for the micronutrient status of the children at baseline were very poor overall (Table 2); 48.1% of the children were anemic (hemoglobin <115 g/L), 55.4% had low serum iron (<9.0 mmol/L), 62.5% had low serum zinc (<10.7 mmol/L), 29.9% had severe (<125 pmol/L) and 38.0% had moderate (125–221 pmol/L) vitamin B-12 deficiency, 19.8% had severe (<0.35 mmol/L) and 72.9% had moderate (0.35–0.70 mmol/L) vitamin A deficiency, and 24.7% had an erythrocyte riboflavin concentration <170 mmol/L. However, only 9.6% had a plasma ferritin concentration <15 mg/L, and none of the samples evaluated showed evidence of folate or copper deficiency.

Antibody titers

Mean (±SE) titers of antibodies to *H. pylori* (IgA, IgG, and IgM isotypes), HAV, rotavirus, tetanus toxoid, and malaria in samples obtained at baseline and 1 y afterward are shown in **Table 3**. Overall, at baseline, a high proportion of children had both IgM (20.0%) and IgG (76.2%) antibodies to *H. pylori* and antibodies to HAV (63.2%) and rotavirus (89.2%). The prevalence of antibody to recombinant malaria proteins was also high, with 72.3% of the children showing higher concentrations than normal (ie, the mean + 2 SD of nonexposed control subjects). However, only 63.2% had a tetanus toxoid IgG concentration >0.1 IU/mL, the minimum threshold needed to protect against this pathogen. The *H. pylori* IgA antibody response was very weak, with only 0.5% of the results defined as elevated. For *H. pylori* IgA and rotavirus complement fixation, the mean antibody titers were significantly

TABLE 3
Elevated antibody titers to *Helicobacter pylori*, hepatitis A virus (HAV), rotavirus, tetanus toxoid immunoglobulin (Ig) G, and recombinant malarial surface proteins, by group and overall for each year[1]

	Meat		Milk		Nonanimal-source food		Control		Overall	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
H. pylori IgA (U)	1.47 ± 0.32 [48]	3.55 ± 0.82[2] [50]	2.75 ± 0.30 [44]	2.54 ± 0.59[2] [50]	3.21 ± 0.47 [47]	2.28 ± 0.30[2] [50]	2.41 ± 0.20 [46]	2.12 ± 0.27[2] [49]	2.45 ± 0.18 [185]	2.62 ± 0.27[2] [199]
>15 U (%)	0	2.0	0	2.0	2.1	0	0	0	0.5	1.0
H. pylori IgG (U)	26.21 ± 3.02 [48]	34.89 ± 3.78 [50]	42.34 ± 4.68 [44]	27.92 ± 4.47 [50]	40.16 ± 4.29 [47]	33.64 ± 4.31 [50]	29.55 ± 3.56 [46]	22.98 ± 3.52 [48]	34.42 ± 2.00 [185]	29.92 ± 2.04 [198]
>15 U	68.8	78.0	86.4	60.0	80.9	74.0	69.6	58.3	76.2	67.7
H. pylori IgM (U)	26.00 ± 4.20 [48]	22.02 ± 3.33 [50]	7.17 ± 1.11 [44]	9.90 ± 1.60 [50]	18.79 ± 4.60 [47]	10.28 ± 1.84 [49]	7.35 ± 1.50 [46]	4.77 ± 0.66 [49]	15.05 ± 1.75 [185]	11.78 ± 1.14 [198]
>15 U	45.8	46.0	6.8	20.0	19.1	20.4	6.5	4.1	20.0	22.7
HAV > 20 mIU/mL (%)	76.1 [48]	78.3 [50]	67.4 [43]	69.8 [50]	52.4 [46]	54.3 [50]	56.5 [46]	54.3 [49]	63.2 [182]	65.3 [199]
Transformed rotavirus	4.46 ± 0.17 [48]	2.10 ± 0.23[2] [50]	2.41 ± 0.27 [44]	2.98 ± 0.24[2] [50]	2.87 ± 0.21 [47]	2.26 ± 0.20[2] [50]	3.33 ± 0.27 [46]	2.29 ± 0.23[2] [49]	3.29 ± 0.13 [185]	2.41 ± 0.12[2] [199]
>1:8 (%)	97.9	78.0	81.8	92.0	91.5	84.0	84.8	79.6	89.2	83.4
Tetanus toxoid IgG (IU/mL)	1.01 ± 0.62 [48]	0.33 ± 0.08 [50]	0.30 ± 0.07 [44]	0.43 ± 0.18 [50]	2.16 ± 0.94 [47]	0.86 ± 0.32 [50]	0.50 ± 0.19 [46]	0.44 ± 0.25 [49]	1.01 ± 0.29 [185]	0.52 ± 0.11 [199]
>0.1 IU/mL (%)	64.6	58.0	56.8	50.0	70.2	46.0	60.9	51.0	63.2	51.3
Recombinant malaria antibody (OD)	1.19 ± 0.04 [48]	1.22 ± 0.05 [35]	0.88 ± 0.07 [45]	0.87 ± 0.08 [47]	0.90 ± 0.08 [49]	1.0 ± 0.09 [50]	0.79 ± 0.08 [46]	0.77 ± 0.07 [49]	0.94 ± 0.04 [188]	0.95 ± 0.04 [181]
>0.53 OD (%)[3]	95.8	94.3	68.9	63.8	65.3	66.0	58.7	61.2	72.3	69.6

[1] $\bar{x}$ ± SE; *n* in brackets. The immunoglobulins were measured by enzyme immunoassay and the rotavirus by complement fixation. OD, optical density.
[2] Significantly different from baseline, $P < 0.05$.
[3] High malarial response.

TABLE 4
Antibody titers from 1998 to 1999[1]

Change in	Meat	Milk	Nonanimal-source food	Control	P^2	Overall	P^3
Helicobacter pylori							
IgA (U)	1.40 ± 0.43	−0.23 ± 0.35	−0.91 ± 0.27	−0.50 ± 0.17	0.16	−0.12 ± 0.17	0.37
IgG (U)	8.43 ± 3.21[a]	−13.63 ± 2.64[b]	−7.13 ± 3.25[a,b]	−6.18 ± 1.72[a,b]	<0.0001	−4.39 ± 1.51	<0.0001
IgM (U)	−3.70 ± 3.84	2.51 ± 0.88	−8.24 ± 3.71	−2.47 ± 1.21	0.030	−3.05 ± 1.44	0.32
Geometric $\bar{x}$[4]	2.68[a]	0.52[a,b]	−1.45[a,b]	−2.65[b]			
Transformed rotavirus	−2.33 ± 0.27	0.59 ± 0.21	−0.57 ± 0.32	−1.07 ± 0.32	0.28	−0.88 ± 0.16	<0.0001
Tetanus toxoid IgG (IU/mL)	−0.67 ± 0.61	−0.00 ± 0.14	−1.40 ± 0.83	−0.04 ± 0.32	0.31[5]	−0.54 ± 0.28	<0.0001
Recombinant malaria antibody (OD)	−0.00 ± 0.04	−0.01 ± 0.04	0.09 ± 0.03	−0.05 ± 0.04	0.83	0.01 ± 0.02	0.52

[1] $\bar{x}$ ± SE. The immunoglobulins were measured by enzyme immunoassay and the rotavirus by complement fixation. OD, optical density. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Determined with analysis of covariance, by control for initial value, group, and school (group) as predictor variables; outcome variable was change.
[3] Determined by paired *t* tests comparing 1998 and 1999 values.
[4] Included because they better reflect the adjusted transformed means used in the analysis of covariance.
[5] Determined by ranking procedure.

different ($P < 0.05$) among groups at baseline. The mean *H. pylori* IgA concentration was lower in the meat group than in the other groups, whereas the opposite was true for rotavirus on complement fixation.

Effects of the intervention

Whereas samples from the meat group showed a significantly different ($P < 0.040$) change in titers of IgG antibody to *H. pylori* from that shown by samples from the milk group, there was no statistically significant difference between these values and the values from samples from the control group or the nonanimal-source food group (**Table 4**). Specifically, the meat group had an increase in total *H. pylori* antibody titers from baseline, while the other 3 groups showed a decrease in antibody titers. The meat group also showed an increase in *H. pylori* IgM antibody titers between the baseline and posttreatment measurements that was significantly different from the decrease in the control group ($P < 0.019$). There were no significant differences between treatment groups for the changes in any of the other antibody titers.

Tetanus toxoid IgG and immunization data

The proportion of children with a low titer of IgG antibody (< 0.1 IU/mL) to tetanus toxoid was low. With the use of Pearson's chi-square test, however, the values were not significantly different between those who had only 1 immunization and those who had all 3 immunizations, at baseline (33.3% and 35.7%, respectively; $P = 0.85$) or 1 y later (60.0% and 45.1%; $P = 0.30$).

Associations between *H. pylori* antibodies and concentrations of hemoglobin, ferritin, and vitamin B-12

No significant associations between *H. pylori* IgA or IgG and hemoglobin, ferritin, and vitamin B-12 concentrations were observed at baseline, even after we controlled for age, sex, SES, HAZ, and WHZ. However, children with anemia had a significantly ($P < 0.0033$) greater proportion of elevated titers of IgM antibody to *H. pylori* (29.6%) than did children with normal hemoglobin concentrations (11.6%). The proportion of children with elevated titers of IgM antibody to *H. pylori* was also significantly ($P = 0.037$) greater among those with severe and moderate vitamin B-12 deficiency (27.8% and 19.1%, respectively) than it was among those with normal concentrations at baseline (12.1%), but this difference became nonsignificant ($P = 0.42$) after we controlled for sex, age, SES, and WHZ. There was no significant association between *H. pylori* IgM and low ferritin concentrations. After 1 y, there were no significant associations between IgA, IgG, or IgM and low hemoglobin, ferritin, and vitamin B-12 status, even after we controlled for age, sex, SES, HAZ, and WHZ.

DISCUSSION

According to their biochemical indicators, the children in this study had a high prevalence of anemia and deficiencies in zinc, vitamin B-12, retinol, and riboflavin at baseline, and yet the results of the battery of antibody tests showed that they also had reasonable to high titers of antibody to nearly all the measured pathogens. However, hemoglobin, zinc, retinol, and ferritin concentrations are altered in the presence of subclinical and clinical infection, and where infections are widespread, those values do not necessarily reflect micronutrient status. The data collected in this study suggest either that these children were not truly micronutrient deficient or that the micronutrient deficiencies observed in them did not impair their ability to mount an antibody response to infection. The data also could mean that such infections are endemic in this population, and thus chronic exposure may lead to sustained concentrations of antibodies not otherwise noted in persons residing in industrialized countries. For example, antibodies to HAV were found at baseline in a much higher percentage of children in our study (63.2%) than in 3–7-y-old children in a small US city on the border between the United States and Mexico, where 16.9% tested positive for this virus (19). Furthermore, 76.2% of the children in our study had elevated *H. pylori* IgG antibody titers at baseline, whereas they have been found in < 10% of Japanese children aged 5–9 y (20) and in 30–40% of children in the general US population. More than two-thirds of the blood samples from the Kenyan children had elevated titers of antibody to rotavirus and to the malarial merozoite surface proteins $MSP1_{19}$, MSP2, Ag512, MSP4, and MSP5 at baseline and after 1 y of supplementary feeding.

Supplementation with meat correlated with a statistically significant increase in *H. pylori* IgM antibody titer compared with the control group over the school-year intervention. Because this isotype is short-lived and because it indicates current or recent infection, the significant increase in the meat group suggests either that improved nutritional status through increased meat consumption

enabled the children to mount higher titers of antibody to *H. pylori* infection or that children who consumed meat had a greater prevalence of *H. pylori* infection than children in the other groups. However, it is not certain that this small yet statistically significant increase in *H. pylori* IgM is biologically meaningful, and because we made a number of statistical comparisons, some may be significant by chance alone. In addition, the fact that the direction of the difference changed with adjustment for school (group) raises the possibility not only of a chance finding, but also of benefit to some groups but not to others. Although the actual route of transmission of *H. pylori* is unknown, we cannot exclude the possibility that the meat was a carrier of *H. pylori*. Our data are interesting because of the interactions of *H. pylori* and iron absorption. Further studies on the immune response to *H. pylori*, under conditions of nutritional deficiencies, are warranted. It will also be helpful in future studies to determine the actual rate of infection in these children, not only with *H. pylori*, but also with the other infectious agents studied herein. Indeed, it remains possible that multiple and repeated exposures may boost the immune system and lead to antibody production even under conditions of nutritional deficiency.

The intervention with meat or milk had no detectable effect on titers of IgG antibody to tetanus toxoid compared with the control, possibly because power was insufficient or because there were no significant differences in the changes in vitamin A status (plasma retinol) between the groups (JH Siekmann, unpublished observations, 2002). Vitamin A is important in this context because tetanus toxoid IgG antibody is reduced in vitamin A–deficient children (21). Thus, several investigators have measured the IgG response to tetanus toxoid after vitamin A supplementation in immunized children, with mixed results. In one study, infants aged 2 mo were given supplements of vitamin A or vitamin E or both, or they were given neither. The infants' IgG response to tetanus toxoid after primary immunization was evaluated up to 18 mo of age (22). No significant difference in serum tetanus IgG concentrations was observed between the groups, but these infants were apparently healthy and had normal plasma vitamin A and vitamin E concentrations at baseline (22). In vitamin A–deficient Bangladeshi preschoolers, tetanus toxoid IgG antibody titers remained low, even after vitamin A supplementation (23), but in a study of older preschoolers in Indonesia, 200 000 IU vitamin A administered 3 wk before tetanus toxoid immunization resulted in significantly enhanced IgG antibody titers (7). In addition, we suggest that future studies explore other readouts to determine the relation between micronutrient status indicators and immune responses, including, for example, IgG subclass responses and cytokine production.

At baseline, 36.8% ($n = 68$) of the Kenyan children had tetanus toxoid IgG concentrations below the protective concentration, which suggests that they had incomplete vaccination or none. Oddly, the proportion of those who had a low titer (< 0.1 IU/mL) of antibody to tetanus toxoid IgG was not significantly different at baseline ($P = 0.85$) or 1 y later (August 1999) ($P = 0.30$) between those who had only 1 or all 3 immunizations. Because completion of the 3-vaccination series results in tetanus toxoid IgG antibody titers greater than the minimum 0.1 IU/mL threshold needed for protection in nearly 100% of Americans for up to 10 y (24), the lack of difference even in the children with all 3 vaccinations suggests that those vaccinations could have been faulty, possibly as a result of improper vaccine storage. In light of the fact that titers of antibodies to most other antigens were high, this possibility may partially explain the failure of the intervention to significantly increase tetanus toxoid IgG antibody titers. Unfortunately, detailed vaccination records for the Kenyan children are unavailable.

H. pylori is a gram-negative bacillus that has a causal role in the pathogenesis of gastroduodenal diseases such as nonulcer dyspepsia, chronic inflammation, and ulcers. Serologic examination for *H. pylori* IgA, IgG, and IgM antibodies using ELISA methods cannot replace gastroscopy for definitive diagnosis, but the presence of such antibodies does provide evidence of previous infections and exposure (25). IgG is a stable, long-term antibody that reflects any past infection and that may return to normal concentrations after successful treatment. *H. pylori* IgG antibodies are thought to be a marker for chronic infection with this bacterium, but they provide no information on the duration of the infection. *H. pylori* IgM antibodies, however, are a more specific marker for recently acquired *H. pylori* infection (26). High concentrations of IgM antibodies to *H. pylori* are interpreted as a sign of acute infection (27).

Overall, the high prevalence of elevated *H. pylori* IgG and IgM antibody titers in our population suggests that a large percentage of the children were currently or chronically infected with *H. pylori*. Because this could be a factor contributing to the high prevalences of anemia (GJ Fuchs, SA Sarker, L Davidsson, unpublished observations, 2001) and vitamin B-12 deficiency (17), we measured the association between elevated (> 15 EIA U) *H. pylori* IgA, IgG, and IgM antibody titers and anemia, low ferritin, and low vitamin B-12 at baseline. IgA and IgG were not significantly associated with anemia, low ferritin, or low vitamin B-12 at baseline or after 1 y. However, a higher proportion of anemic children than of nonanemic children had elevated IgM antibody titers at baseline, even after we controlled for covariates such as age, sex, SES, HAZ, and WHZ. This finding is consistent with the hypothesis that current infection with *H. pylori* is associated with anemia. After 1 y, however, this association was nonsignificant when we controlled for the same covariates. The lack of a significant association between anemia and *H. pylori* IgM after 1 y may be partially explained by an observed increase in hemoglobin concentration and a subsequent decrease in the prevalence of anemia across all groups.

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