The Journal of Nutrition
Community and International Nutrition

Low Plasma Vitamin B-12 in Kenyan School Children Is Highly Prevalent and Improved by Supplemental Animal Source Foods[1]

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Abstract

The high prevalence of vitamin B-12 deficiency in many regions of the world is becoming recognized as a widespread public health problem, but it is not known to what extent this deficiency results from a low intake of the vitamin or from its malabsorption from food. In rural Kenya, where a previous study identified a high prevalence of inadequate vitamin B-12 intakes, this study examined whether plasma vitamin B-12 concentrations were associated with dietary sources of the vitamin at baseline and could be increased by supplementation with animal source foods (ASF). The 4 experimental groups in 503 school children were: *1*) control (no food provided); *2*) *githeri* (a maize and bean staple with added oil); *3*) *githeri* + meat (githeri + minced beef); or *4*) *githeri* + milk (githeri + milk). Feedings were isocaloric. Dietary data were collected at baseline, and biochemical data at baseline and after 1 and 2 y of feeding. Baseline plasma vitamin B-12 concentration was 193.6 ± 105.3 pmol/L and correlated with % energy from ASF ($r = 0.308$, $P < 0.001$). The odds ratio for low plasma vitamin B-12 (<148 pmol/L), which occurred in 40% of children, was 6.28 [95% CI: 3.07–12.82] for the lowest vs. highest ASF intake tertile ($P < 0.001$). Feeding ASF (meat or milk) greatly reduced the prevalence of low plasma vitamin B-12 ($P < 0.001$). The high prevalence of low plasma vitamin B-12 concentrations in these children is predicted by a low intake of ASF, and supplemental ASF improves vitamin B-12 status. J. Nutr. 137: 676–682, 2007.

Introduction

Research in low-income countries has revealed a high prevalence of low plasma vitamin B-12 concentrations. Plasma concentrations of the vitamin are a relatively good indicator of body stores (1,2) and low values have been associated with impaired function in adults and children (3,4). More than 40% of the population groups studied in 9 reports from Latin America had low (<148 pmol/L) or marginal (148–221 pmol/L) plasma vitamin B-12 concentrations (1). In an Indian study, conducted where no or relatively small amounts of animal source foods (ASF)[8] are consumed, 47% of adults had plasma vitamin B-12 concentrations <150 pmol/L (5). Few data are available from African populations. In Nigeria, 9% of 162 girls aged 12 to 16 y had serum vitamin B-12 concentrations <134 pmol/L, with the distribution of concentrations skewed toward the lower end of the reference range for that age (6). In contrast, in Botswana, few children aged 5 to 11 y had low plasma vitamin B-12 concentrations (<148 pmol/L), possibly due to higher ASF consumption (7). In 164 rural South African children aged 2 to 5 y, the intake of vitamin B-12 was found to be extremely low (median intake 0 μg/d) based on usual ASF intake (8). Dietary data from Kenya revealed that vitamin B-12, and other nutrients derived primarily from ASF, was low in the diet (9) and that 87% of school children (aged 7–9 y) consumed inadequate amounts of dietary vitamin B-12 to meet their estimated average requirement (10). School children in the same region of Kenya are the subjects of the current investigation.

The high prevalence of vitamin B-12 deficiency in developing countries may be caused by a low intake of ASF, which is the only dietary source of the vitamin except in the unusual situation where foods are fortified with vitamin B-12. Thus, increasing ASF intake should reduce the prevalence of vitamin B-12 deficiency. A possible alternative explanation is malabsorption of the food-bound vitamin due to infection with *Helicobacter pylori* (11) or bacterial overgrowth (12). In this case, ASF-based interventions might be less effective for improving vitamin B-12 status.

In this study, vitamin B-12 status was investigated as a substudy within the framework of the Global Livestock Collaborative Research Support Program project titled "Role of Animal

[1] Supported by The Global Livestock CRSP under the auspices of the Global Bureau of the US Agency for International Development, grant PCE-G-00-98-000036-00.

[8] Abbreviations used: ANCOVA, analysis of covariance; ASF, animal source foods; CRP, C-reactive protein; HAZ, height-for-age Z; Hcy, homocysteine; MMA, methylmalonic acid; OR, odds ratio; RAE, retinol activity equivalents.

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Manuscript received 22 September 2006. Initial review completed 6 January 2006. Revision accepted 30 November 2006.

Source Foods to Improve Diet Quality and Growth and Cognitive Development in East African Children" (13). The first question addressed was whether plasma vitamin B-12 concentrations were related to dietary intake of the vitamin at baseline. The second question was whether increasing the intake of milk or meat would improve the children's vitamin B-12 status after 1 or 2 y of feeding. Data on plasma vitamin B-12 changes after 1 y of supplementation has been presented elsewhere (14), as have additional details of the goals, methods, and results of the larger Global Livestock Collaborative Research Support Program project (13).

Subjects and Methods

Location and population. The study was conducted in Kyeni South Division of Embu District in Kenya, 150 km northeast of Nairobi and close to the base of Mount Kenya. The site was selected because it was the location of the Nutrition Collaborative Research Support Program, which documented a low intake of ASF and vitamin B-12 in the 1980s (15). The semi-arid, rural region experiences seasonal food shortages due to drought, and farming is the primary occupation. The staple crops and foods are maize and beans. *Plasmodium falciparum* is endemic in the area.

Subjects were 503 school children aged 5 to 14 y. They were recruited through Embu District schools after permission was granted by the Director of the District Ministry of Education on the Central, District, and Zonal levels and the head teachers of the schools. The staple foods consumed by this population are maize and beans. During this study, rainfall was low and poor yields of the staple crops were reported.

Informed consent procedures. Meetings were held in the communities to inform parents about the study. Verbal consent was obtained from parents in parent-teacher meetings at school or at home on an individual basis. Field staff informed children throughout the study that they could choose not to participate at all or to participate in only part of the study. The University of California, Los Angeles Human Subject Protection Committee, the Office of the President of the University of Nairobi, the Kenya Ministries of Education and Health, and the University of California, Davis Human Subjects Review Committee approved the research protocols.

Experimental design. The effect of increasing ASF intake on vitamin B-12 status was assessed by randomly assigning 12 primary schools, at the school level, to 1 of 4 experimental groups: *1*) control: no food provided ($n = 120$); *2*) githeri snack: a vegetarian stew of maize, beans, and vegetables ($n = 133$); *3*) githeri + milk snack ($n = 131$); and *4*) githeri + meat snack ($n = 119$) (16). Feeding took place 5 d/wk when the children were in school, which was 9 mo/y (3 feeding terms of 4 mo, consisting of 3 mo in school with 1 mo out of school) for 2 y. Children were observed during the meal to check that they ate the snack provided to them and any leftovers (which were few) were weighed and recorded.

During the first feeding term, children in the githeri + milk group received 200 mL of milk and those in the githeri + meat group received 60 g of minced beef in addition to the githeri. After the first term, the food given to all 3 groups was increased, with those in the githeri + milk group receiving 250 mL of milk and children in the githeri + meat group receiving 85 g beef (weight before cooking) in addition to the githeri (**Table 1**). Snacks were relatively isocaloric starting at 239–241 kcal (~1000–1010 kJ) and increasing to 291–311 kcal (~1218–1302 kJ) across the intervention groups. After accounting for absences and leftovers, children consumed 76% of the possible amount of energy in the

TABLE 1 Estimated energy and micronutrient content of the food supplements[1]

		% of DRI[3] provided			% of DRI provided	
Nutrient	1st feeding term[2]	4–8 y	9–13 y	Other feeding terms[4]	4–8 y	9–13 y
Githeri						
Energy, *kJ (kcal)*	1003 (239)	—	—	1299 (311)	—	—
Vitamin B-12, *μg*	0	0	0	0	0	0
Vitamin A[5], *μg RAE*	210	53	35	364	91	61
Iron, *mg*	3.16	32	40	3.93	39	49
Riboflavin, *mg*	0.12	20	13	0.15	25	17
Zinc, *mg*	1.35	27	17	1.68	34	21
Githeri + meat						
Energy, *kJ/kcal*	999 (259)	—	—	1215 (291)	—	—
Vitamin B-12, *μg*	0.90	75	50	1.27	106	71
Vitamin A[6], *μg RAE*	112	28	19	112	28	19
Iron, *mg*	2.42	24	30	2.94	29	37
Riboflavin, *mg*	0.12	20	13	0.15	25	17
Zinc, *mg*	2.38	48	30	2.89	58	36
Githeri + milk						
Energy, *kJ/kcal*	1007 (262)	—	—	1219 (292)	—	—
Vitamin B-12, *μg*	0.8	67	44	1.00	83	56
Vitamin A[5], *μg RAE*	244	61	41	412	103	69
Iron, *mg*	1.52	15	19	1.57	16	20
Riboflavin, *mg*	0.44	73	49	0.53	88	59
Zinc, *mg*	1.46	29	18	1.66	33	21

[1] Estimated by Murphy et al. (16) with the WorldFood nutrient database. Includes retinol activity equivalents (RAE) from vegetable spread for *githeri + meat* and *githeri* groups, and RAE from milk and vegetable spread in the *githeri + milk* group. RAE from vegetable spread was calculated based on a content of 70 μg/g.
[2] Term 3, 1998.
[3] DRI, Dietary recommended intake.
[4] Terms 1, 2, and 3, 1999; and Terms 1 and 2, 2000.
[5] Vitamin A is from retinyl palmitate (70 μg/g) added to fortified margarine.
[6] Includes vitamin A in milk and the fortified cooking fat.

githeri group, 89% in the githeri + milk group, and 93% in the githeri + meat group. The amount of vitamin B-12 provided was similar in the 2 ASF groups, starting at 0.90 μg and increasing to 1.27 μg in the githeri + meat group and increasing from 0.80 μg to 1.00 μg in the githeri + milk group.

Dietary information. A 24-h recall questionnaire was administered by trained interviewers to obtain information about the study child's diet on 3 separate occasions prior to the intervention and every 2–3 mo during the study (16). The children's mothers were asked about everything their child ate and drank during the previous 24 h. For recipes, mothers provided information on the amounts of ingredients and the child's portion was estimated using household measures such as spoons and cups. The intake of energy, macronutrients, micronutrients, dietary fiber, and phytate in the usual diet was determined using WorldFood Dietary Assessment System Version 2.0. Analyses here are based on the means of the 3 dietary recalls prior to the intervention.

Clinical and biochemical data. Children received a general medical examination at baseline and after 1 and 2 y of feeding. At these times, 2 tubes of blood (10–12 mL total) were collected from each child and transported on ice to the district hospital in Embu (a 40-min drive). All samples were processed within 6 h of collection. Both tubes of blood were centrifuged and aliquots of serum and EDTA plasma frozen at −20°C. Samples were transported on dry ice to Nairobi and flown to the University of California, Davis for analyses. Persons who performed laboratory analyses were unaware of group assignments. Blood could not be drawn from some children at all time points due to parental refusal and absenteeism.

Plasma vitamin B-12 was determined in duplicate by radioassay (ICN Diagnostics). Children were categorized as having low vitamin B-12 status if their plasma concentration was <148 pmol/L and marginal status if the concentration was 148–221 pmol/L. Plasma vitamin B-12 concentration >221 pmol/L was considered normal (3).

C-reactive protein (CRP) was measured in plasma by radial immunodiffusion (The Binding Site) and elevated CRP defined as >10 mg/L.

Malaria infection was detected by the antigen strip test (Vision Biotech). This test detects the presence of antigens produced by *Plasmodium falciparum*, which accounts for 80–90% of all malaria cases in Kenya (17).

The physicians at the clinical exam diagnosed enlarged spleen, a sign of endemic malaria. Height and weight were measured throughout the intervention and results have been reported elsewhere (18). Only baseline anthropometry is presented here to describe the population. Z scores were calculated based on the CDC growth charts (19).

Statistical analysis. SAS (V. 8.2, SAS Institute) was used for statistical analyses. To meet normality assumptions, plasma vitamin B-12 was transformed by taking its natural log. Baseline characteristics of the population were compared among groups using the SAS GLM procedure.

The intake of ASF and their contribution to total energy intake were calculated to determine whether ASF consumption predicted baseline plasma vitamin B-12 concentration. The association between plasma vitamin B-12 and vitamin B-12 intake was assessed using Spearman correlations. To determine whether children who had lower intakes of ASF were at a greater risk of having lower plasma vitamin B-12, they were categorized into ASF consumption tertiles, or when consumption was low, into 2 consumption categories (none or some). The odds of having low (<148 pmol/L) or low or marginal (<221 pmol/L) plasma vitamin B-12 concentrations were calculated using logistic regression and controlling for age and sex.

A mixed ANOVA model was implemented with the SAS GLM procedure to assess the impact of the intervention on plasma vitamin B-12 concentrations. Explanatory variables included group (nested within the random effect of school), time, group by time interaction, and malaria, enlarged spleen, and elevated CRP as time-dependent covariates. School and subject were included as random effects. When a significant group by time interaction was detected, analysis of covariance (ANCOVA) was performed separately on plasma vitamin B-12 concentration at the end of years 1 and 2 to examine the nature of the interaction and to control for the initial differences in plasma vitamin B-12 between groups. Other covariates included age, gender, malaria infection, elevated CRP, enlarged spleen, and interactions between illness/inflammation indicators. Additionally, if higher-level significant interactions involving both group and time were found, analyses were performed by assigning data from children to 2 separate groups, to examine how the outcome was different in those affected by the variables contributing to the interaction. Presence of intestinal parasites was not used as a covariate in any analyses, because it was shown previously not to be associated with plasma vitamin B-12 in these children (14). For data from a child to be included in these analyses, it was necessary to have biochemical data for at least 2 of the 3 time points ($n = 481$).

Differences in the prevalence of low, and low + marginal, plasma vitamin B-12 concentrations between groups were assessed at each time point by Generalized Estimating Equations utilizing the GENMOD procedure. If children switched schools, they were assigned to the new treatment group as long as they had consumed the snack assigned to that school for at least 6 mo. Unless noted otherwise, values in the text are means ± SD.

Results

Description of children at baseline. The children were 7.6 ± 1.4 y, ranging from 6 to 14 y. The age did not differ by intervention group when assessed by pairwise comparisons (**Table 2**). They were moderately stunted: 32.0% of children had a height-for-age Z (HAZ) score <−2 and 5.5% had a HAZ score <−3. Stunting differed by intervention group at baseline with the greatest number of children <−3 in the *githeri* + meat group. However, wasting was less prevalent: the weight-for-height Z score was <−2 for 3.7% of the children. Indicators of infection (malaria and enlarged spleen) differed by group at baseline, as did low plasma vitamin B-12 concentrations, with the highest prevalence in the *githeri* + meat group ($P < 0.001$). There was a negative correlation, $r = 0.28$ ($P < 0.001$) between age and plasma vitamin B-12 concentration at baseline, suggesting that plasma vitamin B-12 falls with age in these children.

Relation of baseline plasma vitamin B-12 to usual diet. Average energy intake was 1714 ± 440 kcal/d (1841 kJ/d), with 51% from carbohydrates (Table 1). The intake of vitamin B-12 was 0.51 ± 1.48 μg/d (median = 0.31 μg/d), substantially less than the estimated average requirement for children aged 4–8 y (1.0 μg/d) (3). Of the 533 children, 41 (7.7%) reported consuming no vitamin B-12 on any of the recall days. ASF provided only 4.2 ± 4.2% of total energy, of which 21.5% was from meat, 73.8% from milk, and 4.7% from eggs. Meat provided 27.7% of the vitamin B-12 in the diet, compared with 70.0% from milk and 2.3% from eggs.

Plasma vitamin B-12 concentrations were predicted by the % of energy from ASF ($r = 0.308$, $P < 0.001$), milk ($r = 0.266$, $P < 0.001$), meat ($r = 0.172$, $P < 0.001$), and eggs ($r = 0.145$, $P = 0.001$) in the diet. The total amount of vitamin B-12 provided by these foods gave similar predictions for plasma vitamin B-12 concentration to those obtained by expressing intake as % energy from these sources, except that the correlation with total vitamin B-12 from meat was not significant. Milk was consumed in much higher quantities than meat and was the strongest dietary predictor of plasma vitamin B-12 concentration.

The odds ratio (OR) of having a low plasma vitamin B-12 concentration was 6.28 ($P < 0.001$) for children in the lowest vs. highest consumption tertile for "All ASF", 2.15 ($P = 0.002$) for those in the "none" vs. "some" meat group, and 4.99 ($P < 0.001$) for those in the lowest vs. highest tertile of egg

TABLE 2 Descriptive characteristics of subjects at baseline[1]

Variable	Control, $n = 120$[2]	*Githeri*, $n = 133$[2]	*Githeri* + meat, $n = 119$[2]	*Githeri* + milk, $n = 131$[2]	*P*-value[3]
Gender, *% male*	51.2	51.4	52.4	54.7	0.93
Age, *y*	7.42 ± 1.22	7.55 ± 1.54	7.99 ± 1.28	7.51 ± 1.31	0.06
Weight-for-height Z[4]	−0.42 ± 0.87	−0.28 ± 0.84	−0.58 ± 0.91	−0.53 ± 0.82	0.80
Height-for-age Z[4]	−1.39 ± 1.06	−1.54 ± 0.98	−1.71 ± 0.94	−1.49 ± 0.93	0.75
Weight-for-age Z[4]	−1.32 ± 0.99	−1.38 ± 0.92	−1.62 ± 0.97	−1.42 ± 0.86	0.55
MUAC,[4] *cm*	15.80 ± 1.10	15.93 ± 1.11	15.84 ± 1.25	15.84 ± 0.92	0.48
Elevated CRP,[5] *%*	20.5	16.7	23.8	11.2	0.05
Malaria positive,[6] *%*	14.8	12.6	29.9	9.4	<0.001
Enlarged spleen, *%*	45.8	39.7	46.3	25.2	0.001
Plasma vitamin B-12, *pmol/L*	292 ± 144	277 ± 138	212 ± 119	246 ± 108	0.02[7]
<148 pmol/L, *%*	32.5	32.0	56.2	40.2	<0.001
<221 pmol/L, *%*	57.5	64.1	81.8	71.2	<0.001

[1] Values are means ± SD or %.
[2] The number of subjects used in analysis of each variable varied depending on the data available: Control (92–120), *Githeri* (106–133), *Githeri + meat* (86–119), *Githeri + milk* (105–131).
[3] From ANOVA on categorical variables.
[4] CDC reference values (2000) (19). MUAC, mid-upper arm circumference.
[5] Elevated CRP = >10 mg/L.
[6] Positive malaria based on an antigen strip test to detect *Plasmodium falciparum*.
[7] *P*-value tested on log-transformed variable, due to skewness.

consumption (**Table 3**). Using marginal plasma vitamin B-12 as the outcome yielded similar but nonsignificant trends.

Impact of ASF supplements on plasma vitamin B-12 concentration. Controlling only for between-school differences, plasma vitamin B-12 was different among groups at baseline ($P = 0.03$). The githeri + meat group had a lower plasma vitamin B-12 concentration than the githeri group ($P = 0.05$) and tended to have a lower concentration than the control group ($P = 0.09$).

Mixed model ANOVA, controlling for the effect of school on group, indicated that there was a group by time interaction ($P < 0.001$), which allowed exploration of the changes by intervention group. Significant 4-way (group × year × CRP × malaria) and 3-way (group × year × malaria) interactions were found. We assumed that these interactions were due to measuring status at the time of illness (elevated CRP or malaria infection) and not to the treatment itself; mixed model ANOVA was performed controlling for elevated CRP, malaria, enlarged spleen, and school × group interactions. This analysis revealed that the *githeri* + meat and *githeri* + milk groups had lower adjusted plasma vitamin B-12 concentrations at baseline than the *githeri* or control groups ($P < 0.002$ for all comparisons). After 2 y of feeding, the plasma vitamin B-12 in both the meat- and milk-supplemented groups improved further. Concentrations in the meat group were now higher than in the *githeri* group ($P < 0.001$) or control group ($P = 0.003$), whereas in the *githeri* + milk group, they were higher than in the *githeri* group ($P = 0.004$) and marginally higher than in the control group ($P = 0.072$) (**Table 4**).

Because randomization into the 4 feeding groups was across only 12 schools, there were some differences in baseline values among the groups, and the *githeri* + milk and *githeri* + meat groups started out with lower plasma vitamin B-12 concentrations. For this reason, and to control for age and sex, a follow-up ANCOVA was performed, controlling for malaria and elevated CRP, baseline vitamin B-12 concentration, gender, age, enlarged spleen, and interactions. Adjusting the means for these covariates revealed that the *githeri* + meat snack resulted in higher plasma vitamin B-12 concentrations than in the *githeri* and control groups after either 1 ($P < 0.009$ for both) or 2 y ($P < 0.001$ for both). The concentration in the *githeri* + milk group was also higher than in the 2 non-ASF groups at both time points (year 1, $P < 0.009$ compared with both groups; year 2, $P < 0.003$ compared with both groups). Additionally, after year 2, the plasma vitamin B-12 in the *githeri* + meat group was higher than in the *githeri* + milk group ($P = 0.005$) (Table 4).

Mixed model ANOVA using data only from children who were "healthy" (no elevated CRP, malaria infection, or enlarged spleen at time of blood draw) on at least 2 time points resulted in a large loss of sample size (from $n = 481$ to $n = 266$). Because children with malaria or elevated CRP were excluded, only the effect of school on group was controlled for in the analysis. This analysis

TABLE 3 OR of low plasma vitamin B-12 concentration in children by intake of ASF

	Total ASF tertile			Meat		Milk tertile			Eggs	
Intake	T_1	T_2	T_3	None	Some	T_1	T_2	T_3	None	Some
% of energy	0–1.3	1.4–4.2	4.2–37.1	0	0–37.1	0–1.1	1.1–3.5	3.5–25.5	0	2.1–9.7
n[1]	158	161	161	405	75	193	134	153	468	12
OR, low vitamin B-12[2]	6.3 (3.1–12.8)[3]	1.6 (1.0–2.7)	1	2.1 (1.3–3.5)	1	5.0 (2.5–9.8)	1.6 (0.9–2.6)	1	2.1 (0.9–4.9)	1
P	<0.001	0.105		0.002		<0.001	0.17		0.069	

[1] *n* differs for each intake tertile due to variability in number of subjects with plasma vitamin B-12 concentration data.
[2] Low plasma vitamin B-12 concentration = <148 pmol/L.
[3] 95%[CI].

TABLE 4 Plasma vitamin B-12 concentrations in children by year and intervention group analyzed by mixed model ANOVA and ANCOVA[1]

Time	Control	*Githeri*	*Githeri* + meat	*Githeri* + milk
		Mixed model ANOVA[2]		
Baseline	198[a] (186–211)[3]	184[a] (173–196)	136[b] (129–144)	155[b] (145–165)
Year 1	160[a] (148–173)	160[a] (147–174)	183[a,b] (170–196)	206[b] (190–223)
Year 2	193[a] (177–210)	203[a,b] (187–220)	251[c] (232–273)	237[b,c] (219–256)
		ANCOVA[4]		
Year 1	152[a] (132–175)	156[a] (138–177)	221[b] (197–248)	221[b] (195–249)
Year 2	182[a] (169–197)	188[a] (177–200)	309[b] (290–330)	257[ab] (243–272)

[1] Values are adjusted geometric group means [95%CI], total $n = 481$. Means in a row without a common letter differ, $P < 0.009$.
[2] Mixed model ANOVA of plasma vitamin B-12, adjusted for malaria, elevated CRP, enlarged spleen, and random school effect nested within group, and ANCOVA of plasma vitamin B-12;
[3] 95%[CI].
[4] No baseline mean values are presented. ANCOVA is adjusted for age and gender in addition to the variables adjusted for in the mixed model ANOVA.

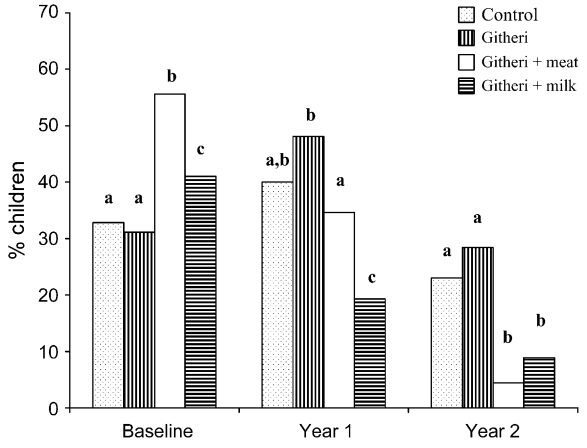

Figure 1 Prevalence of children in each intervention group with low plasma vitamin B-12 concentration (<148 pmol/L) at baseline and after 1 and 2 y of intervention.

revealed the same trend as the previous analysis, in that *githeri* + milk and *githeri* + meat groups tended to fare better than the non-ASF groups ($P = 0.063$); the lack of significant differences likely was due to the smaller sample size and consequent loss of statistical power. A follow-up ANCOVA, which yielded more power by controlling for initial plasma vitamin B-12 concentration, age, gender, enlarged spleen, and school × group interaction, demonstrated the same trend and revealed that plasma vitamin B-12 concentrations were higher in the *githeri* + milk group than in the 2 non-ASF groups ($P < 0.011$) after 1 y, and were higher in the *githeri* + meat group than the *githeri* + milk group ($P = 0.037$) and the 2 non-ASF groups ($P < 0.006$) after 2 y. Children who were excluded from the above analysis as a result of 1 or more positive indicators of infection showed the same trend: both ASF groups had significantly higher plasma vitamin B-12 than either non-ASF group after 1 and 2 y of ASF supplementation.

Change in prevalence of low plasma vitamin B-12 concentrations. The percentage of children with low (<148 pmol/L) plasma vitamin B-12 concentrations in each group changed over time (**Fig. 1**). These changes were analyzed controlling for initial status, age, and gender. Despite the between-group differences in plasma vitamin B-12 concentration at baseline ($P = 0.001$) when the *githeri* + meat group had the highest percentage of children with plasma vitamin B-12 concentrations <148 pmol/L (Table 2), fewer children had low plasma vitamin B-12 concentration after 1 and 2 y, respectively, in the *githeri* + milk group (19.4 and 10.4%) and *githeri* + meat group (20.9 and 29.2%), leaving only 8.9 and 4.5% of children with plasma vitamin B-12 concentration <148 pmol/L. This marked reduction in the prevalence of low plasma vitamin B-12 concentration after 1 y was greater in the *githeri* + milk and *githeri* + meat groups than in the *githeri* and control groups ($P < 0.002$). Additionally, compared with the remaining 28.0% and 23.3% in the *githeri* and control groups, respectively, at the end of 2 y, there were fewer low values in the milk- and meat-supplemented groups than in either of the non-ASF groups ($P < 0.001$). The prevalence before and after 2 y of intervention changed from 55.6 to 4.5% in the *githeri* + meat group and 41.0 to 8.9% in the *githeri* + milk group. This analysis was also run for the percentage of children with low and marginal plasma vitamin B-12 concentrations (<221 pmol/L) with very similar and significantly different changes among the groups.

Discussion

The prevalence of low plasma vitamin B-12 concentrations was very high in these school children. Usual dietary intake of ASF, including milk, meat, and eggs, was correlated with plasma vitamin B-12 concentrations at baseline. Children in the lowest consumption tertile for ASF or milk, and those not consuming meat, had significantly higher odds of their plasma vitamin B-12 concentration being <148 pmol/L. The intervention demonstrated that feeding children supplemental ASF (meat or milk), 5 d/wk in school for 9 mo of the year (3 3-mo feeding terms per year), significantly improved their plasma vitamin B-12 concentration after 1 and 2 y. These interventions almost completely eliminated low plasma vitamin B-12 (<148 pmol/L) concentrations. Because children were receiving protein-bound vitamin B-12 in food and this form of the vitamin will usually not improve status in those who suffer from malabsorption caused by Helicobacter-induced gastric atrophy, for example (20), it is probable that the low plasma vitamin B-12 concentrations in these rural Kenyan school children are caused by inadequate intake and not to malabsorption of the vitamin from food.

There was a negative correlation between plasma vitamin B-12 concentration and age at baseline. This may indicate that there is an age-associated decline in vitamin B-12 status. However, because there were some costs associated with attending school in Kenya at the time of the study, it may simply indicate that poorer families send their children to school at a later age and these families have poorer nutritional status than those who can send their children to school at a younger age.

There was a 4-way interaction between group, time, malaria, and elevated CRP, suggesting that the children who have elevated CRP, malaria, or both were responding differently to the interventions from those who were "healthy." However, repeating the analysis with only healthy children showed similar results to those of the whole group, as did analysis of data from children who were affected by elevated CRP or malaria (data not shown).

Although not a perfect measure of vitamin B-12 status, plasma vitamin B-12 concentrations did improve in the expected way with ASF supplementation. Typically in adults, a plasma vitamin B-12 concentration <258 pmol/L is associated with an increase in methylmalonic acid (MMA), reflecting that vitamin B-12 status is inadequate to support its coenzyme function and with an increase in plasma homocysteine (Hcy) concentration

(21). Although this plasma cut-off has not been confirmed in children, it is possible that the increase in plasma vitamin B-12 concentrations achieved by feeding ASF in this study increased the availability of vitamin B-12 for physiological functions. Although it may have been valuable to measure MMA or Hcy concentrations in this study, MMA may be a less valid indicator of vitamin B-12 status in the developing world; gut bacteria produce propionic acid, a precursor of MMA, which may explain why serum MMA was higher than normal even in Guatemalan children with normal plasma cobalamin concentrations (22). Elevated plasma Hcy also occurs in folate, vitamin B-6, and riboflavin deficiencies (23,24).

A low plasma vitamin B-12 concentration has been associated with anemia, neuropsychiatric abnormalities (25), and altered neurological function (26). As reported elsewhere, supplementation with meat in this study had a positive impact on cognitive performance (27). However, it cannot be determined which meat constituent(s) were responsible for this impact. Randomized, controlled vitamin B-12 supplementation trials are ongoing to evaluate the functional consequences of improving vitamin B-12 status in children with low plasma vitamin B-12 concentrations.

It is generally thought that vitamin B-12 deficiency occurs only in strict vegetarians (vegans), but more recent data show a substantial prevalence of low plasma concentrations in lacto-ovo vegetarians and low meat consumers (28–31). In Australia, there was a stepwise reduction in plasma vitamin B-12 concentration from high meat consumers to moderate meat consumers to lacto-ovo vegetarians to vegans, which was significantly different between all groups (32). In the Australian study, the lacto-ovo vegetarians had an average plasma vitamin B-12 concentration of 210 ± 97 pmol/L, very similar to the average value at baseline in this study (193.6 ± 105.3 pmol/L) for children who consumed very little meat but some milk.

In rural African households of livestock owners, the animal slaughters for familial consumption are rare and much fewer than the number of animals sold (33). However, in this same study of camel, cattle, small ruminant, and pastoral herders in Eastern Ethiopia, 73% of the milk produced by the animals was consumed while only 27% was sold, indicating that consumption of milk was more likely than consumption of meat. In this study, we found also that milk consumption was much more prevalent than the consumption of meat and that it improved plasma vitamin B-12 concentrations in those who consumed it. Ownership of small milk-producing animals might benefit the rural poor even when they do not eat the meat, because milk is also a nutritionally dense food. A dairy goat development project in Ethiopia demonstrated that goat productivity and milk intake by children could be increased by empowering women with technical knowledge and providing micro-credit programs (34).

ASF provide vitamin B-12 and are a good source of other micronutrients, including vitamin A, iron, and zinc. In this ASF intervention, a significant and sizeable effect on plasma vitamin B-12 concentrations was seen. This, coupled with the substantial prevalence of low plasma vitamin B-12 concentrations throughout the developing world, suggests that sustainable ASF production projects such as the one in Ethiopia should be encouraged to increase ASF consumption in nutritionally at-risk groups, where it is culturally acceptable.

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