

# Animal Source Foods to Improve Micronutrient Nutrition and Human Function in Developing Countries

## Kenyan School Children Have Multiple Micronutrient Deficiencies, but Increased Plasma Vitamin B-12 Is the Only Detectable Micronutrient Response to Meat or Milk Supplementation<sup>1,2</sup>

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**ABSTRACT** Animal source foods (ASF) can provide micronutrients in greater amounts and more bioavailable forms compared to plant source foods, but their intake is low in many poor populations. However, the impact of ASF on micronutrient status of undernourished populations has not been assessed. Supplemental meat (60–85 g/d), milk (200–250 mL/d) or energy (isocaloric with the meat and milk, 240–300 kcal/d) were randomly assigned to 555 undernourished school children aged 5–14 y in a rural malaria-endemic area of Kenya, at one school meal daily for one school year. Blood and stool samples were collected at baseline and after 1 y to assess stool parasites, malaria, hemoglobin, serum or plasma C-reactive protein, ferritin, iron, zinc, copper, vitamin B-12, folate and retinol, and erythrocyte riboflavin. At baseline, there was a high prevalence of micronutrient deficiencies (iron, zinc, vitamins A and B-12 and riboflavin), yet plasma ferritin was low in few children, and none had low serum copper. At the end of the year of supplementation, plasma vitamin B-12 concentrations were significantly increased in children fed the Meat or Milk meal; prevalence of severe plus moderate deficiency fell from 80.7% at baseline to 64.1% in the Meat group and from 71.6 to 45.1% in the Milk group, respectively. No significant improvement was observed in the status of other micronutrients compared to the Energy and Control groups, although malaria and other infections may have obscured effects. Supplementation with small amounts of meat or milk reduced the high prevalence of vitamin B-12 deficiency in these children. *J. Nutr.* 133: 3972S–3980S, 2003.

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In developing countries, the widespread prevalence of multiple micronutrient deficiencies is associated with a low intake of animal source foods (ASF).<sup>4</sup> Animal source foods are

nutrient dense and provide high biological value protein, energy and fat and larger amounts of micronutrients in a more bioavailable form compared to plant source foods. The Nutrition Collaborative Research Support Program (NCRSP), a longitudinal observational study in Kenya, Mexico and Egypt, revealed positive associations between children's usual intake of ASF and their physical growth, cognitive development, behavior and school performance (1). This association remained significant after controlling for total energy intake, socioeconomic status (SES), parental education and social factors. The energy and protein intakes of the children were adequate to meet their estimated requirements (2). Thus, the quality of the diet, expressed as percent of energy from ASF, was the main predictor of the children's cognitive and motor development, rather than the quantity of food they consumed. The micronutrient content of ASF may be of primary importance for optimal development (3). However, this remains to be proven.

Strong evidence supporting the importance of ASF comes from studies of subgroups in affluent populations that avoid these foods. In the Netherlands, for example, 4- to 18-mo-old

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<sup>4</sup> Abbreviations used: AGP,  $\alpha$ -1 acid glycoprotein; ASF, animal source foods; CRP, C-reactive protein; EDTA, ethylene diaminetetraacetic acid; GL-CRSP, Global Livestock Collaborative Research Support Program; HAZ, height-for-age Z score; MRDR, modified relative dose response; NCRSP, Nutrition Collaborative Research Support Program; PGHE, Provincial General Hospital Embu; RBC, red blood cells; SES, socioeconomic status; WBC, white blood cells; WHZ, weight-for-height Z score.

infants fed macrobiotic diets gained significantly less weight and height and had lower arm circumferences and weight-for-length than omnivorous controls (4). Children in the macrobiotic group had poorer cognitive function and motor development compared to omnivorous controls, and 12 and 45% suffered from iron and vitamin B-12 deficiency, respectively. Because the mothers of these young children had consumed macrobiotic diets during pregnancy and lactation, it was not possible to separate any effects of prenatal micronutrient deficiencies from low postnatal infant intakes.

Increasing children's intake of meat or milk may improve their micronutrient status. Milk is a good source of vitamin A, calcium, vitamin B-12, riboflavin and folate, although it is low in iron and zinc. Adding milk (sometimes fortified) to diets of young children in developing countries increased their growth in some studies (5,6). To date, no intervention studies have assessed the effects on micronutrient status of feeding supplemental meat to children. Meat is rich in heme iron, zinc, riboflavin, vitamin B-12 and other micronutrients essential for normal growth and function, but low in vitamin A and folate. Furthermore, the effects of consuming supplemental meat versus dairy products on the micronutrient status of undernourished children have not yet been assessed.

These questions were studied within the framework of the Global Livestock Collaborative Research Support Program (GL-CRSP) project titled "Role of Animal-source Foods to Improve Diet Quality and Growth and Cognitive Development in East African Children," described in detail elsewhere in this supplement. It was conducted with rural Kenyan schoolers, a group in which the NCRSP in the 1980s documented a high prevalence of anemia, stunting and an inadequate intake of many key micronutrients (7). In this study, we examined the impact on micronutrient status of feeding, at one meal a day during the school year, a supplementary isocaloric meal containing meat, milk or energy, compared to feeding no supplementary food, on anemia and micronutrient status. Other outcomes, including food and nutrient intake, behavioral and cognitive function, school performance, anthropometry and morbidity, are reported in other articles in this supplement.

## METHODS

**Study area.** The participating school children ( $n = 555$ ) aged 5–14 y lived in three sublocations (Kyen 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. Keny South was selected because it contains an adequate number of schools several kilometers apart and is in the more semiarid region of Embu District where food shortages occur. In 1998 and 1999 rainfall was low, and smaller than usual harvests of maize and beans were reported. *Plasmodium falciparum* malaria is endemic in the region.

The staple foods include maize, beans and dark-green leafy vegetables. In the 1980s, children in this area typically obtained over 75% of their energy intake from maize and beans, 1% from milk (35 g/d) and <1% from meat (11 g/d) (7). The economic situation and food availability have changed little since that time.

**Design.** The effect of consuming ASF was evaluated by random assignment by school (three schools per group) of 555 standard I children in 12 schools, to one of four groups (three intervention and one control). All standard I classrooms in a given school were assigned to the same group for logistical reasons. Although some schools were larger than others, random assignment was carried out without stratification by school size.

The meals were administered once daily in school for an entire school year, which begins in January and is comprised of three 3-mo terms, with three 1-mo breaks. The intervention began in September 1998 and continued through July 1999. The outcomes presented here are changes in micronutrient status indicators over the school year intervention period. Data collectors were not blind to the group assignment, but technicians analyzing the biological specimens at UC Davis were blinded.

**Subject identification and recruitment.** Children were identified through the Embu District schools after full permission was obtained from the District Ministry of Education Director. The headmaster of each school and sublocation chiefs, teachers, head teachers and researchers were involved in the implementation of the school feeding program and in informing the community about the planned study, as described by Neumann et al. (9). Official permission was obtained from the Kenyan Ministry of Education, Human Resource Development and Ministry of Health, and verbal consent was granted by the parents at parent-teacher meetings at school, or at home, on an individual basis. Meetings were held to inform parents in detail about the study. The children were told repeatedly that they could choose not to be in any or all parts of the study. The study was approved by the UCLA Human Subject Protection Committee, the Office of the President of the University of Nairobi, the Kenyan Ministries of Education and Health and the UC Davis Human Subjects Review Committee.

**Subjects.** At baseline, participating children were examined by physicians, and if a child was found to be seriously malnourished, ill or to have a life-threatening condition, arrangements were made for immediate referral, treatment and care in a medical facility. Twenty-six children had severe anemia (hemoglobin <70 g/L) and were advised to go to the health center. At least 13 of these children are known to have gone to a health center where they received iron supplements. Spleen enlargement was noted by palpation, and if the child was ill or deemed to need medication, a prescription was given that the child's care provider could have filled at a nearby pharmacy. It is unknown how many participants utilized the prescription to obtain medication. All children received a single dose of mebendazole for deworming at baseline.

Some children who were assigned to the Milk and Meat groups did not like the taste of meat or milk ( $n = 14$ ). These children were excluded from the study. Another 17 children were dropped from the study because they changed schools.

**Intervention.** The in-school intervention consisted of meat, milk or an energy supplement (Kimbo, a vitamin A-fortified vegetable oil, Unilever Kenya, Nairobi) served together with githeri, a local maize and bean stew prepared with tomatoes, iodized salt, Kimbo and chopped dark leafy greens. Schools were randomly assigned to one of four groups, with roughly equal numbers in each group, resulting in three schools per group. The four treatment groups were as follows: 1) Meat (githeri + ground beef); 2) Milk (githeri + cow's milk); 3) Energy (githeri + Kimbo); and 4) Control (no supplemental food). The children received the supplementary food as a mid morning snack in school, 5 d/wk except during holidays. Consumption was observed and leftovers weighed and accounted for.

**Table 1** shows the Meat, Milk and Energy meals in terms of composition, serving sizes and estimated percent of the recommended energy and micronutrients that they contributed. More details of dietary intervention are provided by Murphy et al. (10) in this supplement. During Term 3 (September–November) 1998 the Meat meal contained 60 g of minced beef, and the Milk meal provided 200 mL of whole cow's milk. The Meat, Milk and Energy meals were estimated to provide 239, 241 and 240 kcal/serving, respectively.

Beginning in January 1999, the diets were changed to provide more energy, milk and meat, with a goal of 300 kcal per serving. Specifically, meat content was increased to 85 g in the meat githeri and the portion size was increased from 185 to 225 g. In the Milk group, the amount of milk was increased to 250 mL and the githeri portion was held constant. The portion of githeri was increased from 185 to 230 g/serving in the Energy group. These changes increased the energy content of the Meat, Milk and Energy meals to 291, 292 and 311 kcal, respectively (Table 1).

The Milk and Energy meals provided more than half the recommended daily intake of vitamin A. The Meat meal provided

TABLE 1

Estimated energy and micronutrient contents of the three meals, and percent of recommended daily intake met by each meal<sup>1</sup>

	Term 3 1998	% recommended/d	Terms 1 & 2 1999	% recommended/d
<b>Meat</b>				
Serving size	185 g (contains 60 g meat)		225 g (contains 85 g meat)	
Energy, kJ	999	14	1215	17
kcal	239	14	291	17
Vitamin A, $\mu\text{g RE}^2$	112	28	112	28
Iron, mg	2.42	24	2.94	29
Riboflavin, mg	0.12	20	0.15	24
Vitamin B-12, $\mu\text{g}$	0.75	63	0.91	76
Zinc, mg	2.38	48	2.89	58
<b>Milk</b>				
Serving size	100 g + 200 mL milk		100 g + 250 mL milk	
Energy, kJ	1007	14	1219	16
kcal	241	14	292	16
Vitamin A, $\mu\text{g RE}^2$	244	61	412	68
Iron, mg	1.52	15	1.57	16
Riboflavin, mg	0.44	73	0.53	88
Vitamin B-12, $\mu\text{g}$	0.96	80	1.16	97
Zinc, mg	1.46	29	1.66	33
<b>Energy</b>				
Serving size	185 g githeri		230 g githeri	
Energy, kJ	1003	14	1299	18
kcal	240	14	311	18
Vitamin A, $\mu\text{g RE}^2$	210	53	364	67
Iron, mg	3.16	32	3.93	39
Riboflavin, mg	0.12	20	0.15	25
Vitamin B-12, $\mu\text{g}$	0	0	0	0
Zinc, mg	1.35	27	1.68	34

RE, retinol equivalents.

<sup>1</sup> Energy and nutrient values determined by Murphy et al. using the WorldFood nutrient database (10). Recommended energy intake assumes a 20-kg child and 85-kcal/kg/d intake, based on data on mean energy intake for girls and boys (42). Recommended micronutrient intakes are for children 4–8 y (35,40).

<sup>2</sup> For vitamin A, only retinol from the oil (fortified at 70  $\mu\text{g}$  retinol/g) is listed for the energy and meat diets. For the Milk meal, the sum of retinol from the fortified oil + retinol from milk is listed.

nearly a third of this level for iron. The Milk meal provided more than 75% of the recommended intake of riboflavin. Both the Meat and Milk meals provided more than 75% of the recommended daily amount of vitamin B-12, and the Meat meal provided more than half the recommended daily amount of zinc.

Children in the Control group did not receive any food in school. However, children in all schools generally bring a lunch from home. At the conclusion of the intervention the parents of children in the Control group received one milk goat for each of their children in the study, as compensation for the food their children missed.

**Food preparation.** The meals were prepared in a hygienic central location that was within close driving distance from all schools. Meals were portioned into bowls with each of the individual participants' names and transported to the schools in insulated containers while still hot. Consumption of the meals was supervised by feeding assistants. In the Milk group, a glass of milk was served after the children had finished eating the githeri without meat. After consumption, the bowls and milk cups were collected and returned to the central food preparation site. Any remaining food from each student was weighed and the amount recorded. The mothers of the participants in all groups were encouraged to maintain the usual diet of all family members, and semiquantitative 24-h recalls were collected monthly to characterize the usual diet of each child and determine whether his or her usual diet had changed at all during the course of the intervention study. These data are reported elsewhere in this supplement (10).

**Anthropometry.** Weight and height were measured monthly following WHO recommended procedures (8,11,12). Only baseline data are presented here. The exact measurement protocol and data on the impact of the diets on anthropometry are described by Grillenberger et al. elsewhere in this issue (12). Weight was measured to the nearest 0.5 kg on an electronic digital scale (Seca, Columbia, MD) and height to the nearest 0.1 cm using a locally manufactured

wooden board fitted with a measuring tape, a fixed-foot plate and a movable headboard. The age of the children was derived from census questionnaires or from the school register. Height and weight measurements were transformed into sex- and age-specific Z scores with the EpiInfo 2000 program (version 1.0.5, Centers for Disease Control and Prevention, Atlanta, GA), which uses the CDC/WHO 1977/1985 reference curves for age, sex, height and weight (13). For girls older than 10 y and boys older than 11.5 y of age ( $n = 13$ ), weight-for-height Z (WHZ) scores could not be calculated due to software and reference limitations.

**Blood collection.** On the day of the baseline clinical examination, ~13 mL of blood was collected from each subject by venipuncture into trace element free (royal blue top) and K<sub>3</sub>EDTA (purple top) vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Due to parental refusal or technical difficulties, it was not possible to obtain blood from all children, and in some cases the amount of blood obtained was insufficient for all the biochemical tests. Children were given fresh fruit, sweets or fruit juice drinks after the clinical exam. They were provided with a small, covered plastic container to collect a stool sample on the same day as the clinical exam and blood draw.

The blood and stool samples were transported from the field sites to Provincial General Hospital Embu (PGHE). On arrival, the stool samples were processed by the formol ether method (14), and blood smears were prepared. Hemoglobin was measured in a drop of venous blood (Hemocue AB, Ängelholm, Sweden) at PGHE. Serum and plasma from the trace element free and EDTA vacutainers, respectively, was aliquoted into cryovials (Nalgene, Rochester, NY). Red blood cells (RBC) from the EDTA vacutainers were washed three times with chilled normal saline (0.9%) and aliquoted into cryovials.

All aliquoted serum, plasma and washed RBC were placed in dark boxes with lids and stored in a freezer at PGHE or at the University of Nairobi Department of Pediatrics (a 2-h trip by car), until the samples

were sent to the United States on dry ice. At UC Davis they were stored at  $-20^{\circ}\text{C}$ .

After 1 y of intervention, the clinical exam and blood and stool sampling were repeated in August 1999 in the same manner as described for 1998.

**Analysis of biological samples.** Stool samples were prepared for microscopic analysis using the formol-ether sedimentation method by clinical chemistry staff at PGHE. A drop of sediment was examined under a microscope for cysts, larvae and ova at  $10\times$  and  $40\times$ . If cysts were seen, a few drops of Lugol's iodine were added to the remaining sediment, which was reexamined at  $10\times$  and  $40\times$  magnification.

Thick and thin blood smears were prepared on a single glass microscope slide, stained with Giemsa and allowed to dry. The prepared slides were transported to the University of Nairobi where they were examined by two experienced lab technicians. Malaria parasites were counted in the thick smear per 200 white blood cells (WBC) and in the thin smear per 100 RBC. The number of parasites per 200 WBC in the thick smear was multiplied by 40 to obtain the density per  $\mu\text{L}$  whole blood. This assumes a mean WBC concentration of  $8000/\mu\text{L}$  whole blood. Because parasite densities were low overall (i.e., not  $>50,000/\mu\text{L}$ ), the percentage of infected RBC was not calculated from the thin smears. Agreement between the two technicians was high, with a  $\kappa$ -value of 0.9039. Unfortunately, the slides prepared in 1999 were damaged to the extent that readings were considered unreliable. Thus, only baseline malaria smear data were available.

At UC Davis, analyses were carried out in the Department of Nutrition and the Clinical Nutrition Research Unit for *P. falciparum* malaria antigen (in vitro antigen-capture strip method, Vision Biotech, Cape Town, South Africa), plasma ferritin (in duplicate by immunoradiometric assay, Diagnostic Products, Los Angeles, CA), C-reactive protein (CRP) (by radial immunodiffusion, The Binding Site, Birmingham, UK), serum iron, zinc and copper [three readings per sample, by inductively coupled plasma (ICP) emission spectroscopy] (15), plasma vitamin B-12 and folate (in duplicate by radioimmunoassay, ICN Diagnostics, Costa Mesa, CA) and plasma retinol (16) and erythrocyte riboflavin (17,18) by high pressure liquid chromatography. RBC riboflavin content was measured as FAD (flavin adenine dinucleotide) plus FMN (flavin mononucleotide), indicators of long-term riboflavin status (19). We also measured  $\alpha$ -1 acid glycoprotein (AGP) on a subsample ( $n = 36$ ) of plasma from baseline by radial immunodiffusion (The Binding Site). This subsample was selected to include children with high and low CRP, retinol and ferritin concentrations.

With each batch of samples, commercial controls were run for CRP (CV 7 and 14%; The Binding Site), plasma ferritin (CV 9 and 18%; Chiron Diagnostics, E. Walpole, MA), vitamin B-12 (CV 5%) and folate (CV 6.5%) (ICN Pharmaceuticals, Costa Mesa, CA). 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 RBC riboflavin (CV 8%).

**Statistical analyses.** Data were hand entered and sorted using Microsoft Excel (Redmond, WA), and all data were rechecked for accuracy to minimize entry errors. Statistical analyses were run using SAS for Windows 8e (SAS Institute, Cary, NC).

Changes in prevalence of elevated CRP, malaria antigen and gastrointestinal parasites overall were measured using McNemar's test and between the intervention groups and the Control, using the SAS CATMOD procedure. Changes in the prevalence of micronutrient deficiencies from baseline to 1 y overall were measured using McNemar's test, and changes in overall concentrations compared using paired *t* tests. Changes in micronutrient status indicators from baseline to 1-y follow-up among the groups were compared using analysis of covariance with the GLM procedure. First, data were transformed to meet the normality assumptions, usually to the natural log (ln). The square root was used for serum iron and plasma vitamin B-12. Only hemoglobin and copper were analyzed without transformation.

Because the four intervention groups had significantly different micronutrient concentrations at baseline for some micronutrient status indicators, change in the transformed variables between 1998 and 1999 was used as the outcome variable, with the baseline

measurement and the school attended included as explanatory variables in the model. The school variable was considered as a random effect and was nested within treatment.

Potential covariates included the following: the baseline variable, school (group), days of school attendance, sex, age, SES (land and livestock ownership, house construction, education and lifestyle-related data collected by interview and standardized to a point scale), baseline weight-for-height Z score, height-for-age Z score (HAZ), malaria smear data, spleen size, stool parasites, malaria antigen data and CRP, for 1998 and 1999. Interaction terms between these variables and intervention group were also included in the model initially but dropped from the final model if not significant. If malaria or CRP by group interaction variables were significant in a model, malaria-positive or CRP-elevated subjects from that year were excluded from the analysis due to the complexity of interpretation. We analyzed data by intention to treat groups. Therefore, those who consumed plain githeri instead of the githeri assigned to them (due to dislike of meat or milk;  $n = 17$ ) and those who switched interventions at any time ( $n = 17$ ) were included in all analyses of covariance. However, for hemoglobin, ferritin and serum iron, those who were given and took iron supplements ( $n = 13$ ) due to initial severe anemia (hemoglobin  $<70$  g/L) at baseline were excluded from analysis.

We defined a CRP concentration  $>10$  mg/L as indicative of infection, and a cutoff of  $>1.2$  g/L to define elevated AGP. The cutoff values used to define low concentrations of the other variables were as follows: hemoglobin  $<115$  g/L, plasma ferritin  $<15$   $\mu\text{g/L}$ , serum iron  $<9.0$   $\mu\text{mol/L}$ , serum zinc  $<10.7$   $\mu\text{mol/L}$ , serum copper  $<11.0$   $\mu\text{mol/L}$ , plasma vitamin B-12  $<125$  (severe) and 125–221 (moderate) pmol/L, plasma folate  $<6.8$  (severe) and 6.8–13.6 (moderate) nmol/L, plasma retinol  $<0.35$  (severe) and 0.35–0.70 (moderate)  $\mu\text{mol/L}$  and RBC riboflavin  $<170$   $\mu\text{mol/L}$ .

The CRP cutoff was selected because moderately elevated serum levels (10–40 mg/L) are associated with mild inflammation and viral infections, and higher concentrations (from 40 to  $>200$  mg/L) occur with acute phase inflammation and bacterial infections (20,21). The cutoff for elevated  $\alpha$ -1 acid glycoprotein was selected based on observations by Paracha et al. (22). The hemoglobin cutoff was selected because individuals of African descent have hemoglobin values that are 3–10 g/L lower than those of whites, irrespective of age and income (23). A plasma ferritin concentration  $<15$   $\mu\text{g/L}$  indicates minimal iron stores (24). The cutoffs to define low serum iron, zinc and copper were selected as follows: the iron cutoff is well below the mean value of 15–16  $\mu\text{mol/L}$  for children aged 5–12 y (25), the zinc value is  $\sim 2$  SD below the adult mean (26) and interpretive guidelines often used for normal serum copper concentrations for adults are 11 to 22  $\mu\text{mol/L}$  (27). The cutoff for severe vitamin B-12 deficiency was selected because values from the ICN Pharmaceuticals kit used are, on average, lower than the levels determined by other methods, and the normally used cutoff is  $<147$  pmol/L (Dr. J. Miller, personal communication). Retinol concentrations  $<0.35$   $\mu\text{mol/L}$  were defined as severely deficient, and 0.35–0.70  $\mu\text{mol/L}$  as deficient, as recommended by WHO/UNICEF and the International Vitamin A Consultative Group (IVACG) (28). The cutoff for riboflavin deficiency was based on the 5th percentile of values in blood samples that we analyzed from 22 fasted, healthy American adults. This cutoff was supported by the fact that after 2 mo of riboflavin supplementation, 95% of women in Guatemala had values above this level, compared to only 29% at baseline (L. H. Allen and M. E. Ruel, unpublished results). Because the malaria antigen detection strips had a high specificity compared to malaria smears at baseline and detected far more cases, only the results from the antigen tests were used as indicators of malaria presence in these analyses.

## RESULTS

**Adherence.** Adherence to the intervention was high. Of those who attended school, 99.38% ate all of the food provided. Absenteeism was similar in the three intervention groups, with children present in 84.9, 84.7 and 84.0% of all school days in the Meat, Milk and Energy groups respectively.

**Anthropometry and morbidity.** No significant differences were observed among intervention groups in anthropometric data at baseline (Table 2). Infections were highly prevalent at baseline, as reflected by elevated CRP (17.8%), malaria infection (31.8%), enlarged spleen (45.4%) and parasites in stool (Table 3). After 1 y, elevated CRP was detected in 8.6% ( $P < 0.0001$ ), malaria in 27.4% ( $P < 0.05$ ) and an enlarged spleen in only 5.8% ( $P < 0.0001$ ) of the children overall. None of the changes in gastrointestinal parasites were significantly different after 1 y. Compared to Controls, the fall in the prevalence of malaria was less in the Meat group ( $P < 0.01$ ), and the prevalence of enlarged spleen was less in the three intervention groups (all  $P < 0.0001$ ). However, the decrease in prevalence of *Entamoeba histolytica* was greater in the Meat ( $P < 0.05$ ) and Milk ( $P < 0.01$ ) groups, and the reduction in prevalence of *Giardia lamblia* and *Iodamoeba butschlii* was greater in the Meat group ( $P < 0.05$ ) than in the Controls. Correlations between decreases in CRP and ferritin ( $r = 0.49$ ), CRP and copper ( $r = 0.23$ ) and copper and ferritin ( $r = 0.35$ ) were all highly significant ( $P < 0.0001$ ). Furthermore, there was a significant, but negative correlation between change in plasma retinol and changes in CRP ( $r = -0.12$ ;  $P = 0.01$ ).

**Micronutrient status and response to supplements.** Median concentrations and quartiles ( $Q_1$ ,  $Q_3$ ) of micronutrient status indicators, and the percentage of deficient children in each intervention group and overall, are presented in Table 4, at baseline and 1-y follow-up. At baseline, the overall prevalence of anemia (hemoglobin  $<115$  g/L) was 48.9%, and 2.7% had severe anemia (hemoglobin  $<70$  g/L) (data not shown). However, the prevalence of low ferritin concentrations was not high. The prevalence of low serum iron concentrations was 52.4%, and of low serum zinc concentrations, 65.6%. The prevalence of low serum copper and plasma folate was almost zero. The children were extremely depleted in vitamins B-12 and A; 30.5 and 37.7% of the children had severe and moderate B-12 deficiency, respectively, whereas the prevalence of severe and moderate vitamin A deficiency was 22.0 and 68.6%, respectively. Overall, 24.3% of the children had RBC riboflavin concentrations  $<170$   $\mu\text{mol/L}$ . Concentrations of hemoglobin, serum iron, plasma vitamin B-12 and retinol increased (all  $P < 0.01$ ), and ferritin, serum iron, copper and RBC riboflavin concentrations decreased (all  $P < 0.01$ ) across all groups by 1-y postintervention.

Mean changes in the micronutrient status indicators by intervention group, with  $P$ -values determined with both simple analysis of variance (maximum number of observations) and analysis of covariance models with significant independent variables, are presented in Table 5. None of the changes in micronutrient status indicators from 1998 to 1999 were significantly different among the intervention groups or between each group and the Control group, except for vitamin B-12. For plasma vitamin B-12, both the Meat and Milk groups showed dramatic increases that were significantly different from the fall in concentrations observed in the Energy and Control groups ( $P < 0.0005$ ). The increases in plasma vitamin B-12 concentration were not significantly different between the Milk and Meat groups ( $P = 0.91$ ). The prevalence of severe vitamin B-12 deficiency fell from 46.8 to 21.4% in the Meat group, and from 30.6 to 9.7% in the Milk group, whereas the prevalence of moderate vitamin B-12 deficiency changed from 33.9 to 42.7% in the Meat group, and from 41.0 to 35.4% in the Milk group. The presence of specific gastrointestinal parasites in either year was not significantly associated with the changes in micronutrient status indicators, except for *E. histolytica* infection in 1998. Specifically, children with *E. histolytica* infection at baseline had a smaller increase in serum iron ( $P = 0.01$ ) and

TABLE 2

*Anthropometric characteristics of the school children by intervention group and overall at baseline (mean  $\pm$  sd)*

Group, n	Age, y	% male	Height-for-age Z	Weight-for-height Z
Meat, 134	7.8 $\pm$ 1.2	50	-1.53 $\pm$ 0.95	-0.37 $\pm$ 0.74
Milk, 144	7.4 $\pm$ 1.3	54	-1.34 $\pm$ 1.00	-0.38 $\pm$ 0.65
Energy, 148	7.2 $\pm$ 1.2	50	-1.36 $\pm$ 1.02	-0.26 $\pm$ 0.82
Control, 129	7.3 $\pm$ 1.2	52	-1.27 $\pm$ 1.11	-0.28 $\pm$ 0.76
Overall, 555	7.4 $\pm$ 1.2	52	-1.37 $\pm$ 1.02	-0.32 $\pm$ 0.74

a greater increase in plasma retinol ( $P = 0.01$ ) across all groups independent of group assignment. Those with an enlarged spleen at baseline had a smaller increase in plasma vitamin B-12 concentrations across all groups, independent of the intervention assignment. Girls had a greater increase in serum iron than boys across all groups ( $P = 0.03$ ), independent of group assignment.

SES, WHZ, HAZ, age at baseline, school attendance and malaria smear data in 1998 were not significantly associated with change in any of the micronutrient status indicators measured and thus were not included in the final models. However, CRP in 1999 was significantly associated with changes in hemoglobin, copper, vitamins B-12, retinol and riboflavin, and CRP in both years was significantly associated with change in ferritin and riboflavin. Malaria in 1999 was significantly positively associated with change in ferritin and copper and negatively associated with change in hemoglobin and retinol.

## DISCUSSION

Providing Kenyan school children with a meal supplemented with either meat or milk increased their plasma vitamin B-12 concentrations and lowered the prevalence of this vitamin deficiency significantly over 1 y. In contrast, in the Energy and Control groups, significantly more children became vitamin B-12 deficient, and there was a fall in plasma vitamin B-12 over the same time period. This important outcome is entirely plausible, given that vitamin B-12 is found exclusively in ASF. It suggests that the high prevalence of vitamin B-12 deficiency in this population at baseline is predominantly related to their low intake of ASF. The high global prevalence of vitamin B-12 deficiency in developing countries where animal product intake is low is becoming increasingly recognized (29,30). Evidence is also accumulating that the lower vitamin B-12 intakes of lacto-ovo vegetarians compared to omnivores can jeopardize adequate vitamin B-12 status (30).

For the other micronutrient status indicators, no significant differences were detected between the groups as a result of the treatments. One potential explanation for these negative findings is the influence of malaria and other infections. In these children malaria reduced the concentrations of hemoglobin, serum iron, plasma vitamin B-12 and retinol and increased the concentrations of serum ferritin, copper and RBC riboflavin, independently of the effects of CRP and other factors such as age, sex and WHZ (31). Malaria also affected prevalence estimates of low hemoglobin, serum iron, serum zinc and RBC riboflavin, controlling for CRP.

It is unclear why the hemoglobin and plasma retinol changes in the Meat and Milk groups were not significantly different from these changes in the Control group. There were no significant malaria by group interactions in the statistical

TABLE 3

Prevalence (%) of elevated CRP, malaria antigens, enlarged spleen and stool parasites at baseline (1998) and after 1 y of intervention (1999), by intervention group and overall

	Meat		Milk		Energy		Control		Overall	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
% infection										
% CRP >10 mg/L	23	13.6	10.9	7.0	16.9	7.6	21.0	6.6	17.8	8.6 <sup>1</sup>
n	126	103	137	114	136	132	124	106	523	455
% malaria antigens	46.8	40.2 <sup>2</sup>	21.5	25.6	31.6	27.1	27.4	15.2	31.8	27.4 <sup>3</sup>
n	126	102	130	86	136	118	124	92	516	398
% enlarged spleen	49.2	9.4 <sup>1</sup>	34.7	9.2 <sup>1</sup>	46.7	4.6 <sup>1</sup>	49.6	0	45.4	5.8 <sup>3</sup>
n	120	107	98	120	120	132	111	106	449	468
Fecal parasites, number examined	124	99	127	113	140	132	119	100	510	444
% <i>Entamoeba histolytica</i>	19.4	15.2 <sup>1</sup>	21.3	11.5 <sup>2</sup>	20.0	17.4	25.2	27.0	21.4	17.6
% <i>Giardia lamblia</i>	15.3	7.1 <sup>1</sup>	15.0	21.2	10.7	15.2	9.2	20.0	12.5	16.0
% hookworm	0	0	0	0.9	0	1.5	0	1.0	0	0.9
% <i>Ascaris lumbricoides</i>	0	8.1	0	2.7	0	5.3	0	3.0	0	4.7
% <i>Escherichia coli</i>	11.3	7.1	8.7	11.5	14.3	13.6	10.1	17.0	11.2	12.4
% <i>Iodamoeba butschlii</i>	8.1	1.0 <sup>3</sup>	7.9	8.0	5.7	8.3	13.4	14.0	8.6	7.9
% <i>Blastocystis hominis</i>	0.8	5.1	0	4.4	0	7.6	0.8	10.0	0.4	6.8

When superscript is in the overall column, it indicates statistical significance overall; when superscript is in one of the group columns, it indicates statistical significance compared to the Control group. CRP, C-reactive protein.

<sup>1</sup>  $P < 0.0001$ .

<sup>2</sup>  $P < 0.01$ .

<sup>3</sup>  $P < 0.05$ .

analyses of change in hemoglobin and retinol. Vitamin A deficiency was confirmed by pupillary threshold testing and the modified relative dose response (MRDR) test during the second year of intervention (32,33). It is unlikely that absorption of micronutrients from the intervention diets was hampered by infection with other parasites. The prevalence of hookworm was essentially zero in our population in both years.

Acute infection, detected by elevated CRP, interferes with assessment of iron, zinc and vitamin A status (26). Specifically, plasma ferritin increases in acute infection because it is an acute phase protein. Serum zinc and vitamin A concentrations fall, and serum copper concentrations increase. The overall decrease in prevalence of elevated CRP during the year was paralleled by a fall in serum copper and plasma ferritin.

No standard algorithms are available to predict increase in serum retinol from dietary intake, and there is essentially no relationship between liver retinol stores and plasma retinol concentration, except when liver stores fall below 20  $\mu\text{g/g}$  liver (34), and plasma retinol concentrations decline. However, when dietary vitamin A in nonpharmacological doses is fed to vitamin A-deficient children, plasma retinol concentrations increase rapidly, even before liver vitamin A stores are restored (35). We observed increases in plasma retinol across all groups in our study and were unable to identify any factor(s) that could explain this change. Recently completed assessment of the MRDR to estimate liver retinol stores, conducted 1 y later in schoolers in the same population who continued to be supplemented, revealed that 75% have depleted liver stores, and 20% are borderline depleted (33). There were, however, no clinical signs of vitamin A deficiency in either year in this population (data not shown). Malaria lowers serum retinol, retinol binding protein and transthyretin, and acute malaria can produce a 50% reduction in serum retinol (36). However, there was no detectable impact of the interventions even on those children who were free of malaria antigens at baseline and the final measurement. Malaria also increases the urinary excretion of retinol (37), which could contribute to depletion of the vitamin during undetected infectious episodes before and during the intervention.

The lower prevalence of elevated CRP and enlarged spleens, and the fall in plasma ferritin and serum copper concentrations, indicates a lower infection prevalence in this population in 1999. The change in prevalence of elevated CRP, malaria and enlarged spleen explain 18.1% of the observed increases in hemoglobin, 8.9% of the changes in serum iron and 6.9% of the changes in plasma retinol concentrations across all groups (path analysis; data not shown). However, these variables do not explain the bulk of the observed changes 1-y post-intervention. It is possible that the much lower prevalence of enlarged spleen at the end of the year was due to the fact that a different physician made this assessment in each year.

There are also no possible diet-related explanations for the lack of significant effects of meat and milk on micronutrient status (apart from vitamin B-12). Based on dietary data derived from the WorldFood nutrient database in Table 1, the Milk and Meat meals provided large amounts of vitamin A, the Meat meal provided ample iron and zinc, and the Milk meal provided more than adequate amounts of riboflavin. Inhibitory factors such as phytate from the maize and beans in the githeri may have reduced absorption of iron and zinc, especially in the Milk and Energy groups. However, the heme iron provided by the meat in the meat githeri would have been more available (~25% absorbed) even in the presence of phytate, assuming other factors were not interfering with absorption (35). The fat content of the githeri diets was sufficient (1.6 g in the Meat and Milk, and 3 g in the Energy meals) such that absorption of fat-soluble vitamins (vitamin A) should not have been impaired.

Our statistical analysis was by intention to treat; therefore we did not exclude the 17 children who disliked meat and milk and were fed plain githeri or the 17 children who switched schools, thereby switching intervention groups. When these cases were excluded from the analyses, the statistical interpretation did not change.

Displacement of other foods by the intervention may also be a factor (10). However, assuming the usual diet did not change, estimates were made of the expected increases in plasma ferritin concentration and liver retinol stores based on the level of iron and retinol in the intervention diets. The additional

TABLE 4

Concentration (medians and quartiles  $Q_1$ ,  $Q_3$ ) of micronutrient status indicators and % deficient at baseline (1998) and after 1 y of feeding (1999), by intervention group and overall

	Meat		Milk		Energy		Control		Overall	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
Hemoglobin g/L, median	114.0	120.0	117.0	123.0	115.0	121.0	115.0	126.0	115.0	122.0
Q <sub>1</sub>	102.3	111.0	103.5	114.0	99.0	109.0	97.0	118.0	101.0	113.0
Q <sub>3</sub>	123.0	127.5	127.0	129.0	124.0	130.0	126.5	131.0	125.0	130.0 <sup>1</sup>
n	126	96	136	110	133	132	124	103	519	441
% <115 g/L	51.6	33.3	46.3	26.4	49.6	33.3	48.4	19.4	48.9	28.3 <sup>2</sup>
Plasma ferritin $\mu$ g/L, median	60.1	48.9	40.5	38.9	53.8	43.8	42.8	39.7	50.4	42.7
Q <sub>1</sub>	39.8	31.3	24.8	25.7	34.0	30.5	23.8	25.0	28.5	28.7
Q <sub>3</sub>	97.3	63.2	69.1	62.8	100.9	65.5	78.2	58.6	82.7	62.8 <sup>1</sup>
n	124	103	128	114	134	132	121	105	507	454
% <15 $\mu$ g/L	2.4	2.9	10.9	4.4	1.5	4.6	10.7	6.7	6.3	4.6
Serum Fe $\mu$ mol/L, median	7.9	9.8	9.5	10.0	9.1	9.9	8.5	10.8	8.7	10.1
Q <sub>1</sub>	6.8	7.9	7.2	7.8	6.4	8.7	6.5	8.5	6.8	8.2
Q <sub>3</sub>	10.1	12.6	12.1	13.7	11.6	13.4	11.4	13.1	11.4	13.4 <sup>1</sup>
n	122	103	134	117	136	132	123	103	515	455
% <9.0 $\mu$ mol/L	63.9	39.8	44	38.5	47.1	28.8	56.1	32	52.4	34.5 <sup>2</sup>
Serum Zn $\mu$ mol/L, median	9.9	8.9	9.8	9.3	10.0	9.4	9.6	10.0	9.9	9.4
Q <sub>1</sub>	8.8	8.1	8.7	8.3	8.8	8.4	8.1	8.9	8.6	8.4
Q <sub>3</sub>	11.5	10.1	11.0	10.1	11.3	10.5	11.3	11.1	11.3	10.62
n	122	103	134	117	136	132	123	103	515	455
% <10.7 $\mu$ mol/L	61.5	83.5	70.1	81.2	64.0	78.0	66.7	67.0	65.6	77.63
Serum Cu $\mu$ mol/L, median	21.9	19.2	20.2	18.4	20.8	18.6	20.3	18.3	20.8	18.6
Q <sub>1</sub>	19.6	17.2	18.1	16.5	18.1	16.3	18.0	15.6	18.4	16.3
Q <sub>3</sub>	24.1	21.4	22.5	20.2	23.7	20.5	23.2	20.5	23.6	20.7 <sup>1</sup>
n	122	103	134	117	136	132	123	103	515	455
% <11.0 $\mu$ mol/L	0.8	0.9	0	0.9	0.7	0	0.8	0.9	0.6	0.7
Plasma vitamin B-12 pmol/L, median <sup>3</sup>	131	189	164	236	195	151	196	181	174	189
Q <sub>1</sub>	98	129	119	171	126	105	133	124	115	127
Q <sub>3</sub>	201	248	241	308	262	221	264	257	245	2712
n	124	103	134	113	132	131	122	103	512	450
% <125 pmol/L	46.8	21.4	30.6	9.7	25.0	35.9	19.7	25.2	30.5	23.6 <sup>2</sup>
% 125–221 pmol/L	33.9	42.7	41.0	35.4	37.1	39.7	38.5	40.8	37.7	39.6
Plasma folate nmol/L, median	31.6	24.3	35.6	28.4	34.9	27.9	30.3	27.7	32.5	27.0
Q <sub>1</sub>	24.9	20.4	28.0	22.2	26.3	22.3	24.0	22.1	25.3	21.8
Q <sub>3</sub>	38.6	28.9	46.5	34.3	42.8	35.3	38.1	35.1	42.2	33.4 <sup>1</sup>
n	124	103	134	113	132	131	122	103	512	450
% <6.8 nmol/L	0	0	0	0	0	0	0	0	0	0
% 6.8–13.6 nmol/L	1.6	2.9	0.8	0.9	0.8	1.5	0.8	1.9	1	1.8
Plasma retinol $\mu$ mol/L, median	0.40	0.67	0.46	0.81	0.50	0.71	0.47	0.78	0.45	0.73
Q <sub>1</sub>	0.33	0.56	0.35	0.62	0.39	0.56	0.37	0.69	0.36	0.60
Q <sub>3</sub>	0.48	0.81	0.58	0.97	0.64	0.89	0.59	0.93	0.58	0.90 <sup>1</sup>
n	96	102	117	115	126	132	103	105	442	454
% <0.35 $\mu$ mol/L	31.3	2.9	23.9	0.9	16.7	0	17.5	0.9	22	1.1 <sup>2</sup>
% 0.35–0.70 $\mu$ mol/L	67.6	53.9	65.0	36.5	66.7	49.2	75.7	25.8	68.6	41.6
RBC riboflavin $\mu$ mol/L, median <sup>3</sup>	211	202	204	186	193	184	185	178	198	186
Q <sub>1</sub>	183	170	177	167	167	158	160	156	171	162
Q <sub>3</sub>	257	224	227	217	223	213	210	196	230	214 <sup>1</sup>
n	94	94	76	76	111	111	89	89	370	370
% <170 $\mu$ mol/L	14.9	26.6	18.4	30.3	28.8	36.9	33.7	39.3	24.3	33.5 <sup>3</sup>

Sample sizes differ by year within groups due to inadequate blood sample available. RBC, red blood cells.

<sup>1</sup>  $P < 0.0001$  for overall change.

<sup>2</sup>  $P < 0.01$  for overall change.

<sup>3</sup>  $P < 0.05$  for differences among groups at baseline.

amount of iron absorbed daily from the heme in the meat supplement would be  $\sim 0.635$  mg, given that 85 g of cooked ground beef contains 2.54 mg of heme iron (38), and roughly 25% of heme iron is absorbed (35). This value, multiplied by the maximum number of days a child could have consumed the intervention ( $\sim 180$  d) gives a total of 114 mg additional absorbed iron over the course of the school year. As this iron apparently did not increase hemoglobin concentrations, it would be stored in the liver as ferritin. In children, each 1  $\mu$ g/L of serum ferritin indicates the presence of  $\sim 14$  mg/kg of storage iron (39). Thus, we might expect an increase in plasma ferritin

of  $\sim 8$   $\mu$ g/L in the meat group. This was not observed, even in those who had normal CRP at both time points (data not shown).

In conclusion, meals containing small amounts of supplemental meat or milk significantly increased plasma vitamin B-12 concentrations, but no significant effects on other indicators of micronutrient status were detected, possibly because of the confounding influence of malaria and other infections. The improved vitamin B-12 status of children fed meat or milk may have several benefits. Because consequences of vitamin B-12 deficiency include megaloblastic anemia, poor motor and

TABLE 5

Unadjusted mean change  $\pm$  *sd* in micronutrient status indicators from 1998 to 1999, with *P*-values determined through simple analysis of variance (maximum observations) and *P*-values from analysis of covariance models including only significant independent variables for each intervention group<sup>1</sup>

Indicator	Meat	Milk	Energy	Control	<i>P</i> -value <sup>2</sup> ( <i>n</i> )	<i>P</i> -value ( <i>n</i> )
Hemoglobin, g/L	7.5 $\pm$ 15.1	6.8 $\pm$ 14.8	8.9 $\pm$ 18.4	11.5 $\pm$ 19.8	0.53 <sup>3</sup> (410)	0.40 <sup>2,4</sup> (317)
Plasma ferritin, $\mu$ g/L	-20.5 $\pm$ 46.4	-7.5 $\pm$ 71.6	-24.0 $\pm$ 70.4	-13.7 $\pm$ 57.8	0.97 <sup>3</sup> (411)	0.92 <sup>3,5</sup> (356)
Serum iron, $\mu$ mol/L	2.3 $\pm$ 3.7	0.9 $\pm$ 6.0	2.0 $\pm$ 5.2	2.1 $\pm$ 4.6	0.61 <sup>3</sup> (418)	0.29 <sup>3,6</sup> (266)
Serum zinc, $\mu$ mol/L	-1.1 $\pm$ 3.5	-0.6 $\pm$ 2.0	-0.5 $\pm$ 2.0	0.6 $\pm$ 3.1	0.14 (427)	0.14 <sup>2</sup> (427)
Serum copper, $\mu$ mol/L <sup>7</sup>	-2.9 $\pm$ 0.5	-1.4 $\pm$ 3.9	-1.9 $\pm$ 8.2	-2.3 $\pm$ 4.0	0.76 (426)	0.65 <sup>8</sup> (269)
Plasma vitamin B-12, pmol/L	47 $\pm$ 66	66 $\pm$ 71	-34 $\pm$ 45	-13 $\pm$ 65	0.0001 (423)	<i>P</i> < 0.0001 <sup>9</sup> (223)
Plasma folate, nmol/L	-6.8 $\pm$ 11.6	-8.8 $\pm$ 32.2	-6.1 $\pm$ 15.0	-3.2 $\pm$ 10.7	0.61 (423)	0.61 <sup>2</sup> (423)
Plasma retinol, $\mu$ mol/L	0.27 $\pm$ 0.19	0.30 $\pm$ 0.25	0.22 $\pm$ 0.23	0.32 $\pm$ 0.19	0.64 (410)	0.91 <sup>10</sup> (358)
RBC riboflavin, $\mu$ mol/L	-18.9 $\pm$ 26.8	-15.0 $\pm$ 28.3	-10.2 $\pm$ 33.7	-5.7 $\pm$ 44.3	0.91 (370)	0.89 <sup>11</sup> (334)

RBC, red blood cells.

<sup>1</sup> Means in table are untransformed, but *P*-values are derived from analyses of covariance on transformed variables.

<sup>2</sup> Analysis of covariance model included only initial value, group and school (group) as predictor variables; outcome variable was change.

<sup>3</sup> Children known to have taken iron supplements (*n* = 13) excluded from analysis.

<sup>4</sup> Model contained malaria99 in addition to variables in footnote 1; cases with elevated C-reactive protein (CRP) in 1999 excluded from analysis (*n* = 34).

<sup>5</sup> Model contained malaria99, CRP98 and CRP99 in addition to variables in footnote 1.

<sup>6</sup> Model contained sex and *E. histolytica*98 in addition to variables in footnote 1; cases with malaria in 1998 excluded from analysis (*n* = 136).

<sup>7</sup> One observation removed due to suspected error.

<sup>8</sup> Model contained CRP99 in addition to variables in footnote 1; cases with malaria in 1999 excluded from analysis (*n* = 153).

<sup>9</sup> Model contained CRP99 and spleen98 in addition to variables in footnote 1; cases with malaria in 1998 excluded from analysis (*n* = 125).

<sup>10</sup> Model contained CRP99 and malaria99 in addition to variables in footnote 1.

<sup>11</sup> Model contained CRP98 in addition to variables in footnote 1; cases with elevated CRP in 1999 excluded from analysis (*n* = 36).

cognitive function and subsequent poor school performance (40), improving vitamin B-12 status may decrease the risk of these and other negative outcomes. Also, there were significant improvements in other outcomes such as weight gain, standardized test performance and cognitive function (12,41).

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