Animal Source Foods Improve Dietary Quality, Micronutrient Status, Growth and Cognitive Function in Kenyan School Children: Background, Study Design and Baseline Findings[1,2]

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ABSTRACT A previous longitudinal three-country study in Egypt, Kenya and Mexico found significant positive associations between intake of animal source foods (ASF) and growth, cognitive development and physical activity. To test for a causal relationship, a controlled school feeding intervention study was designed to test the hypotheses that ASF would improve micronutrient status, growth and cognitive function in Kenyan primary school children. Twelve rural Kenyan schools with 554 children were randomized to four feeding interventions using a local vegetable stew as the vehicle. The groups were designated as Meat, Milk, Energy and Control, who received no feedings. Feeding was carried out on school days for seven terms during 21 mo. Preintervention baseline measures included nutritional status, home food intake, anthropometry, biochemical measures of micronutrient status, malaria, intestinal parasites, health status and cognitive and behavioral measures. The measurements of each child were repeated at intervals over 2 y. Baseline data revealed stunting and underweight in ~30% of children and widespread inadequate intakes and/or biochemical evidence of micronutrient deficiencies, particularly of iron, zinc, vitamins A and B-12, riboflavin and calcium. Little or no ASF were eaten and fat intake was low. Malaria was present in 31% of children, and hookworm, amebiasis and giardia were widely prevalent. The outcomes measured were rates of change or increase during the intervention in cognitive function, growth, physical activity and behavior and micronutrient status. Hierarchical linear random effects modeling was used for analysis of outcomes. J. Nutr. 133: 3941S–3949S, 2003.

KEY WORDS: • *animal source foods* • *growth* • *development* • *micronutrients* • *Kenya*

Nutrient deficiencies, because of poor dietary quantity and quality, are prevalent globally and especially in low income countries. Children and women of reproductive age are particularly vulnerable (1,2). Limited availability, accessibility and intake of animal source foods (ASF)[4] at the household level and lack of knowledge about their value in the diet and role in health contribute to poor diet quality (3). The poor bioavailability of micronutrients in high fiber and phytate plant-based staples, and the low content of some micronutrients in these foods, are also major factors (3). In affluent countries, strict vegetarian diets, and fear of red meat dictated by spiritual and health beliefs, contribute to micronutrient deficiencies (4) and affect the function of an increasing number of children.

[1] Presented at the conference "Animal Source Foods and Nutrition in Developing Countries" held in Washington, D.C. June 24–26, 2002. The conference was organized by the International Nutrition Program, UC Davis and was sponsored by Global Livestock-CRSP, UC Davis through USAID grant number PCE-G-00-98-00036-00. The supplement publication was supported by Food and Agriculture Organization, Land O'Lakes Inc., Heifer International, Pond Dynamics and Aquaculture-CRSP. The proceedings of this conference are published as a supplement to *The Journal of Nutrition*. Guest editors for this supplement publication were Montague Demment and Lindsay Allen.

[2] This research was supported by the GL-CRSP though the Office of Agriculture of the United States Agency for International Development under Grant No. PCE-G-00-98-00036-00 to the GL-CRSP. The project was partially supported by the National Cattleman's Beef Association.

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[4] Abbreviations used: AFA, arm fat area; AMA, arm muscle area; ANOVA, analysis of variance; ASF, animal source foods; BMI, body mass index; CRP, C-reactive protein; HAZ, height-for-age Z-score; NHANES, National Health and Nutrition Surveys; HH, household; MFP, meat, fish and poultry; NCRSP, Nutrition Collaborative Research Support Program; PEM, protein-energy malnutrition; RPM, Raven's Progressive Matrices; SES, socioeconomic status; UHT, ultra-heat treated (milk); WAZ, weight-for-age Z-score.

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The Nutrition Collaborative Research Support Program (NCRSP) (5–8) reported strong statistical associations between the intake of ASF and better growth, cognitive function, activity, pregnancy outcome and morbidity in three parallel longitudinal observational studies in disparate ecologic and cultural parts of the world, Egypt, Kenya and Mexico. Associations remained positive even after controlling statistically for a large number of covariates and confounders such as socioeconomic status (SES), morbidity, parental literacy and nutritional status.

In Embu, Kenya the school-aged children in the NCRSP study of 1984–1987 obtained over 75% of their energy intake from maize and beans, and had mean daily intakes of 35 g milk and 11 g meat (6). These Standard I children had a daily energy intake that averaged only 78% of the recommended level, with 6% of energy from ASF. Iron, zinc, vitamin B-12 and calcium intakes were below two-thirds of the FAO/WHO recommended intakes in 67–100% of children (9). Stunting and iron deficiency anemia were present in 30 and 42% of children, respectively (6).

In the NCRSP studies it emerged that those children who consumed little or no animal products, particularly meat, performed least well on cognitive tests measuring verbal comprehension and abstract and performance perceptual abilities, as evaluated by the Raven's Progressive Matrices (RPM) (10). In addition, those children consuming the fewest animal products were the least attentive in the classroom, less active physically and showed the least amount of leadership behavior in the playground during free play (11,12). The greatest deficits in linear growth were found in those with little or no ASF in their diet (13). Thus, evidence from previous observational studies in 7- to 9-y-old school children in Embu, Kenya, and the similar findings in parallel studies in Egypt and Mexico, strongly suggested a positive link between the intake of ASF and improved cognitive, behavioral and physical development after statistically controlling for an array of covariates (5–7,11,13).

Past studies investigating the effects of supplementation on children with protein-energy malnutrition (PEM) targeted the interventions mainly to preschool children, with some finding clear benefits for children who were already malnourished. These studies, in general, differed widely in their approaches to intervention, making it difficult to isolate the factors and specific nutrients that best accounted for improvements in growth and developmental outcomes (14). Deficiencies of iron, zinc, iodine and, more recently, vitamin B-12 have been reported to affect growth and development adversely (15–17). Because macronutrient and multiple micronutrient deficiencies often coexist, interventions with only one or two micronutrients may not alleviate the functional deficits.

The above findings stimulated the need for a randomized controlled intervention feeding study to test whether or not a causal relationship exists between intake of ASF and health, growth and cognitive function. Thus, a randomized controlled school-feeding intervention trial was designed to answer whether ASF, specifically meat and milk, reduce micronutrient malnutrition and promote growth and cognitive function of school children. Schoolers were chosen as the target group for the study described here because of their accessibility for feeding, measurement and observation. Also, primary school children can still respond functionally to improved diet quality and quantity. A widely held belief that only interventions in the first three years of life are critical for influencing cognitive development is now being challenged (18). Intervention studies utilizing pharmaceutical supplements such as vitamin A, iron and zinc, either singly or in combination have had varying results (19). There have been no previous randomized controlled intervention studies evaluating the response of schoolers to meat and milk.

At issue for the nutrition, educational and livestock communities are policies that may stem from a demonstration of a causal link. The advantages of food-based versus pharmaceutical approaches to combat micronutrient deficiencies are of major interest. Food-based approaches offer more protection, in contrast to single or multinutrient nonfood nutrient supplements. Micronutrient deficiencies are usually multiple and therefore more readily supplied by food than supplements. Also PEM often coexists with micronutrient malnutrition and energy intake may also be inadequate. Food-based approaches are more feasible and sustainable in rural areas of poor countries, especially with the use of locally available and familiar foods and preparation methods (3,20). The main micronutrients of concern to children's growth, function and health are iron, zinc, vitamin A, vitamin B-12, riboflavin and calcium (19,20). Iodine is not included in this context as universal idolization of salt and/or water or administration of iodized oil by mouth or injection are the main methods to supply iodine to populations with iodine deficiency. Also, the impact of milk versus meat supplementation on growth and development needs to be clarified.

Opportune circumstances were available to us in Embu, Kenya, the site of the former Kenya NCRSP funded by USAID (6), to test if the inclusion of more animal products in the diet improves micronutrient status and is advantageous for the health, cognitive and physical development of school children.

Controlled intervention study: 1998–2001

A controlled school-feeding intervention study of two cohorts of ~554 and ~500 school children in rural Embu District, Kenya was designed to test for a causal link among their intake of ASF, rate of growth and development and micronutrient status. To determine the effects of micronutrient-rich ASF, we designed a school feeding intervention adding the following foods to the local plant-based dish Githeri, as the vehicle: meat, milk, oil added as energy and a control with no intervention feeding. As milk and meat are often thought to be equivalent, it was felt important to compare a meat and milk intervention separately, as these differ in their content of some important nutrients (i.e., iron, vitamin A and B-12, calcium and zinc). There is also some evidence that calcium and casein in milk form insoluble compounds with iron and zinc and interfere with their absorption (21). Thus, supplying both meat and milk at one feeding to one group of children may make it difficult to isolate benefits of these two ASF foods that may neutralize one another. We added extra oil to the usual basic vegetable stew (Githeri) to determine whether the same benefits could be gained by merely increasing energy intake. Based on prior findings in Embu, increasing the intake of the usual diet is not expected to show the same benefits as increasing animal products in the diet (6,9), but developmental improvements due to interventions that primarily increased energy intake have been repeated in other countries (15).

The Embu study site was uniquely suited to this intervention study. A cadre of over 100 previously trained, very experienced, local field workers in the NCRSP were available and able to administer all of the assessments. Second, the methodology for measuring food intake, anthropometry, cognitive abilities and behavior on the playground and in the classroom, morbidity, socioeconomic status and literacy had been used extensively in this locale, making the acceptance and implementation of these measurements considerably less burdensome than starting anew. The utilization of identical or nearly identical methods

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from the NCRSP would allow for comparisons of the situation in the mid 1980s with that at the present time (1998–2001), as has already been done for nutrient intake and some aspects of cognition (22). The diet, nutritional status, health and cognitive development of children in the community have been well described (6). Finally, the community was extremely cooperative and excellent rapport had been established with the research team.

Hypothesis. The controlled intervention study among Standard I primary school children was implemented in Kenya in 1998. The underlying hypothesis was that increased intake of ASF will improve the rate of growth, cognitive function, diet quality, micronutrient status and overall health of children compared to those consuming their usual diet. Secondary hypotheses were as follows: *i*) Supplementation with milk versus meat would demonstrate different benefits: the Milk group would show a greater rate of growth in stature than the Meat group because of milk's higher calcium and phosphorus content; *ii*) The Meat group would show the greatest improvement in cognitive function and school performance and reduction in anemia; *iii*) Vitamin B-12, iron, zinc and riboflavin status would improve most in the Meat group. In the Milk group, improvement in vitamin A status and a moderate improvement in vitamin B-12 and riboflavin status, would be seen; *iv*) All supplemented groups would show increased physical activity compared to the controls, but higher meat intake would increase activity the most; *v*) The lowest prevalence of severe morbidity would be found in the Meat group; and *vi*) Weight gain would increase in all supplemented groups compared to the Control group.

Subjects and study design

Two sublocations were selected within Kyeni South location in Embu District, Eastern Province of Kenya, which had ~2,600 households and 18 schools. Twelve out of the 18 primary schools were identified for randomization. Six schools were eliminated because of their very small size and inaccessibility for food delivery, especially in the rainy season, even with a four-wheel drive vehicle (**Fig. 1**).

The cohort I sample was comprised of all children enrolled in Standard I classes in each of the 12 selected elementary schools. Three of the larger schools in the sampling area, each with 2–3 Standard I classes, were randomized among the groups. The remaining smaller schools, each with one Standard I classroom, were randomized to each of the four groups. Each group was comprised of three schools with children aged 6–14 y and a median age of 7.4 y. Cohort I contained ~554 children. After baseline studies from July to August 1998, intervention feeding and data collection took place from September 1998 to December 2000. A second cohort of ~500 Standard I children was enrolled a year later from the Cohort I schools. For Cohort II, after baseline studies from July to August 1999, intervention feeding and data collection took place from September 1999 to December 2001. We felt it necessary to enroll a second cohort because in the first year of the study there was a month-long teachers' strike with disruption of school and a drought with food shortages. The drought and food shortages, however, worsened in the second year. Moreover, we wished to increase the statistical power of the sample, given the modest changes in outcome that we anticipated finding. The second cohort would also serve as a replicate study, except for the repetition of biochemical micronutrient analyses.

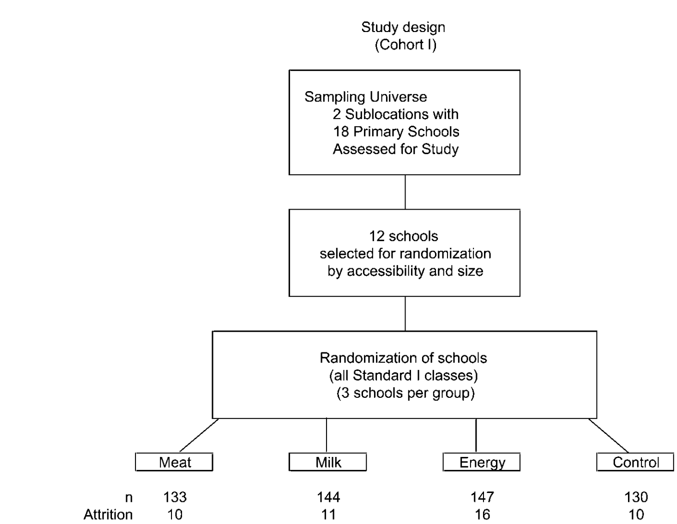

FIGURE 1 Study design and randomization to groups.

A four-condition design, with three classrooms per condition, was utilized. Each of the 12 elementary schools per cohort was randomly assigned to one of the three feeding interventions, or to the Control group. All Standard I classrooms at each school were assigned to the same feeding intervention or the control condition. The number in each of the intervention groups per cohort was relatively equal, 120–130 children. Statistically this is a nested hierarchical design: schools within feeding type groups, classrooms within schools and children within classes and schools (Fig. 1). The schools are all from 2 to >5 km apart with little chance for contamination. Data collection for outcome and covariate measures was carried out longitudinally and at different intervals for each subject.

Exclusions. Children with obvious mental retardation or other chronic handicapping conditions were excluded from data collection, but were fed along with their classmates. Children who switched to schools with a different assigned feeding were excluded from data collection, but were fed along with their classmates when at school. Those with prolonged absences (>3 mo) were likewise excluded from data collection, but not from feeding. Also, six children refused to eat meat, and eight children refused to drink milk. A total of 30 children were excluded from analyses.

Pilot testing and retraining of field staff, and baseline observations of children, were carried out from June to August 1998 for Cohort I, before the start of feeding. The feeding intervention was initiated at the beginning of September 1998. For Cohort II, baseline data was obtained from June to August 1999, and feeding commenced in September 1999 (Fig. 1). During the second school year of study, the children from the first year continued to be supplemented in their Standard II classrooms and then in Standard III. Those who were left back were fed and followed as well.

Human subjects protection assurance. Approval by the UCLA Human Subject Protection Committee (HSPC), the Ethics Committee of the University of Nairobi School of Medicine and the Office of the President, were obtained before commencing the study. Verbal informed consent by parents, assent by children and community permissions were also obtained through community meetings with the investigators.

Feeding intervention

The three food-supplemented groups received midmorning snacks every day they attended school. Because Standard I and II children were in school until 13:00 h, it was determined that a midmorning snack was least likely to influence their regular lunch at 13:00 h when children either went home for lunch or

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ate lunch brought to school. The Control group participated in all of the cognitive, social, food intake, physical, morbidity and biochemical measurements but did not receive a school intervention feeding.

The meal for all three-intervention groups was based on Githeri, a local vegetable stew composed of maize, beans and greens. For the Meat group, finely ground beef with 10–12% fat was added to the Githeri. It was obtained frozen from a reputable meat packer (Farmer's Choice, Nairobi, Kenya). Grinding the meat guaranteed that it was evenly distributed throughout the dish. For the Milk group, a glass of whole ultra-heat treated (UHT) cow's milk was given in addition to the basic Githeri. The Energy group received the Githeri with extra vegetable oil added to increase the energy content. The oil (Kimbo, manufactured by Unilever, East African Industries, Nairobi, Kenya), found to be fortified with retinol, was used in all three types of feeding, but most was added in the Energy group. All three snacks provided to the children were isocaloric. Ingredients were increased by ~25% after a year as the children increased in size. The feedings were designed to offer about one-fifth of the required daily energy intake (**Table 1**). The nutrient composition of the intervention feedings is described by Murphy et al. in this supplement (23). The animal products used were locally available and culturally acceptable. The basic preparation of the food in each condition was similar.

Children were observed during feeding to determine whether they ate the snack provided to them. Food and milk not consumed were measured and leftovers recorded. The vitamin B-12 content of the ultra-heat treated milk was analyzed and found to be comparable to fresh milk.

Quality control and logistics. Steps were taken to ensure the hygienic and nutritional quality of the snacks. Medical examination of all food handlers was performed by the supervisors on a regular basis. The area Public Health Officer visited the kitchen area to ensure high levels of cleanliness and food handling methods. Round the clock supervision was performed at both the kitchen and school levels to ensure standard procedures. Quarterly retraining sessions and regular meetings were held between the staff and the supervisors. Proximate analysis of the snack types was carried out on a regular basis to evaluate nutrient content and any necessary adjustments.

Logistics. The preparation and delivery of the snacks to different schools and children were labor-intensive and challenging. Examples included the need to address rumors about the appropriateness of the snacks prepared and their potential for causing illnesses in the children. Working overnight was a new experience for most of the staff. During the dry seasons there was no water in the pipes, so water had to be fetched from a nearby stream and treated to ensure its safety. During the rainy season pipes became blocked by debris from the forest, hence the persistent need to treat the water. Electricity blackouts were common, forcing the staff to rely on lamps and candlelight to ensure accurate and timely preparation of feedings. Road conditions were poor with excessive dust in dry weather and deep mud and slippery conditions during the rainy seasons. Mosquitoes were present in the open-air kitchen, so the area was enclosed with mesh, and mosquito repellants were provided.

Baseline measures

Baseline measures of the variables to be used as outcomes and covariates (**Table 2**) were collected within 2 mo before the initiation of the school intervention feeding at the start of the school term. Analyses of measures (*t* tests) were carried out to detect any statistically significant differences among the feeding and control groups. The methods for obtaining the baseline

TABLE 1

Nutrient content of school snacks

	Githeri + meat	Githeri + milk	Githeri + extra oil
Y 1: Sept.–Dec. 1998			
Serving size	185g (includes 60 g meat)	100 g + 200 mL milk	185 g + 3 g oil
Energy, *kcal*	239	241	240
kj	1028		
Protein, *g*	19.2	12.7	7.9
Iron, *mg* (total)	2.42	1.52	3.16[1]
Zinc, *mg* (total)	2.38	1.46	1.35
Vitamin B-12, *μg*	0.75	0.96	0.0
Y 2–3: Jan. 1999–Mar. 2001			
Serving size	225 g (includes 85 g meat)	100 g + 250 mL milk	230 g + 3.8 g oil
Energy, *kcal*	313	313	313
kj	1346		
Protein, *g*	21.7	15.2	8.4
Iron, *mg* (total)	2.94	1.57	3.93[1]
Zinc, *mg* (total)	2.89	1.66	1.68
Vitamin B-12, *μg*	0.91	1.16	0.0

[1] Total iron presented. The actual percent absorbed would be ~5%, due to the high phytate and fiber in the plain Githeri.

TABLE 2

Measurements at baseline and throughout the study

	Frequency	Outcome	Covariate
Household[1]			
Census	2	—	X
Socioeconomic status (SES)	2	—	X
Parental information			
Tested reading and literacy	1	—	X
Highest grade completed	1	—	X
Parental height	1	—	X
School measures			
Classroom quality	5	—	X
Videotaping of classrooms	5	—	X
School attendance	5	X	X
Term end examination scores	5	X	—
Child measures[2]			
Health history and physical examination	2	X	X
Hemoglobin, malaria, intestinal ova and parasites	3	X	X
Blood micronutrients	3	X	X
Cognitive tests	5	X	X
Playground and classroom behavior and activity observations	5	X	X
Urinary iodine[3]	1	—	X
Height, head circumference[4]	6	X	X
Weight, arm circumference, triceps and subscapular fat folds[5]	18	X	—
Usual home food intake[5]	18	—	X
Leftovers from snacks	Every school day	—	X
Morbidity[5]	18	X	X

[1] At baseline and y 2.
[2] At baseline and at end of y 1 and 2.
[3] At baseline only.
[4] Quarterly.
[5] Monthly y 1, alternate month thereafter.

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variables, both outcome measures and covariates, were identical to those used in the ongoing data collection in the intervention phase of the study. They were collected at different intervals.

A brief synopsis of the data and methods is provided below. A more detailed description of these methodologies is to be found in the articles in this supplement. The methods were those developed in large part by the NCRSP (6) (Table 2).

Household and family information

Household (HH) census. The household demography or census included the number and age of household members, those deceased, births, birth order of the children and identification of the HH head. The definition of household membership was people who usually live in the HH, cook and eat together and are part of an economic unit. Also, the biological parents of the index child, and the relationships among those who resided in the household on a regular basis (not absent over three consecutive months), were designated.

Socioeconomic status. The SES score is a composite of land ownership and usage for cultivation; income from any source including salaried employment; expenditures; ownership of household goods and implements; forms of transportation; type and structure of the house; parent's occupations; involvement of parents in leadership and community positions; ownership of or access to radio, television and newspapers; water supply; electrification; number and types of animals owned, cash crops, etc. Different weightings were assigned to possessions depending on their relative importance and value. The SES score was validated by community leaders using their own criteria for ranking SES in the former NCRSP study and a statistically significant correlation between the SES scores obtained by each method (6).

Parental information. Parental literacy testing, using graded material (Ministry of Education grade levels) for reading and writing was initiated at baseline and completed during the course of the study. The highest grade of schooling of the parents was also recorded. Parental height was obtained to be better able to interpret the child's stature. For literacy and height measures, nearly 100% of mothers were measured and tested, but only ~70% of the men were available for data collection.

School measures

Classroom quality is an important factor in school performance. Physical amenities were assessed based on observation of the physical amenities, desks, chairs, tables, chalkboards, writing paper, supplies, lighting and types of floor. Most had dirt floors, no electricity and no glassed windows. Classroom size, books, pictures and supplies were recorded. Water supply and latrines were also inspected.

Videotaping of classrooms, 30 min with classes in session, was carried out once per term to assess overall quality, organization, ability of the teacher to control and lead the class and overall classroom ambience. These were coded by three Kenya University students who have attended Kenyan primary schools. The videotaping was performed as unobtrusively as possible and, after the first few sessions, was largely ignored by the children and the teacher. Teachers were asked to rate their students on their academic success and behaviors.

Attendance was assessed for each term based on teacher's reports. Also, the daily school feeding logs at each feeding session were a source of information on daily school attendance.

School examination scores. End of term examination scores were obtained from the Head Teacher's office. These examinations were zone wide and uniform in content and grade level across the schools in the area.

Child measures

Physical status based on health history and physical examination. These examinations were carried out by physicians, a nurse and a clinical officer working with the physician. Obviously retarded or chronically ill children were excluded based on the history and physical examination. Clinical nutritional status was assessed, and vision and hearing were evaluated. Spleen enlargement was measured to assess spleen rates indicative of endemic malaria. Stool samples were collected for detection of intestinal ova and parasites using the formal-ether sedimentation method and Lugol's stain (24).

Blood samples were obtained by venipuncture at baseline and at the end of y 1 and 2 for hemoglobin analysis using the Hemocue apparatus (25) and biochemical analyses of micronutrients only for cohort I. Acute infections were detected by C-reactive protein (CRP) (26). Malaria parasites were detected by thick and thin blood smears (quantitative counts of parasites per red and white blood cells) (24) and malaria falciparum antigen dipsticks (27). These analyses are described in the article by Siekmann et al. in this supplement (28). A single urine sample was collected to measure urinary iodine to assess iodine status. The analyses were carried out at the University of Nairobi in a World Health Organization laboratory.

Growth. Measurements of head and arm circumference, height, weight, triceps and subscapular fat folds were obtained according to Jelliffe and Jelliffe (29), WHO/CDC (30) and WHO and compared to NHANES reference data (30,31). Methods are fully described by Grillenberger et al. in this supplement (32). Indices such as arm fat area (AFA), arm muscle area (AMA) and body mass index (BMI) were derived from the above measurements (33).

Food intake. Usual daily intake was assessed by semi-quantitative 24-h recall from the mother and from the child if present. Data from three consecutive visits, spaced 2–3 wk apart, were averaged to give the usual 24-h intake of nutrients. The WorldFood Minilist, developed for use in Kenya, Mexico and Egypt, was used to convert the data to nutrient intakes (9). The nutrient database for Embu was based on Embu foods with 48 ingredients and dishes actually analyzed for nutrients (Medallion Laboratories, Minneapolis, MN) and the remainder estimated from a variety of appropriate high quality data sources (34). For details, please see the related article by Murphy et al. in this supplement (23).

Cognitive, behavioral and activity measures

The cognitive tests, and behavioral and activity assessments, were those extensively used in the 1984–1987 NCRSP studies in the same population, when over 1000 tests were performed in children and adults (6,11). The same highly trained and experienced field staff performed the testing and observations reported here. The measures have been subjected to repeated scrutiny, and their reliability and validity have been demonstrated (6,11). The cognitive testing was carried out in the school and after class, so that the child was relatively free of distractions. The methods, which included the Verbal Meaning Test (Muinde, N., unpublished results), Digit Span (35) and Raven's Progressive Matrices, are fully described by Whaley et al. in this supplement (36). The RPM is a nonverbal test of performance, abstract meaning, perception and problem solving (fluid intelligence) (10). For all cognitive tests, raw scores were used in the analyses, as standardization is not available in Kenya and standardization based on children in the United States is not appropriate or meaningful (6,11).

Physical activity and behaviors. These were measured by observation techniques using time sampling to obtain estimates

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of child activity and social interaction during unstructured play in the schoolyard and activity and attentiveness in the classroom. Timed observations were used—30 s for observation and 30 s for recording. A total of 30 min per child was required. Strict criteria to define activity and behaviors were used (11,12). Levels of high, medium and low activity were recorded using predetermined criteria. Behaviors of leadership, initiative, solitary play, sustained activity, display of negative or of positive emotion were all strictly defined and recorded. In the classroom, paying attention to schoolwork at hand and to the teacher, talkativeness and playing were used to evaluate on task or off task behaviors or paying attention in class (11). For all of the cognitive testing and observations, frequent quality control, validity and reliability measures and training exercises were carried out.

Morbidity assessment

Morbidity data collection at baseline was incorporated into a health history obtained by the nurse, clinical officer, or a physician. Morbidity information was collected for one entire week every month in y 1 and every other month in y 2. Observations of physical findings were made if a sick child was at home. The child's caretaker was asked about all illnesses, using open-ended and probing questions and a structured questionnaire. Both signs and symptoms were elicited as well as changes in activity and food intake. Signs and symptoms were organized in the questionnaire by general, nonspecific and specific categories, which comprised a diagnosis or illness category. Medications and visits to a health facility were verified by clinic or hospital cards when available. Children suspected of having a severe or life-threatening illness were seen individually by a physician and/or referred immediately to a health facility and project transportation provided if at all possible. A project nurse and physician conducted ongoing training of the enumerators, and there were periodic quality control visits on a 5–10% subset.

For health and ethical reasons, children with severe anemia (hemoglobin ≤70 g/L) were treated with oral ferrous sulfate for 30 d. All children were dewormed every 8 mo during the project with mebendazole to eradicate Helminths (hookworm, ascaris, etc.). If a death occurred in a study child, the physician and/or nurse visited the home, or the clinic or hospital to carry out a verbal autopsy to determine cause of death. The morbidity data was summarized as period prevalence, using time sampling of 1 wk per given mo.

Statistical methods for the intervention study

Once schools were randomized to one of four intervention groups (Control, Energy, Milk or Meat), data collection began with baseline measurements (times before 0) and then continued as the school feeding intervention was initiated. Children were observed from 1 to 18 times at different intervals, depending on the type of measurement.

In addition to the basic outcome measures, the project collected a variety of concomitant data for use as statistical covariates in the interpretation of results. These covariates fell into two broad categories: subject-specific covariates that did not change during the study, such as gender; and time-dependent covariates that were likely to change, e.g., morbidity and blood biochemistry values. Certain outcome measures such as morbidity might well have appeared as covariates in analysis of other outcome measures. The measured outcomes included six classes of responses such as cognitive function, playground and classroom activity and behavior, anthropometry, morbidity, food intake and biochemical micronutrient measures gathered at different time intervals.

Conventional repeated-measures analysis of variance (ANOVA), frequently used to analyze longitudinal data, are based on certain very restrictive assumptions (no missing data, equal covariance among all measurement intervals, equally spaced time points, lack of provision for time-dependent covariates, etc.) that make its use infeasible in this context. In the analyses chosen for this study: *i*) each child is assumed to follow a unique random linear (in time) development course (slope), but with variations at each measured time due to measurement error and random variation; *ii*) the slopes are assumed to be drawn from a population of slopes, with the population means depending on the child's feeding group membership; *iii*) a similar representation is applied to the child's general level (analogous to a regression intercept); and *iv*) there may be a random additive effect attributed to school-to-school variation.

One must allow for differences among children, aside from the effect of feeding intervention. Statistically, this implies that there is natural variability in children's developmental courses. Thus, the study may be described as having a nested or hierarchical design (37); there are schools within feeding groups, children within schools and longitudinal measurements of children. The primary goal of our data analyses was to compare the rates of change across children and feeding groups.

Given all these assumptions, we used standard statistical software, SAS PROC MIXED (38) to compute estimates and standard errors for two types of parameters: *i*) fixed effects including the mean intercepts and slopes for the four feeding groups and *ii*) random effects including the intercepts and slopes of the individual children and school effects. After these analyses, the validity of the models was confirmed using standard statistical methods. The analyses were also repeated including covariates as needed for interpretation. The complete data analysis included estimations of how these conclusions might be affected by various subject-specific and time-dependent covariates.

Baseline findings

Selected baseline findings in cohort I in a number of domains are presented here to show initial similarities and differences among the four groups. They provide information on the households and parents and at the child and group level. Although there were no statistically significant differences among the intervention groups and controls for most of the baseline variables, several blood micronutrient concentrations differed significantly among the groups (see below).

Household attributes. Although not significantly different, the mean SES score for the Milk group was somewhat lower than for the Meat group. The SES scores have not yet been disaggregated to determine which components of the SES score differed among the groups. The average household size was six, and families tend to be nuclear or extended but small (**Table 3**).

Parental education. For maternal schooling and literacy, nearly all mothers were tested (Table 3). Most mothers completed six primary grade levels. The writing ability of mothers was at a mean grade level of 4.6, and for reading, 6.6. For paternal schooling and literacy, about two-thirds of the fathers were available for testing. For schooling, fathers completed about one grade higher than the mothers, completing a mean grade of 7. Their reading and writing literacy abilities were consistently a grade higher than that of the mothers, with a mean grade level of 8 for reading and 6.5 mean grade level for writing (Table 3).

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TABLE 3

Comparison of parent's education, socioeconomic status (SES) and literacy across groups[1]

Groups	*N*	Meat	Milk	Energy	Control	*P*-value
Household SES	494	95 ± 5	83 ± 5	87 ± 6	93 ± 1	
Age of child, *mo*	484	93.6 ± 2.7	88.5 ± 2.6	87.0 ± 2.7	88.1 ± 2.7	0.4
Percentage of male children	494	50	53	51	53	
Percentage of female children	247	50	47	49	47	
Mother	320	5.9 ± 0.3	5.8 ± 0.4	6.2 ± 0.4	6.2 ± 0.4	0.9
Schooling, *y*	441	6.6 ± 0.7	7.5 ± 0.7	6.8 ± 0.7	6.3 ± 0.7	0.7
Reading ability,[1] *grade*	442	4.6 ± 0.5	5.7 ± 0.5	5.1 ± 0.5	5.2 ± 0.6	0.6
Writing ability,[1] *grade*						
Father	226	6.5 ± 0.4	7.6 ± 0.5	6.4 ± 0.5	7.8 ± 0.5	0.2
Schooling, *y*	335	7.5 ± 0.5	8.6 ± 0.5	7.8 ± 0.5	8.5 ± 0.5	0.4
Reading ability,[1] *grade*	335	6.3 ± 0.5	7.3 ± 0.5	6.0 ± 0.5	6.6 ± 0.5	0.4
Writing ability,[1] *grade*						

1 Values are means ± SD, different from Control, $p < 0.05$.

Anthropometric status

Both height and weight are reported as Z-scores (HAZ, WAZ) (28). The mean age of children at baseline was 7.4 y, range 6–14 y. Children are permitted to start Standard I classes at any age, with the poorer children tending to start as late as 14 y.

Stunting (<-2HAZ) was found in 19.4% of the sample as a whole, 23% of the boys and 15.5% of the girls. Severe stunting was found in 4.6% (<-3 HAZ) of the children. The younger the group, the lower the percentage of stunting (**Table 4**).

Underweight (<-2 WAZ) occurred in ~30% of boys and 30% of girls. Mild underweight (from -1 to -2 WAZ) was seen in 42.1% of boys and 31.1% of girls.

Body composition estimates. Children were generally lean, with mean triceps and subscapular fat folds below the 5th percentile, based on reference data for African American Children from NHANES (31). On average, arm fat area was in the 5th percentile and arm muscle area in the 5th–10th percentile (33). A higher percentage of older children was underweight compared to younger children.

Food intake

Foods were converted into nutrients consumed per day, and the usual intake at baseline was estimated by averaging three 24-h recalls taken 3–4 wk apart. Detailed information on food intake is presented by Murphy et al. in this supplement (23).

The children's usual home food intake is important as a covariate and is being used to detect any impact of school intervention feeding on intake at home (**Table 5**). Total energy intake was within the recommended range for a child weighing 20 kg (the mean baseline weight), which is the 20th percentile of weight-for-age. At the 50th percentile of weight for this age, the recommended intake would be 2200 kcal/d (30,39). Total protein intake was also in the normal range, but total animal source protein and protein from meat, fish and poultry (MFP) was extremely low. Fat intake was generally low, 24 g/d or 13% of kcal, as were the intakes of saturated fat and cholesterol (Table 4). Both fiber and phytate intakes were high, 43.8 (± 18.0) g/d and 3,361 (± 1402) mg/d, respectively (9).

Micronutrients

Inadequate micronutrient intakes were highly prevalent, particularly iron, zinc, vitamin B-12, calcium, vitamin A and, to a lesser degree, riboflavin (Table 4). Given the low heme iron in the diet, the bioavailability of both iron and zinc are low (3,9). For most micronutrients, the group differences in intake were not significant. However, vitamins A and B-12 intakes were significantly lower in the Milk and Meat groups compared to the Energy and Control groups (Table 5). The same micronutrients had a high probability of being inadequate in the NCRSP studies of the mid 1980s (9,40). The inadequate intakes were borne out by the baseline biochemical analyses of

TABLE 4

Anthropometric variables at baseline, by group[1]

	Meat	Milk	Energy	Control	*P*-value[1,2]
Height, *cm*	116.5 ± 1.3	115.8 ± 1.3	115.7 ± 1.3	115.8 ± 1.3	0.97
Weight, *kg*	20.1 ± 0.4	19.7 ± 0.4	19.8 ± 0.4	19.8 ± 0.4	0.92
WAZ[3]	−1.3 ± 0.1	−1.2 ± 0.1	−1.0 ± 0.1	−1.1 ± 0.1	0.34
HAZ[4]	−1.6 ± 0.2	−1.3 ± 0.2	−1.3 ± 0.2	−1.3 ± 0.2	0.54
WHZ[5]	−0.4 ± 0.1	−0.4 ± 0.1	−0.2 ± 0.1	−0.3 ± 0.1	0.38
AMA[6]	1558.4 ± 26.8	1566.7 ± 25.8	1575.1 ± 27.1	1560.03 ± 27.4	0.97
AFA[7]	424.5 ± 21.4	435.7 ± 20.7	440.0 ± 21.8	429.3 ± 21.8	0.96
Maternal height, *cm*	156.2 ± 0.6	157.0 ± 0.6	156.6 ± 0.6	156.3 ± 0.6	0.85
Paternal height, *cm*	164.9 ± 1.0	167.9 ± 1.0	165.6 ± 1.1	166.9 ± 1.1	0.25

1 Values are means and ± SD, different from Control, $p < 0.05$, $n = 554$.
2 *t* tests were used.
3 WAZ, weight-for-age Z-score (28).
4 HAZ, height-for-age Z-score (28).
5 WHZ, weight-for-height Z-score (28).
6 AMA, arm muscle area (30).
7 AFA, arm fat area (30).

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TABLE 5

Baseline daily nutrient intakes by groups[1]

Nutrient	Energy	Milk	Meat	Control	All groups
N	135	125	116	116	492
Energy, *kcal*[1]	1698	1781	1758	1741	1762 ± 493
Kj	7132	7480	7376	7312	7400
Protein, *g*	53.6	53.4	54.7	50.7	53.6 ± 17.5
Vitamin B-12, *μg*	0.73	0.39	0.37	0.54	0.4 ± 0.5
Riboflavin, *mg*	1.04	1.02	1.06	1.03	1.1 ± 0.3
Vitamin A, *μg* RE	359	240	274	333	320 ± 322
Calcium, *mg*	268	269	274	307	261 ± 118
Available iron, *mg*	1.44	1.39	1.51	1.40	1.49 ± 5.8
Available zinc, *mg*	1.09	1.02	1.13	0.99	1.5 ± 2.4

[1] Values are means ± SD, different from Control $p < 0.05$, total $n = 492$.

micronutrients in the current study, as described by Siekmann et al. in this supplement (28). Vitamin A intake from ASF was low, with total intake below recommended levels. Most of the vitamin A comes from carotenoid sources. Low riboflavin intake was found in almost a quarter of the children. Inadequate intakes of vitamin B-12 were highly prevalent, which was not surprising as vitamin B-12 is contained almost exclusively in ASF (Table 5).

Total iron intake was 17.7 ± 6.3 mg/d. However, available iron and heme iron, from meat, fish or poultry, were generally very low or negligible, with estimated iron availability only 9–10% (3,9). A similar picture is seen for zinc. Although zinc intake was 7–9 mg/d, estimated zinc absorption is only 11–12% (9) (Table 5). Calcium intake was also low, as it was in 1984. Milk is consumed by schoolers in small amounts, mainly in tea, if at all. The daily calcium intake of 261 ± 118 mg is ~35% of the recommended level. Of interest is that among the toddlers in our more recent Child Survival study in Embu (2001–2002), 28/256 children (10.9%) were diagnosed with full-blown clinical rickets, presumably due to the low intake of milk. Many of these toddlers are siblings of the schoolers in the intervention study. Thus inadequate intake of multiple micronutrients in these rural schoolers remains a serious problem. The poor diet quality has not improved since the 1980s (40).

Biochemical micronutrient status. Baseline biochemical micronutrient determinations confirm the deficiencies predicted from previous food intake data. The main deficiencies found were vitamins A and B-12, iron, zinc and riboflavin (**Table 6**) (28). Vitamin B-12 deficiency was present in 68.2% of the children with 30.5% having severe deficiency and 37.7%, mild-moderate deficiency. The Meat group had the highest prevalence of severe vitamin B-12 deficiency at baseline compared to the other groups. Vitamin A deficiency was present in ~90% of the sample with severe deficiency present in 22%, and mild-moderate deficiency was found in 65–75% of all groups. No clinical signs of vitamin A deficiency were found during the baseline physical examination.

Infections and parasites

The prevalence of malaria, infection, splenomegaly and intestinal parasites was high. C-reactive protein was elevated (>10 mg/L) in 17.8% of all study children. Malaria was found in 31.8% of all children using the faliciparum malaria antigen dip stick (27), and 45% of children had enlargement of the spleen, indicative of endemic malaria. As for intestinal parasites, *Entamoeba histolytica* (amebiasis) was found in the stools of 21.4%, giardia in 12.5% and hookworm and ascaris in 2 and 3%,

TABLE 6

Prevalence of anemia and low micronutrient values at baseline

		Meat 126	Milk 136	Energy 133	Control 124	Overall 519
Parameter	Cutoff[1]	%	%	%	%	%
Hemoglobin, *g/L*	<115	51.6	46.3	49.6	48.4	48.9
Plasma[2] ferritin, *μg/L*	<15	2.4	10.9	1.5	10.7	6.3
Serum[3] iron, *μmol/L*	<9.0	63.9	40	47.1	56.1	52.4
Serum zinc, *μmol/L*	<10.7	61.5	70.1	64.0	66.7	65.6
Serum copper, *μmol/L*	<11.0	0	0.8	0.7	0.8	0.6
Plasma Vitamin B-12, *pmol/L*	<125	46.9	30.6	23.0	19.7	30.5
(severe)	<125–221	33.9	41.0	37.1	38.5	37.7
(moderate)						
Plasma folate, *nmol/L*	<6.8–13.6	0	0.9	0.8	0.8	1
Plasma retinol, *μmol/L*	<13.6	31.3	23.9	16.7	17.5	22.0
(severe)	<6.8–13.6	67.6	65.0	66.7	75.7	68.6
(moderate)						
RBC riboflavin, *μmol/L*	<170	14.9	18.4	28.8	33.7	24.3

[1] Cutoffs for deficient values are provided by Siekmann et al. (28) in this supplement.

respectively. Serum iron values are not interpretable in the presence of malaria (42), but nonetheless low values were seen in 52.4% of all children. Zinc deficiency was present in 66% of children and riboflavin deficiency in ~25%. Both copper and folate concentrations were normal except for 1% having mild folate deficiency. Ferritin concentrations, usually a reflection of iron stores, were seldom low and more often were elevated, probably due to the presence of acute infection and malaria.

In the presence of malaria, as well as acute infection indicated by elevated CRP, serum concentrations of iron and zinc and plasma retinol were significantly lower. However, plasma ferritin and serum copper concentrations, which are part of acute phase reactants to infection, were significantly elevated in the group with elevated CRP. Thus, the presence of malaria and infection can make assessment of micronutrient status difficult (41–43), particularly of iron status.

Anemia. Low hemoglobin concentrations (<115 g/L) indicative of anemia were seen in 48.9% of the sample as a whole, and severe anemia (<70 g/L) in 9.0% (Table 6). The latter children were treated with ferrous sulfate for 30 d and had a moderate improvement in anemia. There are multiple possible etiologies for the anemia. Malaria is endemic in Embu (see above), iron deficiency is likely based on its low dietary availability and vitamin B-12 and vitamin A deficiencies can also cause anemia (43). Hookworm is not common, but a high percent of children had amebiasis, a source of intestinal blood loss (28).

DISCUSSION

The current baseline information reveals the presence of mild-moderate stunting and underweight in about a third of the children, but with less stunting in the girls than the boys. Although energy intake has improved since the 1980s (6), the intake of ASF and dietary fat remains very low. The prevalence of inadequate micronutrient intakes, including vitamins A, iron, zinc, B-12, calcium and riboflavin, remains unchanged. The bioavailability of iron and zinc is low in the face of high dietary phytate and fiber, and dietary heme iron is low.

The dietary information is confirmed by the biochemical findings of vitamins A and B-12, iron, zinc and riboflavin deficiencies. Urinary iodine is in the low normal range, with ~10% of school children in the deficient range.

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Malaria is endemic with nearly 50% having splenomegaly, and a third having positive falciparum antigen dipstick tests for falciparum malaria. Anemia is widespread and has a number of possible etiologies, including multiple nutrient deficiencies and malaria. Although infestation with hookworm has greatly diminished since the 1980s, other infection with intestinal parasites, particularly amebiasis, may contribute to blood loss.

The baseline characteristics of the three feeding intervention groups and the controls at baseline for cohort I were not significantly different, except for a few measures of micronutrient intake, and the fact that the Meat group had lower intakes and blood concentrations of vitamins A and B-12 and lower height- and weight-for-age. The Milk group had a slightly lower SES score than the other three groups, and the Control group was better off in physical size and maternal literacy than the other three groups. Because the study design is randomized, we feel confident that no major problems emerged from these differences, which were controlled for in the statistical analyses.

Policy considerations. This intervention study will provide policy makers with information upon which to make decisions for nutrition interventions and school feeding, smallholder animal production and agriculture. It can help to guide policy decisions on which food-based intervention alternatives are most efficient, feasible and affordable to improve children's micronutrient status, growth, cognitive function and school performance. The school feeding intervention trial can also provide information on logistical and programmatic issues related to the implementation of school feeding programs using local resources and foods.

Thus far, researchers investigating the effects of nutrition on learning have had little discourse with the agricultural sector. Agriculture and livestock agencies need to better support the smallholder subsistence farmer in increasing household mini-livestock to put "meat on the table". This must be affordable and feasible and allow for some income generation. Appropriate nutrition education is essential to promote the use of ASF in child feeding. Investment in children to enhance their ability to learn and grow is an investment in the future of children, the community and nation.

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