Contents

The Food and Nutrition Bulletin *encourages letters to the editor regarding issues dealt with in its contents.*

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Biochemical indicators of nutritional status and dietary intake in Costa Rican Cabécar Indian adolescents

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Editorial comment

Although it is a small local survey, the following paper was accepted by the Food and Nutrition Bulletin *because it is illustrative of a problem that plagues most countries with an impoverished indigenous population. This includes Australia, China, and the United States, as well as developing countries such as Bolivia, Ecuador, Guatemala, Peru, and the hill tribes of Nepal, Vietnam, and Thailand. It is particularly ironic that Costa Rica, which has come so far in improving the health of its people and providing good medical care to nearly all of its population, should have this problem.*

The first large population and health study in Costa Rica was done in the rural zone of Turialba in 1953 [1]. Its findings and that of other surveys over the next 15 years showed that the nutrition and health situation was no better in Costa Rica than in the other countries of Central America. Growth retardation, nutrient deficiencies, and high infant and preschool mortality were characteristic of all these countries [2]. Then in the single decade of the 1970s, the infant mortality rate in Costa Rica dropped from 68 to 19.1 per 1,000, and "health posts emphasizing prevention of communicable diseases, mother and child health, environmental sanitation, and health education covered 84% of the total population" [3]. In the past decade, the overall infant mortality rate in Costa Rica has become the lowest among the mainland Latin American countries and as low as that seen in some industrialized countries.

Yet the paper that follows shows that the improvement has not sufficiently reached small indigenous populations such as the Cabécar Indians. It is a graphic reminder that every country, no matter how good the access to nutrition and health care is for the majority of its population, has an obligation to identify populations left behind. Good health statistics, even for some of the most advanced industrialized and developing countries, can conceal minority groups in desperate need of the health benefits reaching the great majority of the population. As emphasized in the Alma Ata declaration of 1978, nations have an obligation to provide access to health for all of their population [4].

—Nevin S. Scrimshaw

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Abstract

The purpose of this study was to determine the blood levels of selected nutritional status indicators and the dietary intake of Costa Rican Cabécar Indians aged 10 to 16 years. The results showed that 65% of the adolescents had an adequate body mass index (BMI) for their age, and 32% had a BMI < 5th percentile. Likewise, the study revealed a high prevalence of anemia (57%), deficient serum folate levels (54%), deficient vitamin B_{12} levels (31%), and subclinical vitamin A deficiency (10%). Additionally, the youngsters had elevated prevalences of high triglyceride levels (77%), borderline high-density lipoprotein (HDL) cholesterol levels (46%), homocysteine levels > 10 µmol/L (29%), and homozygous mutation of methylenetetrahydrofolate reductase (MTHFR) (49%). The diet was poor, being high in saturated fat and low in polyunsaturated fat, fiber, and several micronutrients. The dietary intakes of more than 55% of the adolescents did not meet 50% of the estimated average requirements (EAR) for zinc, vitamin A, vitamin C, vitamin B_{12}, vitamin B_2, and folate. Furthermore, a high prevalence of parasitosis was found (68%). Our results show an adolescent Cabécar population with a mosaic of nutritional deficiencies and cardiovascular risk factors.

Key words: Adolescents, Cabécar Indians, Costa Rica, indigenous people, nutritional status

Introduction

Five centuries ago, before permanent Spanish colonization occurred, Central American aborigines were pre-agricultural hunter-gatherers who had adapted extraordinarily well to tropical habitats [1]. Colonization had serious negative effects on aboriginal society, well-being and health. Consequently, aborigines are now among the least healthy populations in several countries and some of them have undergone deep cultural changes in recent years [2].

The indigenous people of Costa Rica represent approximately 1.7% of the country's population. They are distributed among 10 groups (Brunca or Boruca, Cabécar, Térraba, Bribri, Huetar, Maleku or Guatuso, Guaymí, Miskito and Sumo, Chorotega, and Teribe) living in rural territories called reservations, and total 63,876 persons [3]. Cabécar people comprise the second largest group, with 10,175 members scattered among seven reservations in the provinces of Limón and Puntarenas. Few studies have been done in this population. In 1985, Mata et al. showed that most of the indigenous people in Costa Rica enjoyed relatively good health and there were no signs or symptoms compatible with nutritional deficiencies [1]. Nevertheless, no recent investigations concerning the health status of these aborigines have been carried out. Currently, indigenous settlements have been forced to leave their natural habitats, thus acquiring rural characteristics. Few of them have access to social and health services, and their rates of birth, infant mortality, and total mortality are high compared with the national average.

Several studies point out that indigenous peoples have increased their intake of industrialized foods, while foods derived from local and natural environments have declined in use [4–6]. The shift away from traditional foods towards a diet composed exclusively of market foods has been characterized by an increase in absolute energy intake and relative contributions of carbohydrate (particularly sucrose) and saturated fat, and a decrease in micronutrients [4]. There is enough evidence to support the statement that some aboriginal groups have several nutritional deficiencies, aggravated by inadequate sanitary conditions [7]. Other groups, particularly those with a long history of acculturation, show a high prevalence of dyslipoproteinemia, impaired glucose tolerance, hyperinsulinemia, obesity, non-insulin-dependent diabetes mellitus, hypertension and cardiovascular disease, particularly ischemic heart disease and stroke [8, 9].

Because several diet-related diseases are believed to have their origins in childhood, serious concern remains about early indigenous lifestyles that may have important implications for health and mortality among some aboriginal groups during youth and into middle age. Considering that almost half of the indigenous people in Costa Rica are between the ages of 5 and 24 years, the purpose of this study was to determine the blood levels of selected nutritional status indicators and the dietary intake of Costa Rican Cabécar adolescents, in order to provide information for designing strategies for disease prevention and health promotion in this minority group.

Methods

Sample characteristics

A sample of Cabécar adolescents aged 10 to 16 years was selected for this study. Subjects were recruited from the four schools in the Indian reservation of Ujarrás, Puntarenas. The adolescents were asked by a research team member to participate in the survey. Although 104 indigenous adolescents wanted to collaborate, only 81% finally consented to participate in the dietary survey. The final sample consisted of 84 adolescents, 35 boys (42%) and 49 girls (58%).

Procedure for consent and data collection

Permission for the study was obtained from the Costa Rican Institute for Research and Education on Nutrition and Health (INCIENSA) and the University of Costa Rica ethics committees. Written parental and adolescent consent was required to participate in the study. For parents who were illiterate or semiliterate, consent was obtained orally in the presence of independent witnesses external to the investigation group. In order to ensure optimal collection of data, the researchers were introduced into the Indian reservation four weeks before the investigation was carried out. Schoolteachers also assisted in the data collection.

Anthropometry

Weight was measured without the subjects wearing shoes or heavy outer clothing. Height was measured with the subject shoeless and facing away from the stadiometer. Standing height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg. Independent duplicate measurements were obtained for height and weight, and the average of the two readings was used in data analysis.

Adolescents with body-mass index (BMI) ≥ 85th percentile were considered to be at risk of being overweight and adolescents with BMI < 5th percentile were considered to be underweight [10]. In the absence of guidelines specifying optimal cutoff values for BMI in childhood, data on BMI for age from US adolescents were used, as recommended in 1995 by the World Health Organization (WHO) Expert Committee [10].

Dietary intake and food availability

Dietary intake was determined with four 24-hour recalls (every other day, including one weekend day) recorded over a period of two weeks. To estimate the portion size of foods, a series of photographs with different sizes of meals commonly consumed in Costa Rica was used [11], as well as three-dimensional food models. The recipes for the prepared foods consumed were obtained by interviewing the adolescents' mothers. To estimate the intake of food served by the school's food service, the weighted-records method was used [12]. In order to complete the nutritional evaluation, the frequency of consumption (daily, 1–3 times per week, 4–6 times per week, 1–2 times per month, never) of 60 foods was studied (including 15 foods derived from the natural environment) using a questionnaire previously validated in Costa Rican aborigines [13]. This questionnaire was administered to each subject one week after the fourth 24-hour recall was done.

The availability of foods in the community and in the home was also evaluated. A questionnaire was designed to record the frequency (daily, 1–2 times per week, 3–6 times per week, 1–2 times per month, never) of the availability of 45 foods in homes and at local business establishments in the community. Additionally, the criteria for availability of foods were explored (e.g., price, habits, storage conditions). In this part of the investigation, all local business establishments at the Indian reservation were visited ($n = 6$), as well as 25 homes. The homes were randomly selected among those of the adolescents included in the study.

To evaluate the quality of the Cabécar diet, a comparison was made with the American Heart Association dietary guidelines [12] and with the estimated average requirement (EAR) [14]. Moreover, the polyunsaturated fatty acids:saturated fatty acids (P:S) relationship and the cholesterol-saturated fat index (CSI) were calculated [15]. The fiber intake was evaluated by using the "age + 5 rule" [16].

Food Processor for Windows version 6.0 (Esha Research, Salem, OR, USA) was used to perform nutrient calculations based on dietary data. The nutritive values of approximately 60 food preparations commonly consumed in Costa Rica were incorporated into this database. This information was supplied by the School of Nutrition, University of Costa Rica. There were no missing nutrient values in the database. All foods included in the Cabécar diet were available in the database, because the consumption of foods derived from the natural environment is low to nonexistent.

Biochemical measurements

A 12-hour fasting blood sample was taken from the antecubital vein using Vacutainer tubes (Becton-Dickinson, Rutherford, NJ, USA), following the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [17]. To avoid light degradation of the vitamin A, the test tubes were covered with special black cloth hoods, as suggested by Dary and Arroyave [18]. Additionally, two test tubes for blood with ammonic heparin and ethylenediaminetetraacetate (EDTA) were taken. The samples were refrigerated (6 ± 2°C) during their transport to the Health Office Laboratory at the University of Costa Rica and the INCIENSA laboratories for analysis.

Serum was obtained by centrifugation at 6,000 rpm for 5 min at 25°C. Removal of the serum from the red cell pack was done in a dark room with a yellow bulb, as suggested by Landers and Olson [19] to avoid the isomerization of retinol.

Total serum cholesterol (TC), high-density lipoprotein (HDL) cholesterol, triglycerides (TG), and glucose were determined by enzymatic colorimetric

reactions using a Vitros 250 dry chemistry system (Ortho-Clinical Diagnostic, Johnson & Johnson, Rochester, NY, USA) at 505 nm and 37°C. Low-density lipoprotein (LDL) cholesterol was calculated by the equation of Friedewald et al. [20]. The respective intra-assay and interassay coefficients of variation were 1.6% and 2.4% for TC, 3.5% and 3.6% for HDL cholesterol, and 1.5% and 2.4%, for glucose. The coefficients of variation for TG were less than 3.3% in both assays. TC and LDL cholesterol concentrations were classified according to the guidelines of the Expert Panel on Blood Cholesterol Levels in Children and Adolescents [21].

Serum levels of vitamins E and A were measured by high-performance liquid chromatography (HPLC), according to the methodology recommended by Beiri et al. [22].

About 50% of the vitamins A and E analyses were done in duplicate. All samples with retinol levels ≤ 0.70 μmol/L or > 1.75 μmol/L were processed again. The coefficients of variation for the assays of α-tocopherol and retinol were < 10%.

Because tocopherol circulates in the bloodstream associated with lipids, the serum α-tocopherol concentration was adjusted for serum lipids by dividing it by the sum of serum total cholesterol and TG concentrations, as suggested by Horwitt et al. [23].

Homocysteine serum levels were measured by a fluorescence polarized immunoassay (FPIA) test using an IMx Analyzer (Abbott Laboratories, Abbott Park, Ill., USA) [24].

Hemoglobin was determined by the cyanmethemoglobin method [25]. Hematocrit was measured by the microhematocrit technique [25]. Serum iron (SI) and total iron-binding capacity (TIBC) were measured with a two-point fixed-time rate assay using a Vitros 250 dry chemistry system (Ortho-Clinical Diagnostic) at 600 nm and 37°C. The respective intra-assay and interassay coefficients of variation were 2.0% and 2.5% for SI and 5.0% and 6.5% for TIBC.

Transferrin saturation (TS) was calculated by dividing SI by TIBC. Serum ferritin (SF) was measured with the Coat-A-Count Ferritin IRMA kit (DPC, Los Angeles, CA, USA), and serum folate and vitamin B_{12} were determined with the Solid Phase No Boil Dual count kit (DPC). For these analyses, duplicated samples were processed. The coefficients of variation for the SF, folate, and vitamin B_{12} assays were 5.3%, 4.3%, and 4.6%, respectively.

Genetic analyses

Genomic DNA was isolated from blood leukocytes using the method of Miller et al. [26]. Identification of the C to T substitution at nucleotide 677 of the enzyme methyltetrahydrofolate reductase (MTHFR) gene was assayed using by method of Frosst et al. [27].

Intestinal parasites

To collect the adolescents' feces, every subject received a sterile container. The presence of worms and protozoa was determined by fresh and lugol-dyed microscopic observations. The Kato concentration technique was used to increase the detection of worm eggs [28].

Statistical analyses

Data were analyzed by using SPSS for Windows (version 10.0) to calculate descriptive statistics, percentiles, to perform Student's t-test and the chi-square test. For between-gender nutrient intake comparison, dietary intakes were adjusted per 1,000 kcal. Partial Spearman correlation coefficients adjusted for energy intake were calculated to determine associations between dietary variables and serum biochemical parameters.

Results

The sample consisted of 35 boys and 49 girls. The

TABLE 1. Anthropometrical and biochemical indicators of nutritional status in Costa Rican Cabécar adolescents ($n = 84$) [a]

Indicator	Mean ± SD
Age (yr)	12.0 ± 1.3
Weight (kg)	29.6 ± 10.2
Height (cm)	137.8 ± 14.6
Body-mass index (BMI) [a]	18.2 ± 4.5
Serum iron (μmol/L)	15.4 ± 5.7
Hemoglobin (g/L)	122 ± 10
Transferrin saturation (%)	22.2 ± 9.1
Ferritin (μg/L)	55.3 ± 34
Total iron-binding capacity (μmol/L)	70.0 ± 9
α-Tocopherol (μmol/L)	2.5 ± 0.6
α-Tocopherol/TC + TG (μmol/mmol)	4.4 ± 1.1
Vitamin A (μmol/L)	1.4 ± 0.3
Vitamin B_{12} (pmol/L)	242.2 ± 145
Folate (nmol/L)	6.9 ± 4.1
Homocysteine (μmol/L)	9.1 ± 2.3
TC (mmol/L)	4.1 ± 0.6
LDL cholesterol (mmol /L)	2.2 ± 0.6
HDL cholesterol (mmol/L)	1.2 ± 0.2
TG (mmol/L)	1.7 ± 0.6
TC:HDL cholesterol ratio	3.5 ± 0.9
Glucose (mmol/L)	4.0 ± 0.8

TC, Total cholesterol; TG, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
a. BMI = weight(kg)/height(m)^2.

mean age was 12.0 ± 1.3 years. The prevalence of underweight (BMI < 5th percentile) was 32%, and the proportion of adolescents at risk for overweight was 3%. The mean values (± SD) and percentiles of biochemical nutritional status indicators are presented in **tables 1 and 2.** The mean values of the studied variables were not significantly different between boys and girls (t-test).

Biochemical status

The prevalence of anemia (hemoglobin < 120 g/L for

TABLE 2. Serum levels of biochemical indicators of nutritional status in Costa Rican Cabécar adolescents ($n = 84$) according to percentile

Indicator	P_5	P_{15}	P_{25}	P_{50}	P_{75}	P_{95}
Hemoglobin (g/L)						
Total	104	112	116	121	130	139
Males	104	114	120	124	130	137
Females	104	108	112	120	129	141
Serum iron (μmol/L)						
Total	7.4	9.6	11.5	14.8	19.5	25.5
Males	6.9	7.8	10.0	13.4	19.5	26.7
Females	8.5	11.0	12.2	15.2	19.6	27.0
Transferrin saturation (%)						
Total	10.6	14.6	16.0	20.0	28.0	39.6
Males	8.8	12.0	15.0	18.0	25.0	46.0
Females	12.3	16.0	16.0	20.5	31.0	41.0
Ferritin (μg/L)						
Total	13	24	36	52	71	125
Males	12	16	27	47	73	166
Females	16	30	37	52	71	89
Folate (nmol/L)						
Total	1.8	2.4	4.0	6.3	9.7	15.2
Males	1.4	2.0	4.2	6.1	8.7	14.9
Females	1.9	2.5	3.7	6.3	10.8	16.1
Vitamin A (μmol/L)						
Total	0.92	1.13	1.19	1.37	1.61	2.18
Males	0.93	1.14	1.21	1.39	1.58	2.54
Females	0.87	1.09	1.19	1.33	1.62	2.16
Vitamin E (μmol/L)						
Total	1.6	1.9	2.2	2.4	2.9	3.7
Males	1.6	2.2	2.3	2.5	2.8	4.0
Females	1.6	1.9	2.1	2.4	2.8	3.7
α-Tocopherol/TC + TG (μmol/mmol)						
Total	2.52	3.32	3.82	4.34	5.09	7.06
Males	2.42	3.31	4.07	4.46	5.57	6.96
Females	2.67	3.02	3.43	4.27	4.86	6.11
Vitamin B_{12} (pmol/L)						
Total	62	81	123	211	362	488
Males	62	80	147	204	346	558
Females	64	82	119	237	380	493
Homocysteine (μmol/L)						
Total	6.33	7.06	7.39	8.33	9.81	13.74
Males	5.93	6.79	8.07	9.27	11.34	13.49
Females	5.52	6.69	7.37	8.06	9.80	15.01

TC, Total cholesterol; TG, triglycerides.

girls and < 130 g/L for boys) was 57%. No significant differences were found in the prevalence of anemia between boys and girls (69% and 45% respectively, $p = .134$). Iron deficiency (SF < 12 μg/L and TS < 16%) was found in 1% of the adolescents, and iron-deficiency anemia (iron deficiency as defined here and hemoglobin levels consistent with anemia) was found in ≤ 2% of adolescents.

About 2.5% of the Cabécar adolescents had depleted iron reserves (SF < 12 μg/L), and 12% had marginal levels (SF between 12 and 23 μg/L) (**table 3**). The prevalence of SF values < 12 μg/L was similar for boys and girls.

The prevalence of deficient serum folate levels (< 6.8 nmol/L) was 54% **(table 3).** The prevalence was slightly higher, but not significantly so, in boys. Marginal serum folate levels (6.8–13.6 nmol/L) were found in 38% of the adolescents. The proportion of boys and girls with marginal serum folate levels was not significantly different.

Subclinical serum vitamin A deficiency (0.70–1.04 μmol/L) was found in about 10% of the adolescents. The prevalence was slightly higher, but not significantly so, in girls. More than 30% of the Cabécar adolescents had deficient vitamin B_{12} levels (≤ 148 pmol/L). This prevalence was higher, but not significantly so, in girls. Marginal vitamin B_{12} levels (148–701 pmol/L) were found in close to 70% of the adolescents. Only 1.3% of the adolescents had vitamin E deficiency (< 1.2 μmol/L), and 2.4% had marginal levels of this vitamin (1.2–1.6 μmol/L).

The mean values of TC, HDL cholesterol, and LDL cholesterol were not significantly different between boys and girls (data not shown). However, the mean value for TG was significantly higher in girls than in boys (1.82 ± 0.77 and 1.49 ± 0.61 mmol/L, respectively; $p = .045$). The TC:HDL cholesterol ratio averaged 3.5, with no differences between the sexes. **Table 4** shows the classification of Cabécar adolescents according to serum lipid and glucose levels. The proportions of adolescents with borderline serum TC (4.42–5.17 mmol/L; 18%) and high levels of serum TC (≥ 5.2 mmol/L; 7%) were not significantly different between boys and girls. Likewise, the proportions of girls (10%) and boys (11%) with borderline levels of serum LDL cholesterol (2.86–3.35 mmol/L) were not significantly different. Approximately 57% of girls had borderline HDL cholesterol levels (0.91–1.17 mmol/L), and 32% had high HDL cholesterol levels (> 1.17 mmol/L). These prevalence levels were significantly higher than

TABLE 3. Proportion of Cabécar adolescents with deficient, marginal, and adequate serum levels of selected nutritional status indicators

Indicators	Total ($n = 84$)	Boys ($n = 35$)	Girls ($n = 49$)
Ferritin (μg/L)			
Deficient (< 12)	2.4	2.9	2.1
Marginal (12–23)	12.2	17.6	8.3
Adequate (> 23)	85.4	79.4	89.6
Folate (nmol/L)			
Deficient (< 6.8)	54.5	56.3	53.3
Marginal(6.8–13.6)	37.7	37.5	37.8
Adequate (> 13.6)	7.8	6.3	8.9
Vitamin A (μmol/L)			
Deficient (< 0.70)	1.2	0	2.0
Marginal (0.70–1.04)	9.6	8.8	10.2
Adequate (> 1.04)	89.2	91.2	87.8
Vitamin B_{12} (pmol/L)			
Deficient (< 148)	31.2	25.0	35.6
Marginal (148–701)	68.8	75.0	64.4
Adequate (> 701)	0	0	0
Vitamin E (μmol/L)			
Deficient (< 1.2)	1.3	1.5	1.1
Marginal (1.2–1.6)	2.4	2.8	2.0
Adequate (> 1.6)	93.3	95.7	96.9
Homocysteine (μmol/L)			
Adequate (< 10)	70.7	60.0	81.5
Increased risk of coronary artery disease (≥ 10)	29.3	40.0[a]	18.5

a. Significant at $p < .05$ level (t-test); other values not significant.

those observed in boys. The prevalence of high TG levels (≥ 1.47 mmol/L) was 77%, with no differences between the sexes.

Glucose levels averaged 4.0 mmol/L, with no differences between the sexes. More than 3% of all subjects had glucose levels between 6.11 and 6.94 mmol/L. The proportion of boys (5.7%) with glucose intolerance was higher than the proportion of girls (2%), but the difference was not significant.

Homocysteine values ranged from 4.95 to 15.21 μmol/L. The mean homocysteine concentration was not significantly different between boys and girls (9.5 ± 2.1 and 8.8 ± 2.4 μmol/L, respectively; $p = .169$). The proportion of boys with homocysteine levels above 10 μmol/L (40%) was significantly higher ($p = .02$) than the proportion of girls with these levels (18.5%).

The distribution of the three genotypes in the studied population was as follows: homozygous normal (CC) genotype, 4%; heterozygous (CT) genotype, 47%; and homozygous mutant (TT) genotype, 49%. The allele frequency of the T-mutation in the subjects was 0.725 (data not shown).

Dietary intake

The reported mean energy intake was 1,280 ± 253 kcal. As expected, the reported total energy intake was significantly higher in boys than in girls ($p = .037$), although the micronutrient-dense diet was similar in composition in both sexes. The micronutrient reported intake adjusted per 1,000 kcal was significantly greater in girls than in boys only for folic acid and vitamin B_{12} ($p < .05$). The mean cholesterol intake was about 60 mg, and the total fiber intake was approximately 18 g/1,000 kcal.

Table 5 presents the daily intake of vitamins and minerals by percentiles. Approximately 50% of the study subjects had an intake of ≤ 160 μg of folate, ≤ 221 mg of calcium, ≤ 7 mg of iron, ≤ 3 mg of zinc, ≤ 7 mg of α-TE (α-tocopherol equivalent) vitamin E, and ≤ 0.5 μg of vitamin B_{12}. Only 25% of the adolescents had a daily intake of > 352 mg of magnesium, > 899 μg RE of vitamin A, and > 65 mg of vitamin C. About 15% of the adolescents studied had a daily intake of ≤ 0.2 mg of vitamin B_2, and a similar proportion had an intake of ≤ 7 mg of vitamin B_3.

TABLE 4. Classification of Cabécar adolescents according to serum lipids and glucose levels based on the National Cholesterol Education Program guidelines

Value	Total ($n = 84$)	Boys ($n = 35$)	Girls ($n = 49$)
TC (mmol/L)			
< 4.42	74.4	73.5	75.0
4.42 – 5.17	18.3	20.6	16.7
≥ 5.2	7.3	5.9	8.3
LDL cholesterol (mmol/L)			
< 2.86	84.8	84.6	85.0
2.86 – 3.35	10.6	11.5	10.0
≥ 3.36	4.5	3.8	5.0
HDL cholesterol (mmol/L)			
< 0.91	10.6	11.5	10.0
0.91 – 1.17	45.5	26.9	57.5[a]
>1.17	43.9	61.5	32.5[a]
TG (mmol/L)			
< 1.02	14.6	20.6	10.4
1.02 – 1.46	8.5	11.8	6.3
≥ 1.47	76.8	67.6	83.3
TC:HDL cholesterol ratio			
≤ 4.49	83.3	80.8	85.0
≥ 4.50	16.7	19.2	15.0
Glucose (mmol/L)			
< 6.11	96.4	94.3	98.0
6.11– 6.94	3.6	5.7	2.0
≥ 7.0	0	0	0

TC, Total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein. TG, triglycerides.
a. Significant at $p < .05$ level (*t*-test); other values not significant.

TABLE 5. Cabécar adolescents' daily dietary intake of selected vitamins and minerals according to percentile

Nutrient	P_{15}	P_{50}	P_{75}
Folate (μg)	66	160.5	258
Calcium (mg)	107	221	377
Iron (mg)	5	7	11
Magnesium (mg)	124	193	352
Zinc (mg)	2	3	5
Vitamin A (μg RE)	75	193	899
Carotene (μg)	25	48	795
Vitamin B_1 (mg)	0.6	0.8	1.0
Vitamin B_2 (mg)	0.2	0.5	0.8
Vitamin B_3 (mg)	7	8	13
Vitamin B_{12} (μg)	0.1	0.5	1.1
Vitamin C (mg)	11	26	65
Vitamin E (mg α-TE)	4.4	6.2	9.2

RE, Retinol equivalent; TE, tocopherol equivalent

Table 6 presents the percentage of EAR satisfied by the Cabécar adolescents' diet. Individual dietary intake analyses indicate that approximately 75% of the Cabécar adolescents had a zinc intake lower than 50% of the EAR. Between 40% and 65% of the adolescents did not meet 50% of the EAR for vitamin A, vitamin B_2, vitamin B_{12}, vitamin C, and folate. Likewise, around 30% of the Cabécar adolescents reported intakes of iron and vitamins B_6 and E lower than 50% of the EAR, and more than 15% reported intakes of these nutrients between 50% and 69% of the EAR.

Inadequate intakes of the following micronutrients were reported: zinc, vitamin A, vitamin C, vitamin B_{12}, vitamin B_2, and folate. More than 55% of the sample did not meet 50% of the EAR for these micronutrients.

The rank distribution of Cabécar adolescents based on energy consumption derived from the macronutrients is shown in **table 7**. No significant differences were observed between boys and girls. The average percentages of total energy obtained from carbohydrates, protein, and total fat approached 61%, 13%, and 28%, respectively. Nevertheless, 76% of the adolescents obtained more than 55% of their total energy from carbohydrates, and approximately 35% obtained less than 10% of their total energy from protein.

Approximately 27% of the adolescents obtained more than 30% of their total energy from fat. Of these, approximately 10% obtained more than 40% of their total energy from fat (data not shown). About 58% of the adolescents obtained more than 10% of their total energy from saturated fat. Of these, approximately 20% obtained more than 15% of their total energy from saturated fat. More than 90% of the adolescents obtained less than 7% of their total energy from polyunsaturated fat. Approximately 35% obtained between 10% and 15% of their total energy from

TABLE 6. Distribution of nutrients among Cabécar adolescents ($n = 84$) according to nutritional adequacy ranks for estimated average requirements (EAR) [a]

Nutritional adequacy rank (%)	Vitamin A	Vitamin B_1	Vitamin B_2	Vitamin B_3	Vitamin B_6	Vitamin B_{12}	Folate	Vitamin E	Vitamin C	Iron	Zinc	Magnesium
< 30	55.0	0	25.0	1.7	10.0	15.0	30.0	13.3	31.7	13.3	38.3	8.3
30–49	10.0	3.3	18.3	15.0	18.3	50.0	16.7	21.7	16.6	15.0	38.4	13.4
50–69	3.3	21.7	18.4	28.3	18.4	13.3	16.6	28.3	6.7	21.7	10.0	20.0
70–89	1.7	21.7	11.7	23.3	13.3	10.0	16.7	15.0	6.7	20.0	6.6	16.6
90–100	1.7	8.3	8.3	1.7	15.0	34.0	5.0	3.4	5.0	6.7	6.7	5.0
> 100	28.3	45.0	18.3	30.0	25.0	8.3	15.0	18.3	33.3	23.3	0	36.7

a. Based on EAR data [14]

TABLE 7. Distribution of Cabécar adolescents according to cholesterol, fiber, cholesterol-saturated fat index, and energy derived from macronutrient ranks[a]

Nutrient	Total (n = 84)	Boys (n = 35)	Girls (n = 49)
Energy from carbohydrates (%TE)			
< 55	24.2	30.8	17.6
55–60	25.3	26.9	23.6
>60	50.5	42.3	58.8
Energy from proteins (%TE)			
< 10	33.0	30.8	35.3
10–15	52.4	57.7	47.1
>15	14.6	11.5	17.6
Energy from total fat (%TE)			
< 20	23.3	23.1	23.5
20–30	49.1	42.3	55.9
>30	27.6	34.6	20.6
Energy from saturated fat (%TE)			
≤ 10	41.8	30.8	52.9
10–15	38.4	38.4	38.3
>15	19.8	30.8	8.8
Energy from polyunsaturated fat (%TE)			
< 7	94.6	92.3	94.6
7–10	5.4	7.7	5.4
>10	0	0	0
Energy from monounsaturated fat (%TE)			
< 10	42.3	34.6	50.0
10–15	34.9	34.6	35.3
>15	22.8	30.8	14.7
Cholesterol (mg)			
< 100	86.9	84.5	85.3
100–300	13.1	11.5	14.7
>300	0	0	0
CSI			
≤25	87.9	84.6	91.2
> 25	12.1	15.4	8.8
Total fiber			
< "Age + 5" rule	56.8	65.3	48.2
≥ "Age + 5" rule	43.2	34.7	51.8

TE, Total energy; CSI, cholesterol-saturated fat index.
a. No significant differences were found between values for boys and girls for all nutrients ($p < .05$, t-test).

monounsaturated fatty acids, and more than 40% obtained less than 10% of their total energy from monounsaturated fatty acids. All adolescents reported a cholesterol intake of less than 300 mg/day. The cholesterol-saturated fat index (CSI) of the diets of more than 85% of the adolescents was 25 or less.

More than 55% of the sample had inadequate fiber intakes. They did not meet the minimum dietary fiber intake according to the "age + 5 rule" (ie, add 5 grams to adolescents' age to obtain the minimum recommended fiber intake).

The most frequently consumed foods were beans, rice, chicken, eggs, sugar, sausage, sweets, roots and tubers, fruits, and palm shortening. More than 70% of the adolescents ate at least one of these foods four to six times per week. In contrast, approximately 60% consumed milk, cheese, vegetables, or meats only once a week or less. The foods with a frequency of consumption lower than once a week were soybean oil, cookies, pastries and organ meat. Foods derived from the natural environment were eaten rarely.

The most available foods in the aborigines' homes were rice, beans, palm oil, avocado, bananas, mangoes, oranges, palm peach (*Bactris gasepaes*), plantains, salt, brown sugar, and tubers such as cassava (*Manihot esculenta*), taro (*Colocasia esculenta*), sweet potato (*Ipomoea batatas*), and malanga or blue taro (*Xanthosoma violaceum*). According to the informa-

tion reported by the indigenous people, these foods are generally available at home because they are affordable, are always available at the stores on the reservation and do not require special storage conditions. In contrast, perishable foods such as meat, milk, and vegetables have a limited availability, because these foods are expensive, are scarce in the commercial establishments of the community, and require special storage conditions for preservation. The foods always available at the local stores of the reservation include basic grains (rice and beans), white bread, sugar, flour pastries, brown sugar candy, palm shortening, roots and tubers, eggs, carbonated beverages, condiments, canned foods and other foods such as chocolate bars, candies, biscuits, plantain and potato chips, and fried corn flour snacks. Perishable foods such as meat, chicken, milk, cheese, fruits and vegetables are available only once or twice every two weeks.

Partial Spearman correlations adjusted for energy intake among the biochemical parameters and some dietetic variables are presented in **table 8**. Strong positive correlations ($r > 0.310$) were observed for energy intake and serum levels of folic acid, vitamin E, vitamin B_{12}, vitamin A, and LDL cholesterol. Strong correlations were also seen among serum levels of folic acid and intakes of vitamin A ($r = 0.330$), folate ($r = 0.312$), and iron ($r = 0.364$), as well as among serum concentrations of vitamin B_{12} and intake of folic acid ($r = –0.380$), vitamin C ($r = –0.345$), and iron ($r = 0.402$). Hemoglobin levels correlated modestly with the intake of vitamin A ($r = 0.278$) and vitamin C ($r = 0.234$), and weakly with the intake of iron ($r = 0.156$). Energy intake from carbohydrates correlated negatively with the serum levels of HDL cholesterol and positively with those of TG, but these correlations were weak ($r < 0.15$). Total energy intake was strongly correlated with intakes of carbohydrates (0.897), protein (0.679), total dietary fiber (0.752), total fat (0.809), saturated fat (0.757), monounsaturated fat (0.776), polyunsaturated fat (0.804), and cholesterol (0.331). Likewise, energy intake correlated strongly with the intakes of vitamin A (0.672), B_1 (0.715), B_2 (0.693), B_3 (0.759), B_6 (0.795), B_{12} (0.529), folate (0.677), iron (0.877) and vitamin E (0.678).

Prevalence of parasites

The prevalence of intestinal parasites was 68%. The most frequently found parasites were *Entamoeba coli* (42%), *Endolimax nana* (44%), *Entamoeba histolytica* (24%), *Iodamoeba butschlii* (20%), and *Lamblia intestinalis* (18%). The presence of tapeworms was seen in 2% of the samples. There were no significant differences between adolescents with parasites ($n = 57$) and those without parasites ($n = 27$) in serum levels of folate, hemoglobin, vitamin B_{12}, vitamin A, ferritin, iron, and TS (data not shown).

Discussion

The deficient status of ferritin, hemoglobin, folic acid, and vitamins A and B_{12} in Cabécar adolescents may be associated with a low-energy-density diet. Our data showed a strong positive association between energy intake and these biochemical indicators. This is probably due to the strong relation existing between energy and micronutrient intakes. Our results and those reported by Nicklas et al. [29] show that the intakes of most micronutrients increase proportionally to energy intake.

The low energy intake observed in this study could be questioned because of the methods used to determine it. Willett [30] has pointed out that the 24-hour-recall method can underestimate intake of energy and nutrients if it is applied for only one day. However, he also states that the estimation of intake over at least four nonconsecutive days by this method (as applied in this study) is a reasonable compromise for assessing current individual intake of energy and several nutrients.

The low reported energy intake may be a direct effect of the family's socioeconomic dynamics. On one hand, the money earned by an indigenous man working on the plantations frequently does not reach the family economy[31], since he uses it for what he subjectively believes to be necessary, including alcohol consumption. On the other hand, women have stopped working the land and are limited to household chores, expecting the man to provide the money to purchase food. This dynamic becomes a vicious circle in which the man does not earn enough money, the job restricts food purchases, and the diet is limited to the minimum and least expensive foods.

Because of the families' low purchasing power and the low availability of food from animal sources in the community (once or twice every two weeks), the Cabécar diet is characterized by a predominance of vegetable foods. However, the amount consumed per day is not enough to satisfy the dietary recommendations for fiber, folate, vitamin A, and iron.

The diminished quantity and low bioavailability of dietary iron (predominantly nonheme iron) would seem to be the primary cause of the elevated prevalence of anemia in the study population. Moreover, the deficiency of vitamin A may have a negative effect on normal hematopoiesis. Several studies indicate that vitamin A deficiency reduces the availability of iron for synthesis of the heme protein [32].

The presence of parasites is also a factor that is widely associated with the development of anemia [33]. However, the particular parasites identified in this study are not associated with the development of anemia. The presence of similar levels of hemoglobin and ferritin among adolescents with and without parasites may suggest that dietary deficiencies are the

TABLE 8. Spearman partial correlation coefficients between serum biochemical parameters and some dietary variables adjusted for energy intake in Cabécar Costa Rican adolescents ($n = 84$)

	Serum biochemical parameter									
Dietary variable	Homo-cysteine	Folic acid	Vitamin E	Vitamin B_{12}	Vitamin A	Hemo-globin	TC	HDL cholesterol	LDL cholesterol	TG
Energy	0.053	0.397*	0.326*	0.343*	0.336*	0.128*	0.056	−0.070	0.245*	0.021*
Vitamin A	0.049	0.330*	0.225*	0.381*	0.001	0.278*	0.122	0.070	0.043	0.085
Vitamin B_6	−0.035	0.236*	−0.311*	0.274*	0.048	0.140	0.092	0.017	0.052	0.192
Vitamin B_{12}	−0.060*	−0.250*	0.051	0.231*	0.194	0.078	0.216	0.043	0.198	0.058
Folate	−0.014*	0.312*	0.197	−0.380*	0.011	0.087*	0.016	0.138	0.103	0.043
Vitamin C	0.031	0.270*	0.263	−0.345*	0.016	0.234*	0.095	0.138	0.050	0.149
Vitamin E	0.076	−0.148	0.105	−0.187	0.137	0.118	0.099	0.187	0.201	0.076
Iron	0.067	0.364*	0.268	0.402*	0.423	0.156*	0.031	0.174	0.076	0.014
Energy from protein	−0.010	0.131	0.060	0.111	0.155	0.194*	0.122	0.056	0.498	0.125
Energy from carbohydrate	0.030	0.004	0.092	−0.026	−0.205	−0.076	−0.129	−0.133*	0.072	0.114*
Energy from total fat	0.045	0.049	0.126	0.089	0.194	0.016	0.101	0.180	0.047	0.042
Energy from saturated fat	0.068	0.037	0.155*	0.018	0.278*	0.052	0.141*	0.082	0.001	0.232
Energy from polyunsaturated fat	0.072	0.086	0.012	0.066	0.106	0.046	0.001	0.092	0.006	0.284
Energy from monounsaturated fat	0.029	0.115	0.038*	0.126	0.156	0.028	0.070	0.139	0.037	0.081

TC, Total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.
*Correlation is significant at the $p < .05$ level.

main cause of anemia in Cabécar adolescents.

In Costa Rica, wheat flour has been fortified with iron and folic acid since 1997, corn flour has been fortified with iron and folic acid since 1999, and milk has been fortified with vitamins A and D and folic acid since 2001. Nevertheless, based on our findings it appears that this strategy is insufficient for improving the status of these nutrients in the indigenous population studied due to the limited familial budget allocated to buy these foods and the limited access they have to them on the reservation.

Vitamin B_{12} deficiency is worrisome in this population because of its association with megaloblastic anemia [34]. Nevertheless, Knittingen et al. [35] have suggested that populations are capable of adapting to chronically low levels of vitamin B_{12} by means of genetic mechanisms, such as polymorphism of the enzyme MTHFR. This 677 C→T point mutation seems to protect against megaloblastic anemia by retaining cellular folate. In the sample studied, the prevalence of adolescents with the homozygous mutant was very high (49%), as seen other Costa Rican indigenous populations [36], and markedly greater than the prevalence reported in the Yupka Indians from Venezuela (15%) [36] and some tribes of Brazilian Amazonians (7.8%) [37].

Folic acid and vitamin B_{12} nutritional deficiencies and the presence of the MTHFR polymorphism are factors that epidemiologic evidence points to as strong modulators of homocysteine levels. Boushey et al. [38] have suggested that high levels of homocysteine are equivalent to hypercholesterolemia as risk factors for cardiovascular disease. Although homocysteine reference intervals are not well established, most researchers believe that "recommended values" are concentrations lower than 10 μmol/L [39]. Continuing with the current dietary pattern, it is possible that in the medium term, Cabécar adolescents may show a prevalence of homocysteine levels higher than 15 μmol/L, such as those reported for Australian aboriginals (24%) [40]. This is particularly important because of the high prevalence of the MTHFR polymorphism in this group, since it has been established that the homozygous or TT mutation of the gene in this enzyme increases the risk of developing hyperhomocysteinemia, especially in subjects with low serum folate levels [41, 42].

Because high TG levels and low HDL cholesterol levels are also cardiovascular risk factors, the proportion of adolescents with this lipid profile is worrisome. The Bogalusa Heart Study suggests that more than 70% of adolescents with adverse lipid profiles tend to remain so as young adults [43]. The trend toward low HDL cholesterol and high TG levels is similar to the lipid pattern that has also been observed in the Pima Indians and the Tarahumara Indians, which have a high prevalence of cardiovascular disease [44, 45].

Multiple evidence suggests that a high carbohydrate intake is positively associated with increased TG levels and inversely associated with HDL levels [30, 46]. The observed lipid profile of more than 50% of the Cabécar adolescents could be explained, at least partially, by their high carbohydrate intake (> 60% of total energy). HDL decreases when the intake of any kind of carbohydrate is increased, because endogenous TG synthesis and very low-density lipoprotein secretion are increased [30, 46].

It is interesting that despite the high intake of saturated fatty acids (56% of the sample obtained more than 10% of total energy from saturated fatty acids), the prevalence of borderline and high LDL cholesterol levels was less than 15%. Some studies have indicated that serum lipoprotein responses to saturated fatty acids vary among individuals and that the variation in responsiveness may be regulated, at least in part, by apolipoprotein E polymorphism [47].

Several limitations should be noted when interpreting the results of our study.

First, our results are based on cross-sectional data and the sample included only adolescents enrolled in schools. Therefore, youngsters deserting the educational system for social or economic reasons were not included.

Second, the 24-hour recall in the population studied may have generated an underestimation of the intake of some nutrients. Despite that, the biochemical parameters confirm some of the findings observed in analyzing the diet. In addition, the survey to determine food availability in the home reinforces the scarcity of food in this population. The nonassociation between dietary intake and biochemical parameters found among the adolescents may be a consequence of the methodological difficulty in measuring nutrient intake. However, it has been postulated that over a "ceiling" level of dietary components, variability in biochemical parameters reflects individual metabolic variations rather than differing dietary intake [48].

Third, the anthropometric appraisal was carried out using BMI values based on the Health Examination Survey and the first National Health and Nutrition Examination (NHANES I) in the United States. The influence of genetic and environmental factors on the indigenous population studied may cause an underestimation of the prevalence of underweight adolescents. The elevated obesity and overweight rates in all age groups in the United States tend to push the BMI values upward. This is one of the reasons why the WHO committee of experts indicates that such types of references do not provide a desirable pattern to be used as a healthy goal for adolescents internationally. Nevertheless, the same committee indicates that for uniform reporting purposes and in the absence of other data specifying optimum cutoff values for BMI in adolescents, BMI-for-age data for US adolescents

may be used on a provisional basis [10].

Fourth, the sample size is small, so its explanatory power is limited. However, our results do show that the nutritional status of this population is currently in a deteriorated state. This agrees with what has been reported in various countries [4, 7], although it contrasts sharply with the nutritional status of Costa Rican indigenous populations reported in the 1980s [1].

Our results demonstrate an adolescent Cabécar population with a mosaic of nutritional deficiencies and cardiovascular risk factors. The transition from their traditional diet to a Western-style diet appears to be manifesting its first effects. Consequently, it is necessary to define strategies to improve the quality of the Cabécar adolescents' diet in order to prevent the onset of diseases associated with nutritional deficits or noninfectious chronic disorders. Developing these strategies has been difficult in various industrialized nations [49], so less-developed countries would presumably have difficulty with these strategies also, at least in the short term.

Poverty and neglect are the factors that initiate the inequity in health status experienced by the Cabécar Indians and by many other indigenous peoples in Latin America. Equity in health status is built providing people with access to the resources, capacities, and power they need to act on the circumstances of their lives that determine their health [50]. Therefore, it is necessary for governments to assign greater importance to primary health care and prevention in health-determining sectors, such as employment, income maintenance, social welfare, housing, and education, as proposed by the Toronto Declaration, 2002 [50].

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Impact of a homestead gardening program on household food security and empowerment of women in Bangladesh

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Abstract

This paper assesses the additional benefits of a homestead gardening program designed to control vitamin A deficiency in Bangladesh. In February and March 2002, data were collected on the food security and social status of women from 2,160 households of active and former participants in the gardening program and from control groups in order to assess the impact and sustainability of the program. The proportions of active and former-participant households that gardened year-round were fivefold and threefold, respectively, higher than that of the control group (78% and 50% vs. 15%). In a three-month period, the households of active participants produced a median of 135 kg and consumed a median of 85 kg of vegetables, while the control households produced a median of 46 kg and consumed a median of 38 kg ($p < .001$*). About 64% of the active-participant households generated a median garden income of 347 taka (US$1 = 51 taka), which was spent mainly on food, and 25% of the control households generated 200 taka in the same period (*$p < .001$*). The garden production and income levels of formerly participating households three years after withdrawal of program support were much higher than those of the control households, illustrating the sustainability of the program and its ability to increase household food security. Significantly more women in active- and former-participant households than in control households perceived that they had increased their economic contribution to their households since the time the program was launched in their subdistricts (> 85% vs. 52%). Similar results were found for the level of influence gained by women on household decision-making. These results highlight the multiple benefits that homestead gardening programs can bring and demonstrate that these benefits should be considered when selecting nutritional and development approaches targeting poor households.*

Key words: Bangladesh, food security, homestead gardening, women empowerment

Introduction

Vitamin A deficiency is a major public health problem in more than 70 countries, including Bangladesh. Horticultural approaches have been considered among the long-term sustainable strategies to reduce the prevalence of micronutrient deficiencies, including that of vitamin A [1, 2]. However, it has recently become evident that the bioavailability of provitamin A from dark-green leafy vegetables, and to some extent from fruits and tubers, is much lower than was previously assumed [3, 4]. Nevertheless, gardening programs continue to be widely adopted strategically to improve vitamin A status of women and children in developing countries, including Bangladesh [5–7]. This widespread adoption is reasonable because homestead gardening is a traditional and sustainable activity of most rural households in developing countries, and garden produce can be an important source of multiple micronutrients, such as vitamins A, C, and B-complex and iron from fruits and other plant sources [8]. In addition, a number of studies have suggested other potential benefits of homestead gardening programs, including the improvement of household food security and female status as well as increased income [9, 10]. However, little documentation exists that focuses on these additional advantages of gardening programs originally designed to control vitamin A deficiency.

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Gardening in Bangladesh

Homestead gardening in Bangladesh is traditionally the responsibility of women. Gardening activities are mainly seasonal in Bangladesh, where about 70% of vegetables and fruits are produced in the winter, and very little produce is stored or preserved by households [11, 12]. It has been estimated that less than 10% of homestead gardens grow vegetables year-round. The Food and Agriculture Organization (FAO) of the United Nations has estimated vegetable and fruit production in Bangladesh to be less than 30% of the national needs [13]. Given the understanding that homestead gardening has the potential to reduce the prevalence of vitamin A deficiency in children and women [1], and that the production and availability of vegetables and fruits were inadequate in Bangladesh, Helen Keller International initiated a nationwide homestead gardening program through partnership with local non-governmental organizations (NGOs) to reduce vitamin A deficiency in poor rural households [9].

The program, "NGO Gardening and Nutrition Education Surveillance Project" (NGNESP), was launched in 1993 and aimed at encouraging poor households with very limited land to start gardening, producing vegetables year-round, and increasing the number and production of vitamin A–rich crops grown per garden. The main participants in the NGNESP are poor rural women. By February 2000, the NGNESP had worked with 51 local NGOs as partners and covered more than 860,000 households in 210 of the 460 subdistricts of Bangladesh [9]. Based on the number of beneficiaries and total budget of the project since 1993, the cost of the NGNESP has been estimated at US$7.66 per household during the three years of project support.

Status of rural women in Bangladesh

The social context in Bangladesh is characterized by female seclusion and subordination, which combine to relegate women to a restricted role, mainly involving domestic work [14, 15]. From childhood, most rural women have been taught to be obedient wives; their primary role is to bear children and maintain household responsibilities. The fact that they are expected to eat only after their husbands and sons have finished [14, 16] speaks to their place in the family hierarchy. Poverty, oppression, and illiteracy, combined with limited exposure to new information, have reduced the ability of many rural women to learn about life outside their homesteads and immediate surroundings; as a result, they are seldom consulted on the majority of household decisions. Under these conditions, most rural women are forced to depend on their husbands, who exercise complete control, which further damages any residual self-confidence they may have. According to Abecassis [17], many rural women in Bangladesh have completely internalized their inferior status; their self-image is very low, their creativity is stifled, and they believe that their inferiority is both real and inevitable. Ironically, the extremely poor women, who have no money, cannot afford to observe seclusion. As a result, they enjoy much more freedom, as they must leave home to work. Many of these poverty-stricken women have learned how to earn money inside or outside household compounds and have been contributing to household economic well-being. These women are learning that they are valuable to society and to themselves and are gaining some sort of self-esteem and influence over business and general household decisions [14].

NGNESP evaluation

A study was conducted in the winter of 2002 (February and March) to determine the economic and social impact of the NGNESP on its beneficiaries and to evaluate the sustainability of the program. Prior to data collection, the study protocol was approved by a research committee of local and international staff of Helen Keller Worldwide in Bangladesh.The data presented in this paper focus on the way the NGNESP has influenced household food security and the empowerment of women in program areas.

Methods

Study design and data analysis

Administratively, Bangladesh is divided into divisions, subdivisions, districts, subdistricts, unions, and villages. According to the level of household participation in the program, three groups comprising 720 households each—the active-participant, former-participant, and control households—were included in the study. The "active-participant group" consisted of households that had been receiving technical and material assistance from Helen Keller International for less than three years, and the "former-participant group" consisted of households that had completed the program and had been operating without Helen Keller International assistance for at least three years. Beneficiary households in the active and former groups were selected based on whether they had ever participated in the program, but regardless of their current gardening activities. In order to avoid a potentially confounding spillover effect, the control group of households was selected from within the target subdistricts from unions where NGNESP activities had not yet been implemented. For the control group, only households that had similar socioeconomic conditions at the time NGNESP beneficiaries were selected were considered. Although the selection criteria varied slightly among

subdistricts, the majority of NGNESP beneficiaries had been chosen from landless or low-income households having a total land size of less than 50 decimal (0.2 ha), generally with a day laborer as household head. A total of 2,160 households (3 × 720) were randomly selected from 39 of the 210 subdistricts where the NGNESP has been implemented, and thus they represent the current national project population.

The interviews were conducted in February and March 2002. Structured questionnaires were used to collect data on the impact of the program on household food security and female social status. Data on homestead gardening-related activities were collected for the three-month period prior to the interview. Because the majority of vegetable crops promoted through the NGNESP have a growing season of three months or less, a three-month time frame allowed us to capture most household gardening activities. The sustainability of the program was determined by comparing the active- and the former-participant households.

The World Bank, the FAO, and the US Agency for International Development (USAID) define food security in terms of access at all times to sufficient food to meet dietary needs for a productive and healthy life [18]. Household food security has three components: availability, accessibility, and utilization of food. Availability implies uninterrupted supplies of food, and accessibility refers to both physical and economic access to it. Utilization relates to the quality of food and the ability of households and its individual members to use it, including whether the health conditions of the individuals allow for appropriate absorption of the nutrients ingested. Data on homestead garden production, estimated in kilograms by the homestead caretakers, including the adoption of year-round production practices, were used to assess the effect of the project on food availability. The caretakers can estimate the production in kilograms fairly accurately, because sales of produce tend to measured in kilograms. The ability of households to access food was measured by the consumption levels of garden produce (estimated at harvest), the amount of cash generated from gardening activities, and by the extent to which garden income was spent on food and productive assets. The level of fruit and vegetable crop diversification in homestead gardens, the number of vitamin A–rich crops grown, and the type of foods purchased by households using garden income were used to estimate the program's effect on the quality of foods accessible to households. The list of vitamin A–rich crops compiled by Darnton-Hill and colleagues [19] was used to determine whether a crop was vitamin A-rich (> 250 retinol equivalents [RE]/100 g). The social impact of the program was determined by the changes, as perceived by women, in their ability to contribute to household livelihoods and participate in household decision-making.

Data analysis was performed with the Statistical Package for Social Sciences (SPSS, version 9.02 Windows, Chicago, IL, USA). Analysis included descriptive statistics and analytical models. For categorical variables, significance of the differences between group medians was determined by the chi-square test. When the main outcome measures were continuous variables with normal distributions, the significance of the differences in group means was determined by analysis of variance (ANOVA). In cases of non-normal distributions with continuous variables, the significance of a difference between groups was determined by the Mann-Whitney test for paired comparisons and the Kruskal-Wallis test for multiple comparisons. A p value $< .05$ was considered to indicate statistical significance.

Results

Characteristics of households

The average household size before the NGNESP was introduced in the subdistrict did not differ among the three groups. The active-participant households had an average of 5.8 members, whereas both the former-participant and the control households had an average of 5.9 members. A household was defined as the number of people living in the same compound (enclosed group of buildings belonging to the same family) and eating from the same cooking pot. The data also show that 84%, 83%, and 77% of the gardeners in the active-participant, former-participant, and control groups, respectively, were women. Farming was the primary income source for all three categories of households before the advent of the NGNESP (**table 1**). A significant number of households in all three groups had no main income at the onset of the program in the subdistrict. These findings suggest a relatively similar economic status among the three groups of households at the time of NGNESP introduction.

TABLE 1. Main source of income for households before NGNESP activities were launched in the subdistrict

Main source of income	% of households		
	Former participants ($n = 683$)	Active participants ($n = 711$)	Controls ($n = 603$)
No regular income	30.4[b]	43.9[a]	21.6[c]
Farming	33.1[a]	25.9[b]	34.2[a]
Small business	11.7[a]	5.8[b]	12.3[a]
Day labor	6.3[b]	10.4[a]	8.9[ab]
Fixed wages	6.0[a]	2.2[b]	5.9[a]
Other	12.5	11.8	17.1

Percentages in rows followed by different letters are significantly different ($p < .05$).

Homestead gardening practices

More households in the active-participant and former-participant groups than in the control group managed a garden and practiced year-round gardening (**table 2**). The program beneficiaries, as compared with the control households, also had diversified their vegetable and fruit crops more and grew more vitamin A–rich crops. The data presented in **table 2** also suggest that households in the completed group had, to a large extent, maintained the level of gardening activities after assistance from the NGNESP had been terminated, illustrating the sustainability and value of NGNESP activities.

Production and consumption of garden produce

Table 2 shows that active-participant households produced a median of 135 kg of vegetables and 24 kg of fruits, and households in the former-participant group produced a median of 120 kg (range, 50–220 kg) of vegetables and 24 kg (range, 12–50 kg) of fruits. In contrast, the control households had a median production of 46 kg of vegetables and 14 kg of fruits during the same time period. The quantities of vegetables and fruits produced by households in both the active-participant and the former-participant groups were significantly different from those produced by the control households ($p < .001$), but there were no differences between the quantities produced by the two groups of beneficiaries.

During the three-month period prior to data collection, the consumption of vegetables and fruits from homestead gardens by households in the active-participant and former-participant groups was significantly higher than the consumption by the control households during the same period. When the two groups of beneficiaries were compared, we found that the households in the active-participant group consumed more vegetables than those in the former-participant group, but no significant difference was detected in fruit consumption. The consumption figures of garden produce represent a daily per capita consumption* of vegetables of 160 g for the active-participant group, 132 g for the former-participant group, and 71 g for the control households. Considering that the recommended World Health Organization (WHO) and FAO daily per capita consumption of vegetables is 200 g [20], the data suggest that active-participant households are obtaining 80%, and the former-participant households 66%, of their required daily per capita consumption of vegetables from homestead gardening activities, compared with the 35% of required daily per capita consumption by the control households during the period covered in the study.

Homestead garden income and expenditure

During the three-month period prior to data collection, significantly more households in the active- and former-participant groups than households in the control group generated income by selling part of their garden produce (**fig. 1**). The amount of money earned by beneficiary households (490 and 347 taka) from gardening activities was also higher than that

* Estimation of the daily per capita consumption: consumption per month (g)/30/household size (5.9).

TABLE 2. Homestead gardening practices, production, and use of garden produce by households in the previous three months ($n = 2,160$)

Variable	Former participants	Active participants	Controls
Managing a garden (%)	96[a]	100[a]	85.6[b]
Year-round production (%)	50.4[b]	77.8[a]	15.4[c]
Crop diversification (no.)			
Vegetable crops	6.3 (4.3)[b]	9.4 (3.6)[a]	3.5 (2.3)[c]
Fruit crops	5.3 (3.1)[a]	5.6 (2.7)[a]	4.4 (4.2)[b]
Vitamin A–rich vegetables	4.9 (2.0)[a]	5.3 (2.4)[a]	1.8 (1.4)[b]
Production (kg)			
Vegetables	120 (50–220)[a]	135 (80–207)[a]	46 (20–90)[b]
Fruits	24 (12–50)[a]	24 (20–90)[a]	14 (7–34)[b]
Consumption (kg)			
Vegetables	70 (49–110)[b]	85 (60–110)[a]	38 (20–65)[c]
Fruits	18 (10–39)[a]	20 (10–40)[a]	12 (6–25)[b]

Crop diversification data are means (± SD). Consumption and production data are medians (25th–75th percentiles). Numbers in rows followed by different letters are significantly different according to analysis of variance (ANOVA) or the Kruskal-Wallis test ($p < .05$).

earned by control households (200 taka) in the same period. When the two groups of beneficiaries are compared, the former-participant households are found to generate more income from gardening than those in the active-participant group (**fig. 1**). The fact that the former participants had been involved in the program longer than the active participants leads us to believe that they had acquired enough experience to establish better channels for selling produce and to focus on high-market-value crops. This could explain the difference in income generated by the two groups of participants.

With regard to income expenditure, the results presented in **table 3** show that food was the item most frequently purchased by the three categories of households using homestead garden income. Among the households that managed a garden, significantly more households in the active- and former-participant groups than in the control group purchased food or paid for education, clothing, productive assets, and health care from their gardening activities. The food items purchased included oil, salt, spices, fish, rice, and meat.

Changes in the ability of women to contribute to the household

Women respondents were asked to compare their current level of contribution to the household in terms of money and/or garden produce with their level of contribution prior to the establishment of the program in the subdistrict. Whereas more than 85% of women in both the active- and the former-participant groups believed that they had considerably increased their contribution to the household, only 52% of women in the control group believed they had done so (**fig. 2**). About 92% of women in the active-participant group and 77% in the former-participant group considered gardening to be one of the main activities that allowed them to increase their household contribution. Gardening was cited by only 31% of women in the control

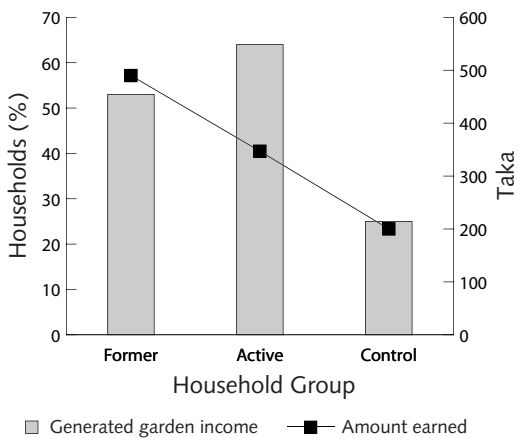

FIG. 1: Percentage of households that generated garden income (n = 2160) and their median income (n = 1018) in the three-month period prior to data collection

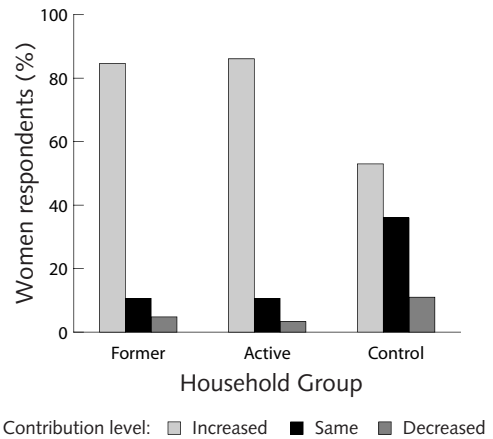

FIG. 2. Contribution of women to the household as perceived by the respondents at the time before and after NGNESP establishment in the sub-district

TABLE 3. Expenditures and food items purchased with garden income among households with homestead gardens in the former-participant, active-participant, and control groups during the three-month period prior to data collection

	% of households				% of households		
Expenditure	Former participants (n = 691)	Active participants (n = 720)	Controls (n = 616)	Food item	Former participants (n = 193)	Active participants (n = 256)	Controls (n = 110)
Food	28.0^b	35.6^a	17.8^c	Oil	26.2^a	28.9^a	12.9^b
Education	30.8^a	35.4^a	7.3^b	Salt and spices	21.8^a	26.8^a	12.3^b
Clothing	27.3^a	26.3^a	8.4^b	Rice	10.8^a	13.0^a	8.3^a
Productive assets	24.0^a	18.2^a	7.4^b	Fish	7.8^b	15.3^a	6.8^b
Health care	15.3^a	14.6^a	5.0^b	Pulses	12.6^a	8.5^a	5.0^a
Housing	6.9^a	4.6^a	1.4^a	Meat	11.0^a	11.2^a	3.4^b
Social activities	3.5^a	2.8^a	1.8^a	Vegetables	0.1^b	0.7^b	1.6^a

Percentages in rows followed by different letters are significantly different (chi-square test, $p \leq .05$).

group as a reason for their increased contribution.

Changes in the level of influence of women on household decision-making

The influence of women respondents on household decision-making before and after the introduction of the NGNESP in the subdistrict was assessed on the basis of a set of questions representing different socioeconomic aspects of their livelihoods that are traditionally under male control. "Full power" is the category assigned to women who are capable of making final decisions either after consultation with their husbands or alone; "some power" designates women who may be consulted but do not have the power to make final decisions; and "no power" (not included in **table 4**) refers to women who are not even consulted. The data show that more women in the former-participant group than women in the active-participant and control groups said they had gained power even before the introduc-

TABLE 4. Changes in the level of influence of women in household decision-making as perceived by women respondents before NGNESP and in 2002 when data were collected* (n = 2,016)

	Women's decision level (% of respondents)					
	Full			At least some*		
Indicator (type of decision)	Before NGNESP	2002	% *increase*	Before NGNESP	2002	% *increase*
Participating in group meetings						
Former participants	8.6	51.2	*595*[b]	33.2	83.4	*251*[b]
Active participants	2.0	32.8	*1,640*[a]	10.3	96.2	*933*[a]
Controls	4.0	18.3	*475*[c]	16.4	42.3	*257*[b]
Deciding how to use household land						
Former participants	10.6	34.5	*325*[b]	55.7	92.1	*165*[b]
Active participants	3.8	26.9	*707*[a]	37.5	86.8	*231*[a]
Controls	7.0	16.0	*266*[c]	33.9	56.8	*167*[b]
Making small household purchases						
Former participants	14.1	49.1	*348*[b]	75.1	97.8	*130*[a]
Active participants	6.7	41.7	*622*[a]	65.0	94.6	*145*[a]
Controls	7.6	21.8	*286*[c]	60.0	83.1	*138*[a]
Making large household purchases						
Former participants	11.1	23.3	*209*[b]	52.3	83.9	*160*[ab]
Active participants	5.8	22.7	*391*[a]	41.0	81.1	*197*[a]
Controls	6.5	12.3	*189*[b]	31.7	47.5	*149*[c]
Deciding on type and quantity of vegetables or fruits to be consumed in household						
Former participants	34.4	80.5	*324*[a]	89.0	99.1	*111*[a]
Active participants	28.5	77.3	*271*[b]	87.8	99.4	*113*[a]
Controls	26.7	53.7	*201*[b]	72.5	86.6	*119*[a]
Visiting stores or large markets						
Former participants	8.6	23.0	*267*[b]	30.4	65.6	*215*[b]
Active participants	2.8	11.1	*396*[a]	16.6	64.0	*385*[a]
Controls	7.1	10.5	*147*[c]	17.9	30.9	*172*[c]
Determining woman's daily workload						
Former participants	25.2	65.0	*257*[a]	76.1	95.9	*126*[a]
Active participants	23.0	64.0	*278*[a]	71.1	98.0	*137*[a]
Controls	18.2	36.6	*197*[b]	63.9	80.6	*126*[a]
Visiting woman's parental home						
Former participants	12.1	43.6	*360*[a]	73.3	93.5	*127*[a]
Active participants	11.1	34.6	*311*[b]	62.4	92.7	*148*[a]
Controls	10.3	23.3	*226*[c]	49.1	67.6	*137*[a]

* "At least some" represents the total percentage of women with full power or some power.
Percentages in columns followed by different letters are significantly different ($p \leq .05$) according to the Kruskal-Wallis test for each indicator.

tion of the NGNESP in their subdistricts. This finding might be due to the fact that recall by the former participants was less reliable than that by women in the active-participant and control groups because of their longer history with the program at the time of data collection. The data presented in **table 4** also show that more women in all three groups, including the control group, had full or at least some power in decision-making at the time the data were collected than they did during the period prior to the NGNESP. However, the relative percentages of increase for women beneficiaries (those in the active-participant and former-participant groups) are significantly higher than those for women in the control group. For the two groups of beneficiaries, more women in the former-participant than in the active-participant group reported having full power for making certain decisions, and the proportion that had either full or some power was comparable in the two groups of beneficiary women.

Discussion

As stated at the beginning of this paper, household food security was determined by assessing the availability of food to households, the ability of households to access food, and the utilization of food by households. In this study, households that participated in the NGNESP had more food available to household members. Although homestead food production was measured for only a three-month period, the assessment was conducted in the winter, which is the main gardening season for most households. More households under the NGNESP were recorded as practicing year-round production; meaning that most control-group NGNESP households garden even in the off-season. Therefore, NGNESP participating households that garden year-round would be expected to have greater production in the off-season compared to the control households that garden only in the winter. A year-round supply of food to households illustrates that the program has been instrumental in augmenting food availability to the households, thus contributing to food security. Whereas former-participant households generated more income than active-participant households, fewer former participants produced year-round. This may indicate that the former-participant households optimized their garden production in a way that was most suitable for them and made optimal use of opportunities for selling produce.

Increased homestead production does not necessarily translate into increased household consumption. Therefore, we considered the amount of homestead garden produce consumed by households, rather than production, as the indicator to measure the capacity of households to access food. The much higher consumption of vegetables and fruits observed among beneficiary households in this study suggests an improvement in the ability of poor households to access food, especially vegetables and fruits. In addition, the higher proportion of households that generated income and increased the amount of income per garden under the NGNESP, and the fact that garden income was largely spent on food, also illustrate that the gardening program strengthens the ability of households to access food. The productive asset holdings of households are often considered a reliable indicator for determining household vulnerability to adverse conditions and food insecurity. The fact that more beneficiary households were found to acquire productive assets using income earned from selling garden produce indicates an increase in their ability to access food under different conditions, and thus an improvement in food security.

As pointed out, the utilization of food adds a qualitative dimension to household food security in the form of nutritional security. Having enough food in terms of calories does not necessarily guarantee a household's food security; the quality of food needs to be considered as well. Because of the nature of this study, details on the quality of food accessible to households through gardening activities could not be directly determined. However, increased vegetable crop diversification in homestead gardens has been found to be associated with increased nutritional quality of garden produce in terms of its iron, vitamin A, vitamin C, and fiber contents [8]. In this regard, the higher number of vegetable and fruit crops, especially vitamin A–rich crops, grown by households under the NGNESP suggests an improvement in the nutritional quality of the garden produce accessible to households. In addition, the fact that more households under the NGNESP used garden income for health care and to purchase other nutritious foods, such as fish, meat, and pulses, also suggests an improvement in the quality of food accessible to the households.

As stated earlier in this paper, more than 860,000 households have directly participated in the NGNESP since its onset in 1993. Considering the median garden production of former-participant households and a 4% dropout rate (**table 2**), the total amount of produce produced by the 860,000 active and former beneficiary households in a three-month (winter) period can be estimated at 99,072 metric tons of vegetables and 19,814 metric tons of fruit. This production represents a significant contribution to the national requirement of vegetables and fruits. Therefore, it can be considered that this program has also made, at the macroeconomic level, a significant contribution to the availability of vegetables and fruits and, through income generation, to the reduction of poverty in Bangladesh. These findings illustrate that the NGNESP can be credited with improving not only the availability of food to households but also the ability of households to access

quality foods and, therefore, their overall food security. And as shown by the comparison of former- versus active-participant and control households, the benefits gained by households participating in the NGNESP were sustained after the withdrawal of support after three years.

By participating in the NGNESP, poor rural women are learning new skills in improved gardening practices. They are participating in training and nutritional education sessions and are exchanging ideas with other women. These actions have resulted in the improvement of gardening activities in terms of production and income generation and in an increase in the contribution of women to household economic well-being. More important, women beneficiaries believed that their ability to contribute to the household livelihood had improved since they became involved with the NGNESP.

Although it is unclear to what extent the perception of an increased economic contribution to the household by poor women translates into self-esteem and empowerment, a number of authors have argued that there is at least some gain when women believe that they are significantly contributing to household economic well-being [14]. In addition, studies carried out in Bangladesh have suggested that women who make themselves economically valuable through their activities are more likely to win respect among household members, which in turn leads to the enhancement of their social status within the household [17]. In this regard, the observed increase in decision-making power by NGNESP beneficiaries in this study can be credited, in part, to the intrahousehold respect that women gained based on their economic contribution to the household. The increase of decision-making power among the controls, though smaller than that among the beneficiary women, may be attributed to a combination of socioeconomic factors, including participation in gardening activities. About 61% of women in the control group who claimed to have increased their decision-making power attributed the increase to their involvement in gardening. The remaining 39% of the women gave reasons such as their involvement in income-generating activities other than gardening, the increase of a woman's status with age, and the fact of having sons for their increase in decision-making power. This study also showed that the longer women are involved in the activities (women of former-participant versus active-participant households), the more household decision-making power they acquire.

This evaluation has shown that homestead gardening programs can play an important role in increasing household food security, household income, and the empowerment of women, in addition to increasing vitamin A intake. Therefore, improving traditional gardening practices through homestead food-production programs is an important strategy for combating micronutrient deficiencies as well as poverty, and should be part of a mix of strategies that address these problems. In order to implement such programs on a large scale, partnership with suitable local institutions, as it is done through the NGNESP [9], is needed.

Whereas the increased intake of vegetables and fruits increases vitamin A intake [5, 9] and hence contributes to reducing vitamin A deficiency [21], the bioavailability of vitamin A from fruits and vegetables is lower than assumed [4, 22]. Therefore, in order to further improve dietary quality and come closer to meeting the needs for micronutrients, animal foods should be added to the diet. These can also be produced at the homestead; Helen Keller International has added an animal husbandry component to its homestead food-production program. In addition, possibilities for food fortification and growing crops with increased micronutrient content and/or reduced content of inhibitors of absorption, such as phytate, should be sought.

Future research and evaluations of homestead food-production programs should assess year-round production and household food distribution, which appear to be deficient in poor households with limited resources [23, 24]. In addition, future studies should investigate how a program such as the NGNESP changes women's empowerment and whether it also affects their share in the household's food distribution. Such information can be used to design strategies to promote equal sharing of benefits within households and to strengthen household food security and the empowerment of women in future programs.

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Review Paper

Maintaining high vitamin A supplementation coverage in children: Lessons from Niger

Victor M. Aguayo, Shawn K. Baker, Xavier Crespin, Harouna Hamani, and Aissa MamadoulTaïbou

Abstract

In 1997, the reduction of child mortality became a policy priority for the Government of Niger because Niger's child mortality rate was the highest in the world. The Ministry of Public Health, Helen Keller International (HKI), and UNICEF spearheaded a coalition-building process linking vitamin A deficiency (VAD) control to national child survival goals. An evidence-based advocacy strategy was developed around the child survival benefits of adequate and sustained VAD control with one unambiguous message: "VAD control can avert over 25,000 child deaths per year." As a result, in 1997 Niger became one of the first countries in Africa to effectively integrate vitamin A supplementation into National Immunization Days (NIDs) for polio eradication. The challenge was then to provide children with a second annual dose of vitamin A. This led in 1999 to the first ever National Micronutrient Days (NMDs) in Africa. NMDs are mobilization campaigns in which caregivers are actively encouraged to take their children for the delivery of vitamin A supplements. Since 1999, the combination of NIDs and NMDs has ensured that over 80% of children 6 to 59 months of age receive two vitamin A doses annually. The success of NIDs/NMDs has relied on five pillars: leadership and ownership by the Ministry of Public Health; district-level planning and implementation; effective training and flexible delivery mechanisms; effective social information, communication, and mobilization; and responsiveness and flexibility of Ministry of Public Health and development partners. This successful approach has been widely disseminated, notably through the West African Nutrition Focal Points Network.

Key words: Child survival, Niger, supplementation, vitamin A

Introduction

For several decades, vitamin A deficiency (VAD) has been recognized as the leading cause of preventable pediatric blindness in developing countries [1]. A better understanding of the public health importance of VAD began when four independent meta-analyses revealed that in areas where VAD is prevalent, mortality rates in children 6 to 59 months of age can be reduced by 23% to 34% following vitamin A repletion [2–5].

Current global estimates suggest that 127 million preschool-age children have VAD and therefore are at an increased risk of death, mainly from diarrhea, measles, and malaria; an estimated 26% to 33% of vitamin A deficient children worldwide live in sub-Saharan Africa [6, 7]. The recognition of VAD control as a low-cost/high-impact child survival intervention in countries where VAD is endemic led numerous countries in sub-Saharan Africa to launch broad-based, high-potency vitamin A supplementation programs to cover 4–6 months of children's vitamin A needs twice yearly. This paper reviews the chronology, principles, and perspectives of the implementation of twice-yearly broad-based vitamin A supplementation programs in Niger.

Chronology of program development

Niger is one of the poorest countries in the world. The Demographic and Health Survey (DHS-I) conducted

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in Niger in 1992 revealed a national mortality rate of 320 deaths per 1,000 live births among children under five years of age; 80% of these deaths occurred in children 6 to 59 months of age [8]. This under-five mortality rate was at that time the highest in the world. Moreover, child mortality rates had showed no positive trend in the previous 25 years. Following the release of the DHS-I report, the reduction of child mortality became a policy priority for the Government of Niger and its development partners.

In 1993, an independent meta-analysis of eight population-based trials enrolling more than 165,000 children worldwide showed that in areas where VAD is prevalent, child mortality is reduced by an average of 23% following vitamin A repletion [2]. This significant reduction in childhood mortality, which is attributable largely to the reduction in mortality from measles [9, 10], severe diarrhea and dysentery [11], and possibly falciparum malaria [12], made VAD control one of the most cost-effective and high-impact child survival interventions in regions where VAD was prevalent.

In the light of these findings, in 1995 the control of VAD became an integral part of the Ministry of Public Health's national sectoral policy. In 1996, routine vitamin A supplementation was integrated into the *Journées d'Accéleration du PEV*—a catch-up campaign added to the Expanded Program of Immunization (EPI); this approach ensured the coverage of 71% of infants 6 to 11 months of age (the EPI target group). However, only 19% of children 12 to 59 months of age benefited from high-potency vitamin A supplementation [13].

The Ministry of Public Health of Niger, Helen Keller International, and UNICEF decided to join forces to demonstrate that the Government of Niger with its development partners could deliver vitamin A supplements to children 6 to 59 months of age through National Immunization Days (NIDs) for polio eradication. In 1997, Niger became one of the first countries in sub-Saharan Africa to ensure the effective integration of vitamin A supplementation into NIDs, allowing for the annual provision of a high-potency vitamin A supplement to over 80% of children 6 to 59 months of age in 1997 and 1998 (**fig. 1**).

In 1998, the Micronutrient Initiative (MI) and UNICEF generated worldwide country-level VAD prevalence estimates to increase policy attention to the control of VAD in countries where country-level VAD survey data were not available [14]. These estimates were developed using interpolation models built upon a data set that included 42 VAD surveys (39 of them subnational) in 36 countries worldwide (1987–95). The models that maximized the concordance between the observed and predicted values for countries with VAD survey data were used to generate VAD country-level estimates for countries where, as in Niger, national-level VAD survey data were not available. According to these and later calculations, an estimated 25–50% of children in Niger were vitamin A deficient.

These VAD estimates and the momentum created by the successful integration of vitamin A supplementation into NIDs in 1997 and 1998 (advocacy is effective only if one can demonstrate that what *needs* to be done *can* be done) were the foundation for an advocacy coalition-building process linking effective VAD control to national child survival goals. A targeted, evidence-based policy advocacy strategy was built around the child survival benefits of effective and sustained policy and program action for VAD control. Two unambiguous policy advocacy messages were developed: "In Niger, effective and sustained VAD control can avert over 25,000 child deaths per year" and "In Niger, effective and sustained VAD control can reduce child mortality by an estimated 29% from 1992 mortality levels."

From a programmatic perspective, the challenge was to ensure that children 6 to 59 months of age be provided with two high-potency vitamin A doses per year: one annual dose delivered in conjunction with NIDs, and a second annual dose provided through a new delivery mechanism in the form of a national mobilization campaign around micronutrients. This led in 1999 to the first National Micronutrient Days in Africa (and the first nationwide mass vitamin A supplementation campaign independent of a national immunization campaign). National Micronutrient Days (NMDs) are mobilization campaigns in which caregivers are actively encouraged to take their children to designated centers or outreach posts for the delivery of vitamin A supplements. Since June 1999, NMDs have been organized every six months, either in conjunction with NIDs or as stand-alone institutionalized campaigns managed by the district-level health system. Since June 1999, NMDs have ensured that more than 80% of children receive a second dose of vitamin A annually. Moreover, since

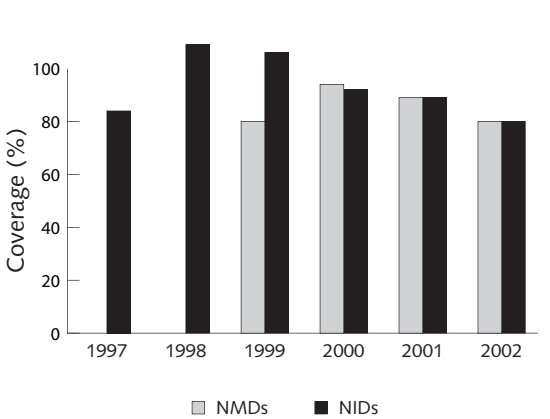

FIG 1: Vitamin A supplementation coverage (%) in children 6–59 months old through National Immunization Days (NIDs) and National Micronutrient Days (NMDs). Niger, 1997–2002

December 1998 the combination of NIDs and NMDs has ensured that over 80% of children 6 to 59 months of age receive two high-potency vitamin A doses annually (**fig. 1**) and has allowed for the provision of vitamin A and iron-folate supplements to more than 50% of eligible postpartum (vitamin A) and pregnant (iron-folate) women. (**fig. 2**).

Key features of the program

In Niger, a sustained coalition for child survival between the government and its development partners has ensured the effective and sustained integration of vitamin A supplementation into NIDs and NMDs on the basis of five features.

Leadership and ownership by the Ministry of Public Health

The Ministry of Public Health has led the planning, implementation, monitoring, and evaluation of NIDs/NMDs since their inception through the Ministry of Public Health-based National Coordination Committee for NIDs/NMDs, under the presidency of the Deputy Secretary General of the Ministry of Public Health. The National Coordination Committee has three subcommittees: the Technical Committee, the Social Mobilization Committee, and the Logistics Committee. The same organizational chart (i.e., a coordination committee consisting of three subcommittees for technical, social mobilization, and logistic issues) exists in each of the country regions ($n = 8$) and districts ($n = 42$).

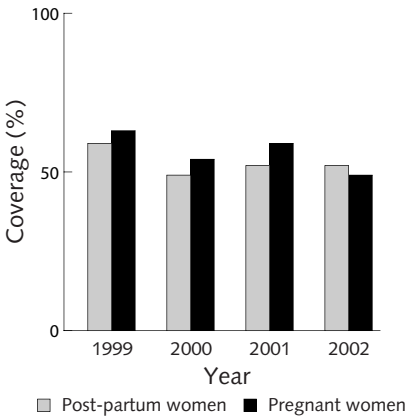

FIG 2: Supplementation coverage (%) of post-partum (vitamin A) and pregnant (iron + folate) women through National Micronutrient Days (NMDs). Niger, 1997–2002

Women in the early post-partum period (< 40 days after delivery) received a high-potency vitamin A supplement (200,000 IU). Pregnant women received a 90-day supply of iron + folate supplementation (60 mg elemental iron and 400 μg folic acid per supplement).

District-level planning and implementation with oversight and coordination at the central level

Each district takes the lead in the planning and implementation of the district plan of action for NIDs/NMDs. District-level planning is led by the district with technical assistance from the central level when assistance is needed. The planning phase involves all district-level administrative and traditional authorities, the heads of all health centers in the district, the leaders of the district health committees, and the leaders of women, youth, and religious groups. Once finalized, district plans of action are submitted to the regional coordination committee. The regional committee reviews all district plans in the region and consolidates them into a regional plan of action for NIDs/NMDs. The eight regional plans are then reviewed by the National Coordination Committee and consolidated into a national plan for NIDs/NMDs. Once the national plan is finalized and the necessary governmental and nongovernmental resources have been mobilized and allocated by the Ministry of Public Health (including the vitamin A supplements), resources are passed on from the Ministry of Public Health (central level) to the eight Regional Health Directorates (intermediary level), who in turn allocate them to the Health Districts (peripheral level) for the implementation of the district plans of action for NIDs/NMDs. Vitamin A supplements are donated by the Canadian Agency for International Development (CIDA) to UNICEF through the Micronutrient Initiative.

Effective training and flexible delivery mechanisms

A cascade approach ensures the effective training of supplementation and supervision agents at all levels. A training of trainers takes place at the central level, where two master trainers per region are trained; region-level master trainers train two trainers per health district; district-level trainers train the district supplementation and supervision agents, a body consisting of over 5,000 district-level health workers and volunteers (with no medical training). Training and supervision tools are developed at the central level and adapted at the district level to the specific needs and realities of the districts. To respond to the uneven geographical distribution of the population and coverage of the national health system (only 48% of the population lives within 5 km of a health facility), flexible delivery mechanisms have been conceived for the distribution of vitamin A supplements at NIDs/NMDs. Districts have adopted a combination of three approaches: the fixed strategy, in which supplement distribution takes place in the existing health facilities (fixed posts); the advanced strategy, in which supplement distribution takes place in health posts created for the occasion (advanced posts) in rural areas located within 5 to 10 km from a fixed

post; and the mobile strategy, in which supplementation is implemented by mobile distribution teams in populations located more than 10 km from a fixed or advanced health post, and distribution may take place in a centrally located site or door-to-door.

Effective social information, communication, and mobilization

A country-wide mobilization campaign is designed and implemented to mobilize the population around vitamin A supplementation at NIDs/NMDs. This social mobilization campaign uses nationwide mass communication media (television and radio), as well as regional and district-level communication channels such as visual supports (fliers, posters, banners), local theater/mobilization groups, and radio and television spots in region- or district-appropriate languages. The involvement of policy makers, decision makers, and opinion leaders in rallying the population around vitamin A supplementation at NIDs/NMDs is crucial. This involvement takes place at all levels. At the regional and district levels, the involvement of local administrative authorities and traditional and spiritual leaders with their public endorsement and support of vitamin A supplementation ensures mass social participation at NIDs/NMDs. At the central level, this involvement includes debriefing sessions with the Prime Minister, the President of the National Assembly and the Head of State, followed by press conferences and press releases by the Minister of Public Health and the representatives of major development partners. The launch day of NIDs/NMDs has been declared a holiday to encourage the active participation of employed caregivers in NIDs/NMDs. The President of the Republic of Niger launches the campaigns himself; all government ministers and a large delegation of the diplomatic corps accompany the president at this ceremony. This high-profile event is widely disseminated through the national television and radio networks.

Responsiveness and flexibility of Ministry of Public Health and development partners

In order to make the most efficient use of resources, it is important to take advantage of opportunities to integrate vitamin A supplementation into other programs. It has been critical that the Ministry of Public Health and its partners be able to respond to opportunities, and, conversely, act quickly to maintain coverage if other distribution mechanisms are not available. In 2003, NIDs for polio eradication were planned for the entire country; however, six weeks before their implementation, new surveillance data led to the decision to restrict the polio eradication campaign to 13 districts. The Ministry of Public Health and its partners were able to react quickly enough to ensure micronutrient distribution independently of the immunization campaign in the remaining 29 districts.

Perspectives

Programmatically, the challenges are now to ensure that twice-yearly universal vitamin A supplementation is sustained as a regular ("routine") strategy of increasing cost-effectiveness, and to ensure that twice-yearly universal vitamin A supplementation does not delay, displace, or weaken the implementation of other VAD control strategies, but that it drives an integrated, effective, and sustained nationwide assault on VAD that includes the following four other key components.

Improved infant and young child feeding

The meta-analysis by Beaton et al. [2] showed that the mortality reductions in children 6 to 24 months of age made up more than 70% of the total mortality reduction in children 6 to 59 months of age following vitamin A repletion. Optimal infant and young child feeding is therefore crucial for the effective control of VAD. Breastmilk is vital in keeping an adequate vitamin A intake in infants in the first six months of life and possibly throughout infancy [15]. In Niger, breastfeeding indicators reveal a suboptimal situation, as only 2% of infants 0 to 3 months of age are exclusively breastfed [16]. In West Africa, Gambia, Ghana, and Mali have proved that well-designed community- or facility-based programs can bring about significant improvements in the rates of early initiation of breastfeeding, exclusive breastfeeding, and prolonged breastfeeding.

Maternal postpartum vitamin A supplementation

When the vitamin A content of human milk is suboptimal due to the suboptimal vitamin A status of the mother, vitamin A supplementation of women in the early postpartum period becomes key in improving women's vitamin A status and the vitamin A content of breastmilk [17]. Although it is a policy of the Ministry of Public Health, maternal postpartum vitamin A supplementation coverage in Niger is still low, as only an estimated 16% of mothers are provided with a high-potency vitamin A supplement within the 40 days following delivery (28% of women living in urban areas and 14% of women living in rural areas) [18]. NMDs have been used both as an awareness-raising and as a delivery mechanism for maternal postpartum vitamin A supplementation. Since 1999, over 50% delivering within the 40 days (the traditional lying-in period) prior to an NMD have received a high-potency vitamin supplement.

Improved vitamin A dietary intake

Dietary improvement approaches need to be an integral part of a sustainable strategy to control VAD. In the past 10 years, significant progress has been achieved globally in the design and implementation of dietary approaches, particularly the new generation of projects that integrate production, nutrition education, and behavior-change communications strategies [19]. In Niger, a behavior-change communications strategy focusing on increasing liver consumption resulted in significant improvements in liver intake [20]; similarly, a homestead food-production approach focusing on increasing the production of micronutrient-rich crops—including the introduction of orange-fleshed sweet potatoes—resulted in significant improvements in production and consumption [21].

Vitamin A fortification of locally available foods

Fortification of widely consumed foods with vitamin A can be crucial for improving the vitamin A status of the general population, and that of women of reproductive age in particular. In Niger, the production of centrally processed foods is limited (most processed foods that are consumed are imported) and the private sector is weak. A National Food Fortification Committee was created in January 2003 to encourage and monitor food-fortification initiatives. The Committee includes representatives of the Ministry of Public Health, Rural Development, Finance and Economy, and Agriculture and Industry, as well as representatives of the Chamber of Commerce, the National Consumers' Associations, food processing companies, the World Health Organization (WHO), UNICEF, the Food and Agriculture Organization (FAO), and Helen Keller International (HKI). A formerly state-owned peanut oil refinery in Maradi, which was out of operation since 1990 and privatized in 2001, began production of peanut oil in 2002. Annual production is currently about 20,000 metric tons, with a capacity for 65,000 metric tons. This is the sole large-scale producer of cooking oil in the country, and it is being targeted for vitamin A fortification through a public–private partnership, as the national committee and the refinery owner have both agreed to pursue vitamin A fortification of this oil. National food-consumption surveys show that an estimated 85% of women of reproductive age consume cooking oil regularly (three to seven times per week).

Conclusions

African and other world leaders have made a commitment to reduce mortality rates in children by two-thirds between 1990 and 2015 [22]. Epidemiological evidence shows that in sub-Saharan Africa, the effective control of VAD has the promise to be among the most cost-effective and high-impact policy and program actions towards this goal. In Niger, a sustained coalition for child survival between the government and its development partners has ensured high coverage (more than 80%) of vitamin A supplementation twice yearly since December 1998. This successful program in Niger—one of the poorest countries in sub-Saharan Africa—along with those in Ghana and Zambia [23] shows that among the many challenges that African countries will need to face in the coming years, VAD control is one that can be overcome. The need is urgent, and the solutions are known, effective, and affordable.

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Milk fortified with iron or iron supplementation to improve nutritional status of pregnant women: An intervention trial from rural Vietnam

P. Thuy Hoa, Nguyen Cong Khan, Christine van Beusekom, Rainer Gross, Wolney L. Conde, and Ha Dui Khoi

Abstract

Anemia is still the major nutritional problem among pregnant women in Southeast Asia. The objective of this study was to measure hemoglobin status and reduction of underweight in a group of pregnant women who received iron-fortified or nonfortified milk, and another group who received iron supplements (tablets) or placebo. The 44 women in the iron-fortified milk group received 15 mg of iron per day per 400 ml of milk, and 41 women received placebo. The 40 women in the iron supplement group received 60 mg of iron per day, and 43 women received nonfortified milk. During this intervention trial, all women were supervised from the 14th to the 18th week of gestation until delivery. Blood was sampled at 0, 5, 10, and 16 weeks of intervention. After the 16th week of intervention, the changes in hemoglobin (ΔHb) concentrations in both treatment groups (the iron-fortified milk and the iron tablet groups) were not significantly different (ΔHb: –0.5 ± 0.9 and –0.3 ± 0.9 g/L, respectively), but the changes were significantly greater in the nonfortified milk and placebo groups (ΔHb: –1.2 ± 0.9 and –1.1 ± 0.8 g/L, respectively; $p < .01$*). The change in transferrin saturation (ΔTS) in the iron-fortified milk group (ΔTS: 3.4 ± 12.9%) was greater than that in the placebo and nonfortified milk groups (ΔTS: –10.1 ± 9.8% and –11.6 ± 10.7 %, respectively) (*$p < .01$*). The weight gain of the subjects during intervention did not differ significantly in the fortified and nonfortified milk groups (Δweight: 5.0 ± 2.0 and 5.8 ± 2.1 kg, respectively), but was higher than in the iron tablet group (Δweight: 4.6 ± 3.1 kg;* $p < .05$*) and the placebo group (Δweight: 3.8 ± 2.5 kg;* $p < .001$*). Iron supplementation and fortification were seen to be effective in promoting weight gain in pregnant Vietnamese women. For women who are underweight, the administration of iron-fortified milk has additional benefits to those of supplementation, most likely due to additional energy and nutrient inputs.*

Key words: Anemia, efficacy, iron-fortified milk, iron supplementation, pregnancy, weight gain

Introduction

Worldwide, anemia affects more than two billion people [1]. Pregnant women are at special risk, and the prevalence of anemia in this vulnerable group in Southeast Asia has been reported to be as high as 60% to 70% [1]. In Vietnam, the prevalence of anemia among pregnant women was reported to be 52.7%, with iron deficiency being the major cause [2]. As a result, the risk of reproductive failures such as miscarriage, stillbirths, premature birth, low birth weight, and maternal mortality is increased [3]. Many countries implement iron-deficiency control programs to increase the iron intake during pregnancy by distributing iron tablets. However, despite these efforts, no further reduction of anemia can be observed [1]. In Vietnam, iron supplementation for pregnant women was introduced in selected districts in 1991 and has been slowly expanded to most parts of the country. Nevertheless, compliance is still low because of factors such as poor motivation of the health staff and the pregnant women, the poor taste of the tablets, and negative side effects [4].

Iron supplementation is regarded as a short-term intervention [1]. In addition to supplementation, fortification and changes in food consumption are seen as medium-and long-term solutions for the control of

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iron-deficiency anemia. In Vietnam, many pregnant women suffer not only from micronutrient deficiencies but also from low energy and protein intake [5, 6]. Despite this, at the time of the study there was no existing iron supplementation or fortification program at the national level. As a result, the question arose as to whether fortification of food with iron reduces anemia and provides other benefits, in addition to those of iron supplementation, to the undernourished mother and her newborn during and after pregnancy. A study was carried out to compare the efficacy of two approaches to enhancing the iron nutritional status of pregnant women: iron supplementation with tablets (the traditional approach) and the use of iron-fortified cow's milk (the novel approach).

Subjects and methods

Population

The study was conducted between 1996 and 1997 in 12 communes in Dong Hung District, Thai Binh Province, in the rural delta area of the Red River in northern Vietnam. Agriculture is the main occupation of the adult household members of the study population. The criteria for the selection of this area for study were homogeneity of socioeconomic and ecological conditions, acceptance by the community, and the absence of an existing iron-supplementation program. In the health center of each commune operated by the national Ministry of Health, women between the 14th and 18th weeks of pregnancy were asked whether they were willing to participate in the study. The eligibility criteria were age 20 to 32 years; no more than two prior pregnancies; no stillbirths, premature births, or hemorrhage in previous pregnancies; no manifestations of chronic or infectious diseases, including hookworm infection; hemoglobin (Hb) > 70 g/L; and no planned travel or plans to move out of the area during the study period. Mothers who did not sign an informed consent did not participate in the study. At the end of the recruitment process, a total of 202 women were chosen to participate in the study.

Ethical considerations

The data collected have been used for study purposes only. The women were informed about the purpose of the study and the research institution before agreeing to participate. Assurance was given that cooperation was voluntary. The ethical committee of the National Institute of Nutrition approved the research protocol.

Study design

The study had four intervention groups. For practical reasons, it was possible to implement only one type of intervention per commune (block randomly adjusted). Each group started with 44 subjects, based on a between-group difference in hemoglobin of 5 g/L, with a significance level of $p = .05$, a power of 0.9, and a dropout rate of 20%. Participants were randomly assigned to treatment and control groups.

The women in the four groups received daily interventions. Group 1 received 400 ml of milk fortified with iron (IM); group 2 received the same volume of nonfortified milk (M); group 3 received one daily iron-folic acid supplement in pill form (IS); and group 4 received one placebo tablet (P).

Table 1 shows the contribution of energy and selected nutrients from the milk or tablets. Group 1 received 15 mg of iron daily as ferrous fumarate from the fortified milk. All milk powder (iron-fortified or nonfortified) was also enriched with vitamin C and folic acid. The iron-fortified and nonfortified milk had the same white color and identical smell and flavor. Both types of milk powder were specially produced and packaged for the study (Friesland Dairy Foods Company, Leeuwarden, Netherlands). Group 3 received a tablet daily containing 200 mg of ferrous sulfate (60 mg of elemental iron) and 250 μg of folic acid according to World Health Organization (WHO) recommendations [7]. The iron supplement and the placebo tablet had the same red color and shape. The two treatments could not be distinguished by sight. The tablets were provided by UNICEF and produced by Weiders Farmas Rytiske A/S, Norway. The pharmaceutical factory No II "Dopharma" of the Ministry of Health, Hanoi, Vietnam, produced

TABLE 1. Daily nutritional contribution according to the type of intervention in the four groups

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Contribution	With iron	Without iron	Iron	Placebo
Energy (kcal)	120	120	0	0
Protein (g)	6.8	6.8	0	0
Elemental iron (mg)	15	0	60	0
Folic acid (μg)	200	200	250	0
Vitamin C (mg)	17.5	17.5	0	0

the placebo for the single blind study.

Since Vietnamese women rarely consume cow's milk, it was necessary to start the study with an adaptation phase of one week. During this week, 101 subjects received gradually increasing amounts of milk: 100 ml for the first 2 days, 200 ml on the 3rd day, 300 ml on the 4th day, and 400 ml at on the 5th and 6th days. Non-milk-drinking Southeast Asian communities suffer widely from lactose intolerance [8, 9]. The daily gradual increase of milk was intended to help achieve cultural acceptance; however, for practical reasons it was not possible to plan a long enough exposure to realize a biological effect, i.e., a shift in the flora of the colon. Fortunately, most women did not have symptoms even when given the full amount of milk from the beginning.

Study organization

Each survey team consisted of four study workers who were trained before the beginning of the study. The task of each study worker was to prepare and distribute the milk or tablets, to interview the women, and to motivate them to take the distributed commodities regularly. Each survey team was appointed to cover four or five mothers. Between 2 and 3 p.m. every day, the subjects arrived at the home of their study worker. The study workers prepared the milk shortly before the time of administration. The women then drank the milk or took the tablet with water in the presence of the study worker, to ensure compliance.

During the first month of intervention, the designated supervisors of the research team visited the study workers on a weekly basis at random. The supervisors verified the information collected by the study workers at random (e.g., intake of milk or tablets, side effects) by asking the women in the study. The collected information was then compared with the results from the form completed by the study worker. On the first round, one error was found in 4.1% of the forms. All errors discovered were corrected. Based on the errors found and on inquiries, the supervisors assisted the study workers in organizational and methodological matters. From the second month on, the supervisors coached and monitored the study workers on a monthly basis.

Measurements

At baseline and at weeks 5, 10, and 16 of the study, 3 ml of venous blood was collected from each woman before she received the milk or supplement. The cyanmethemoglobin method was used to determine hemoglobin concentration [10] immediately after blood collection. After the hemoglobin determination, the serum was stored at –20°C for about 5–6 days. Serum iron (SI) was determined according to the recommendations of Gibson [11]. Total iron-binding capacity (TIBC) was determined according to Ramsay's recommendations [12]. Both biochemical analyses were carried out in the laboratory of the National Institute of Nutrition of the Ministry of Health. All analyses were performed in duplicate. Serum transferrin saturation (TS) was calculated according to Gibson's recommendations [11]. Anemia and iron-deficiency anemia were assessed by using the WHO classifications [10].

Height and weight measurements were performed at baseline following the recommendations of Gibson [11]. Weight was also measured at weeks 5, 10, and 16 and before delivery. Body weight was measured to the nearest 0.1 kg by an electronic weighing scale (SECA 770 alpha, SECA, Hamburg, Germany) with the woman wearing light clothing. Body height was measured to the nearest 0.1 cm by a microtoise (UNICEF, Copenhagen, Denmark). Pregestational weight was recorded from the subjects' health cards.

The women were asked to collect stool samples in small plastic containers, which were distributed at the beginning of the study. Within a week after collection, the samples were analyzed for hookworms according to the Kato-Katz method [13].

Food intake was assessed by 24-hour recall, repeated on three consecutive weekdays at the initiation of the study, as described by Gibson [11]. The Vietnamese food-composition table was the basis for the calculation of energy and nutrient intakes [14].

Statistical analysis

Data were entered by using SPSS for Windows software, Version 7.5 (SPSS, Chicago, IL, USA). The following statistical analyses were performed: analysis of variance (ANOVA) or analysis of covariance (ANCOVA), with hemoglobin concentration adjusted to the initial values, to analyze between-group differences in nutritional status; binary logistic regression to model the relationship between deteriorated iron status and adequate weight gain during pregnancy, adjusted for initial hemoglobin and prepregnancy weight (as a socioeconomic marker); and multinomial logistic regression for unordered multiple traits, adjusted for initial hemoglobin and prepregnancy weight, to model the relationship between adequate or inadequate iron status and weight gain during pregnancy [15]. The hypothesis under study was tested by independent *t*-tests to compare the four intervention groups with regard to the distribution of baseline variables that could influence changes in iron status and weight gain (e.g., baseline anthropometric data, iron status data, pregnancy indicators, nutrient intake, age, and family income); and by paired sample *t*-tests to assess in each group changes in iron status and weight during the follow-up period. The three first statistical analyses were implemented by SPSS, Version 7.5; the last two

tests were done with the Stata Version 6.0 software package (Stata, College Station, TX, USA).

Results

Table 2 shows the number of women excluded from data collection according to intervention group, along with the reasons for exclusion. Of the 202 women initially enrolled, a complete data set was obtained for 168. No significant differences between the excluded and the studied individuals were seen.

Table 3 compares the groups at baseline with regard to selected anthropometric data, pregnancy indicators, and nutrient intake data. No statistically significant differences were found among the groups.

Table 4 shows changes in iron status (hemoglobin concentration and TS) and weights during the trial. At the initiation of the study, there were no significant differences among the groups in the three measured indicators of nutritional status. At the end of the intervention, the hemoglobin concentrations of all four groups had decreased significantly ($p < .001$, paired t-test). However, the final hemoglobin concentrations and the changes among groups differed significantly ($p < .001$, ANCOVA adjusted for initial hemoglobin). The decrease in hemoglobin concentration in the iron-fortified milk and supplement groups was significantly less (–0.5 ± 0.9 and –0.3 ± 0.9 g/L, respectively) than in the unfortified milk and placebo groups (–1.2 ± 0.9 and –1.1 ± 0.8 g/L, respectively).

At baseline, the TS values showed no significant differences among groups (ANOVA). As with hemoglobin concentration, there was a significant difference among groups in TS changes ($p < .001$, ANOVA). The TS value decreased in the iron-fortified milk group and increased slightly in the supplement group (–2.7 ± 9.4% and 3.4 ± 12.9%, respectively), but the nonfortified milk

TABLE 2. Number of subjects and reasons for dropout according to intervention group

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Reason	With iron	Without iron	Iron	Placebo
Change of residence	0	2	0	1
Illness	2	1	1	1
Miscarriage	0	2	0	0
Premature delivery	1	3	4	2
Delivered before blood was taken	1	0	1	1
Refused taking of blood	2	2	2	1
Hemolysis	0	1	1	2
Total	6	11	9	8

TABLE 3. Comparison of selected anthropometric data, pregnancy indicators, and nutrient intake data between groups at baseline[a]

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Characteristic	With iron ($n = 44$)	Without iron ($n = 41$)	Iron ($n = 40$)	Placebo ($n = 43$)
Age (yr)	25.0 ± 3.7	25.8 ± 4.3	25.5 ± 3.8	25.3 ± 3.7
Pregestational weight (kg)	44.0 ± 3.9	44.0 ± 4.4	43.6 ± 3.4	43.8 ± 3.4
Weight at beginning of study (kg)	45.4 ± 5.0	45.2 ± 4.0	45.1 ± 3.6	46.0 ± 4.3
Height (cm)	153.2 ± 5.4	153.0 ± 5.1	152.9 ± 3.9	152.3 ± 4.5
Pregestational BMI (kg/m^2)	18.8 ± 1.4	18.7 ± 1.3	18.7 ± 1.7	19.2 ± 1.7
No. of children	0.5 ± 0.5	0.6 ± 0.5	0.5 ± 0.5	0.5 ± 0.5
No. of pregnancies	1.5 ± 0.6	1.7 ± 0.7	1.8 ± 0.8	1.7 ± 0.8
Duration of gestation (wk)	15.6 ± 1.4	15.6 ± 1.5	16.2 ± 1.6	16.6 ± 1.6
Energy intake (kcal/day)	2,188 ± 856	2,027 ± 812	2,124 ± 729	2,071 ± 664
Protein (g)[b]	36.2 ± 15.3	30.9 ± 11.8	35.6 ± 14.1	35.1 ± 13.3
Food iron intake (mg/day)	10.3 ± 4.4	9.9 ± 3.9	10.1 ± 3.7	9.7 ± 3.6
Food vitamin C intake (mg/d)	50.4 ± 33.6	47.8 ± 45.1	42.7 ± 31.2	41.1 ± 29.6

BMI, Body-mass index.

a. Values are means ± SD. There are no significant differences between groups ($p > .05$).

b. Protein was calculated with estimation of NPU (net protein utilization) = 60

TABLE 4. Hemoglobin, transferrin saturation, and maternal weight (mean ± SD) of the four intervention groups during pregnancy at baseline and after 16 weeks of intervention

	Hemoglobin (g/L)			Transferrin saturation (%)			Weight (kg)		
Group	Baseline[a]	Week 16[b]	Change[b,c]	Baseline[a]	Week 16[d]	Change[c,e]	Baseline[a]	Week 16	Change[b,e]
IM	117.4 ± 6.7	112.1 ± 8.4[f]	–0.5 ± 0.9	26.3 ± 7.8	23.7 ± 6.7	–2.7 ± 9.4	45.4 ± 4.9	50.4 ± 4.6[g]	5.0 ± 2.0
M	117.5 ± 10.6	105.2 ± 11.3[g]	–1.2 ± 0.9	26.1 ± 8.0	16.0 ± 5.8	–10.1 ± 9.8	45.2 ± 4.4	50.9 ± 5.5[g]	5.8 ± 2.1
IS	116.3 ± 8.9	113.3 ± 8.8[g]	–0.3 ± 0.9	24.6 ± 10.2	28.0 ± 9.0	3.4 ± 12.9	45.1 ± 3.6	49.7 ± 4.0[g]	4.6 ± 3.1
P	115.5 ± 7.5	104.1 ± 10.0[g]	–1.1 ± 0.8	26.0 ± 11.1	14.4 ± 7.1[g]	–11.6 ± 10.7	46.1 ± 4.2	49.9 ± 4.9[g]	3.8 ± 2.5

IM, Iron-fortified milk; M, nonfortified milk; IS, iron supplement; P, placebo.
Differences between groups:
a. Not significant (paired sample *t*-test)
b. $p < .001$ (ANOVA).
c. $p < .001$ (ANCOVA adjusted for initial hemoglobin).
d. Difference between baseline and 16th week.
e. $p < .01$ (ANOVA).
f. $p < .05$ (paired *t*-test).
g. $p < .001$ (paired *t*-test).

and the placebo groups both showed marked decreases in TS concentration (–10.1 ± 9.8% and –11.6 ± 10.7%, respectively).

The mean weights of the women in the four groups, which did not differ at baseline (ANOVA), increased significantly, as expected, with the advance of pregnancy beyond 16 weeks ($p < .001$, paired *t*-test). The weight increases differed significantly among groups ($p = .004$, ANOVA). The highest weight increases were found in the nonfortified- and fortified-milk groups (5.0 ± 2.0 and 5.8 ± 2.1 kg, respectively), followed by the supplemented group (4.6 ± 3.1 kg) and the placebo group (3.8 ± 2.5 kg). **Figure 1** shows the risk of developing anemia during pregnancy according to the four types of intervention, taking into consideration the initial hemoglobin levels. In all groups, the risk increases with reduced initial hemoglobin concentration. However, supplementation and fortification reduce the risk of anemia drastically, even if women are already anemic at the beginning of pregnancy. **Figure 2** shows the probability of adequate weight gain after intervention (at least 20% of prepregnancy weight) adjusted for prepregnancy weight. The probability of an adequate weight gain increases rapidly in women with a prepregnancy weight below 50 kg. However, the probability of an adequate weight gain despite a lower prepregnancy weight is highest in the fortified-milk group, followed by the nonfortified-milk group.

Discussion

Undernutrition and micronutrient deficiencies are still widespread among Vietnamese women. In 1994, it was estimated that about 30% of reproductive age women in rural area suffered from chronic energy deficiency (body-mass index < 18.5 kg/cm^2) [16]. In mothers with children under five years of age, this rate exceeded 40% [5]. The anthropometric data collected from the pregnant women in this study showed the same pattern

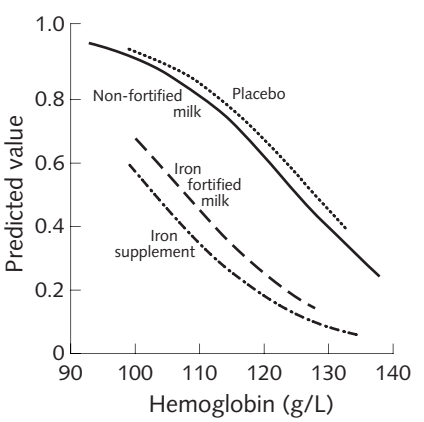

FIG. 1. Probability of anemia (hemoglobin < 110 g/L) adjusted for initial hemoglobin after intervention, according to the four different types of intervention

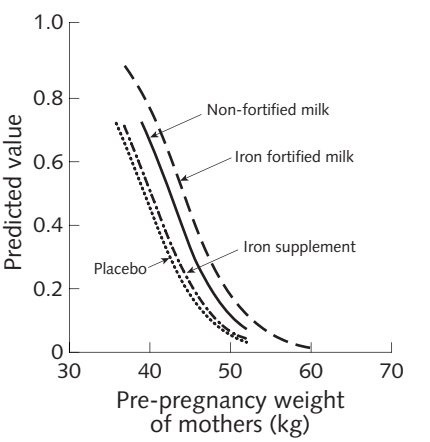

FIG. 2. Probability of adequate weight gain (> 20% of pre-pregnancy weight) adjusted for prepregnancy weight after intervention, according to the four types of intervention

of high prevalences of undernutrition (**table 3**).

At the baseline, the prevalences of anemia (Hb < 110 g/L) and of iron deficiency (TS < 16%) were 19% and 16%, respectively. The anemia prevalence was lower than that observed in Indonesian women in the first and second trimesters of pregnancy [17, 18]. During pregnancy, women have increasing requirements for iron. Iron is needed for the increasing maternal red cell mass (the demand is equal to 500–600 mg of iron) and for the growing fetus and placenta (with a demand of 350–450 mg of iron). When the normal physiological loss of iron from skin, stool, and urine is added to these demands, there is a cumulative need for iron during pregnancy of 1,100 to 1,400 mg, or 4 to 5 mg daily [10]. However, the need for iron is not evenly distributed throughout pregnancy. There is no increase or only a slight increase in the iron requirements in the first half of pregnancy, followed by a marked increase in the second half, leading to a daily demand for iron uptake in the third trimester as high as 8 to 10 mg daily.

In Vietnam, the average diet contains about 9 to 10 mg of iron daily, with an estimated net absorption rate of 5% to 10% [15]. The findings of this study confirm these low iron intakes in pregnant women (**table 3**). Therefore, the low iron intake from food and the increased iron requirements at the later stage of pregnancy result in a decreasing hemoglobin concentration in the blood (**table 3**).

According to the findings in this study, iron depletion can be compensated for or slowed down by iron supplementation and fortification, depending on the iron status at the beginning of the pregnancy. **Figure 2** suggests that the probability of anemia is slightly lower in the supplemented than in the fortified-milk group. However, the difference is relatively low, considering that the fortified group received only 25% of the additional daily iron dose as compared with the tablet-supplemented group. Therefore, the question remains whether women really need the recommended daily dose of 60 mg of iron for the control of anemia.

A weight gain of 9 to 12 kg during pregnancy has been recommended for pregnant women with an adequate pregestational weight [19, 20]. In Vietnam, mean weight gain of pregnant women in rural areas has been reported as 6.6 kg [14]. These published data are consistent with the mean weight gain of the placebo group (6.1 kg) in this study. Taking the weight gain recommendations into consideration, less than a quarter of the women (22.8%) achieved this goal in the present longitudinal observations. However, the prevalence of insufficient weight gain during pregnancy differed significantly between groups. About one-third of the women in the iron-fortified milk and the nonfortified milk groups achieved the recommended weight increase. In the iron supplementation group, one-fifth of the women (20%) met the weight gain recommendation, whereas only 9% of the women in the placebo group did so. As shown in **fig. 2**, the probability of adequate weight gain during pregnancy depends not only on diet, but also on socioeconomic factors and the prepregnancy weight of the woman. In particular, low-weight pregnant women benefit from fortified food.

Conclusions

The administration of milk fortified with 15 mg of iron per day and iron supplementation with 60 mg of iron per day in tablet form improved the iron status of pregnant Vietnamese women in this study. These findings suggest that doses even lower than 60 mg of iron per day are sufficient to prevent a drastic increase in anemia rates as pregnancy advances. However, if a woman enters pregnancy with anemia, or even with empty iron stores, supplementation and fortification, even under controlled conditions, seem to be insufficient to eliminate iron deficiency during gestation. This underscores the importance of preventing anemia among women of childbearing age before pregnancy.

Since the women suffered not only from low iron intake (and possibly also from other micronutrient deficiencies), but also from low energy consumption, the additional energy and nutrients obtained from the milk contributed to the weight gain of the mothers in the fortified-milk group during pregnancy. The distribution of fortified milk to pregnant women is far more expensive than the distribution of iron supplements alone. However, in the Vietnamese situation, in which mothers have a high prevalence of acute undernutrition, supplementation alone was insufficient to address weight gains during pregnancy. Rather, the increased availability of fortified food commodities such as milk at the household level should be considered.

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Cultural and environmental barriers to adequate iron intake among northern Kenyan schoolchildren

Bettina Shell-Duncan and Thomas McDade

Abstract

The purpose of this study was to examine the context of iron deficiency and feeding patterns of iron-rich foods among northern Kenyan school-aged children. A nutrition survey was conducted among 300 subjects in two Rendille communities, Korr and Karare. The objectives were to determine the prevalence of iron deficiency as it relates to parasitic infection, dietary intake, and sociodemographic factors, as well as cultural food proscriptions influencing child feeding. Sociodemographic and qualitative data on food beliefs and child-feeding practices were obtained from the primary caretaker of each subject. From pediatric subjects, 24-hour dietary recall data were obtained with the help of the primary caretaker, and capillary blood from a fingerstick was used to detect iron deficiency based on measures of hemoglobin, the zinc protoporphyrin-to-heme ratio, C-reactive protein, and transferrin receptor. With an overall prevalence of 31.2%, iron deficiency was found to be associated with dietary iron intakes constrained by diverse economic, cultural, and environmental factors among Rendille children. In Karare, where children's iron intake approached recommended levels, iron deficiency was found to be attributable to low bioavailability of iron (only 4.3% of total iron intake), rather than low dietary intake per se. By contrast, in Korr the average daily iron intake was estimated at only 65% of recommended allowances, indicating that iron deficiency was the outcome not merely of low bioavailability, but rather of overall inadequate iron intake. Sociodemographic analysis showed a significant interaction between sex and economic status, revealing that girls in economically sufficient households were 2.4 times as likely to have iron deficiency as boys. This difference in risk parallels culturally defined gender-based proscriptions for child feeding: girls are believed to benefit from "soft foods," including rice, maize porridge, and tea, whereas boys benefit from "hard foods," including meat, blood, and beans. Consequently, in households economically able to purchase iron-rich foods, these foods are being preferentially fed to boys. Economic development may result in improved iron status for boys, but it will be unlikely to benefit girls in the absence of a dietary modification intervention. A modification of culturally acceptable "soft foods" to include iron-rich foods may provide a sustainable approach to controlling and preventing iron deficiency in this population.

Key words: Bioavailable iron, food prescriptions, hemoglobin, iron deficiency, parasitic infection, school-age children, transferrin receptor, zinc protoporphyrin

Introduction

Iron deficiency is reportedly the most common micronutrient deficiency worldwide, and in developing countries the burden rests not only on women and infants, but also on school-aged children [1, 2]. Iron-deficiency anemia has serious costs, including impaired learning and school performance [3], growth faltering and reduced physical fitness [4], and increased risk of infectious morbidity [5], conditions that may also occur during milder preanemic forms of iron deficiency [6]. Iron deficiency arises when iron absorption is insufficient to meet the body's needs. The insufficiency may be attributed to low overall iron intake, or to increased need for iron from chronic blood loss due to parasites such as hookworm and *Schistosoma* [1]. In addition, iron absorption is influenced by the bioavailability of iron. Whereas heme iron (present in animal foods) has high bioavailability, the absorption of nonheme iron

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(present in plant foods) is influenced by meal composition. Enhancers, such as ascorbic acid, promote the absorption of nonheme iron, whereas inhibitors, such as phytates and polyphenols, decrease the absorption of nonheme iron.

As DeMaeyer and colleagues [5] point out, the treatment and control of iron deficiency is technically quite simple, requiring only an increase in iron intake. Dietary modification can improve iron status in poor communities in two key ways: by increasing the overall iron content of the diet by modifying household food acquisition and allocation practices, and by increasing the bioavailability of ingested iron by promoting the consumption of iron absorption enhancers or reducing the ingestion of inhibitors. Numerous factors, however, make dietary modification difficult to attain. It is well recognized that economic constraints pose formidable barriers for both avenues of dietary modification. The most efficient manner of improving the overall dietary iron content or the bioavailability of iron in communities with predominantly starch-based diets is to increase the consumption of meat, which not only provides heme iron but also increases the absorption of nonheme iron [5, 7, 8]. In many communities high cost is an obstacle to obtaining animal foods. However, in households that do have access to heme iron, barriers to access to iron-rich foods may be cultural, influencing food selection and household food distribution. Therefore, efforts to modify dietary intake need to consider both environmental and cultural factors influencing dietary iron intake.

The purpose of this study is to investigate the epidemiology of iron deficiency among northern Kenyan children and to identify barriers to iron intake. We evaluate not only the biomedical and socioeconomic context of iron status, but also the cultural factors contributing to observed patterns of iron deficiency. The results of this research are used to explore the possibility of dietary modification as a sustainable approach to preventing iron deficiency.

Subjects and methods

In July 1999, research was conducted in Marsabit District in northern Kenya among a population ethnically identified as Rendille. The Rendille are traditionally nomadic, subsisting through camel pastoralism in the Kaisut Desert. This desert is one of the harshest and least productive regions of East Africa, receiving on average less than 250 mm of annual rainfall [9]. It is also characterized by high levels of endemic disease stress, with respiratory infection, malaria, and diarrhea being the leading sources of morbidity [10]. Recently, in response to a series of droughts that diminished large portions of the livestock, many Rendille have settled in permanent towns in the Kaisut Desert and have shifted to alternative forms of subsistence, including dryland agriculture, milk marketing, trade, and blacksmith artisanship. Settlement of former nomads is accompanied by major changes in diet, away from an iron-rich diet of blood, milk, and meat to a maize meal-based diet [9]. This study investigates the prevalence of iron deficiency among settled Rendille schoolchildren and evaluates the cultural ecology of dietary iron intake.

Blood samples and anthropometric measurements were obtained from 5- to 10-year-old Rendille children in two rural villages, Korr and Karare. Following the construction of community maps and a complete census of the 5- to 10-year-old population in each village, 300 children were selected in a 30-strata sampling design. All children in the desired age range were considered elgible once oral consent was obtained from a parent or primary caretaker. The strata represented the town center and surrounding *menyattas* (circular compounds of houses containing extended families). The children's ages were determined by reports from their parents or primary caretakers using a local event history calendar, and by the date of birth recorded on the clinic card. Discrepancies were resolved by relative ranking against other children of known age in the community. The study protocol was reviewed and approved by the Human Subjects Division at the University of Washington and the Ethics Committee at Kenyatta Hospital in Nairobi.

Assessment of health and iron status

Sterile, disposable microlancets were used to collect free-flowing capillary blood to assess iron status and inflammation. Iron status was determined by combined measures of hemoglobin, the ratio of zinc protoporphyrin to heme (ZPP:H), and transferrin receptor (TfR). This multiple criteria model has been previously assessed [11]. Because hemoglobin and ZPP:H may be altered in the presence of infection [12, 13], C-reactive protein (CRP) was used to identify individuals with inflammation.

Hemoglobin concentrations in capillary blood were determined in the field using the HemoCue B-Hemoglobin system (HemoCue, Mission Viejo, CA, USA). Calibration was checked daily by measuring a sample with a known hemoglobin concentration determined by ICSH (International Council for Standardization in Haematology) recommended reference methods [14]. Anemic subjects were identified by subnormal hemoglobin according to the World Health Organization (WHO) age-specific cutoff values adjusted for ethnicity and altitude [15].

ZPP:H was measured from whole blood collected in two heparinized capillary tubes, which were then sealed and stored for up to two weeks in a portable, car-battery-powered refrigerator. The tubes were transported to the Clinical Nutrition Laboratory at

the University of Washington and analyzed for ZPP:H using the ProtoFluor-Z Hematofluorometer (Helena Laboratories, Beaumont, TX, USA). A cutoff value of 80 μmol/mol is recommended for identifying elevated ZPP:H for all ages above one year [16].

TfR and CRP were determined from capillary blood dried on filter paper. At least two drops of whole blood were collected on filter paper (Schleicher & Schull #903, Keene, NH, USA), allowed to dry for approximately four hours, and sealed in plastic bags with desiccant. The samples were refrigerated prior to transport to the Laboratory for Human Biology Research at Northwestern University, where they were stored at –20°C until analysis. Prior research has demonstrated that CRP is stable in dried blood spots for at least 14 days when stored at room temperature or 4°C, and for up to one year when stored at –20°C [17]. CRP levels were assayed following the ELISA protocol developed by McDade et al. for whole blood spots [16, 17]. TfR concentrations were measured by a commercially available ELISA kit (TF-94, Ramco Laboratory, Stafford, TX, USA), modified for whole blood spots [18]. Current plasma/serum protocols suggest a cutoff value of 8.5 for identifying iron deficiency [12, 19]. This corresponds to a whole blood spot TfR concentration of 6.7 mg/L [18].

Thick and thin smears were prepared on glass slides for the determination of malarial parasites. The slides were fixed and stained with Giemsa stain and screened for malaria parasites at the Laboratory of Medicine at the University of Nairobi. Only the presence or absence of malaria parasites was reported.

Urine samples were collected on the day of nutritional assessment to screen for microhematuria, which often arises from schistosomiasis [20, 21]. Hematuria was tested with Hemastix reagent strips (Bayer Corporation, Elkhart, IN, USA), which generally detects free hemoglobin levels from 0.015 to 0.062 mg/dL.

A general assessment of nutritional status was obtained from anthropometric measurements performed by a single trained and experienced observer using standard techniques described by Jelliffe and Jelliffe [22]. Height was measured to the nearest millimeter with an anthropometer while the subject stood on a level platform. A Seca (Hanover, Md., USA) electronic digital LED scale was used to measure weight to the nearest 0.1 kg, with the subject wearing light clothing.

Dietary intake and child feeding

Twenty-four-hour dietary recall data were obtained from children and their caretakers according to methods described by Buzzard [23]. During the dietary intake interviews, an enamel cup commonly used in northern Kenya was used as a reference for the quantities of food consumed. This cup was then used to determine the equivalent weights of various food portions in ounces or grams. For combined foods such as stews and tea, recipes were obtained through participant observation, i.e., through observing and assisting in cooking in selected households, and interviewing. Portions were measured by using local utensils and converted into ounce or gram equivalents. Finally, open-ended interviews centered on the mother's perception of the child's food preferences and aversions, as well the mother's beliefs and self-reported practices regarding child feeding.

Sociodemographic data

A pretested questionnaire was used to interview the primary caretaker of each selected child regarding socioeconomic and demographic information, including the child's attendance at school, the child's birth order, the mother's age and level of education, whether the household was headed by a male or a female, prolonged absence of the husband (more than six months in the past year), household size, number of dependents, and the economic status of the household. Following earlier developed methods described in detail elsewhere [24], economic status is, for this analysis, dichotomized into poor vs. economically sufficient. Briefly, several items were used to create this index, including wage income, livestock holdings (quantified as total livestock units, with one unit set equal to 1 cow, .8 camels, or 10 goats or sheep), garden size, farm production, marketing or bartering of items such as milk, firewood, charcoal, and alcoholic beverages. Using earlier determined equivalence factors, holdings were converted into total livestock units, and families classified as "not poor" were those that owned more than 4.5 total livestock units per capita.

Data analysis

Iron deficiency was identified by a multiple-criterion model defined as elevated ZPP:H in the presence of normal CRP and/or elevated TfR [11]. Iron-deficiency anemia was defined as iron deficiency in the presence of subnormal hemoglobin (hemoglobin in highland Karare < 110 g/L for age 5 or < 115 g/L for ages 6–10; hemoglobin in lowland Korr < 100 g/L for age 5 or < 105 g/L for ages 6–10). Iron deficiency was defined as elevated ZPP:H (> 80 μmol/mol) in the absence of inflammation (CRP ≤ 1.5 mg/L) and/or elevated TfR (TfR > 6.7 mg/L). Preanemic iron deficiency, or iron-deficiency erythropoiesis, was identified by iron deficiency in the presence of normal hemoglobin.

Anthropometric measurements were entered into EpiInfo (version 1.0.5, Centers for Disease Control and Prevention, Atlanta, GA, USA) to calculate sex-specific height-for-age and weight-for-height Z-scores (WHZ). For children aged 5–10 years, there is no accepted

indicator for wasting [25]. We used a WHZ below –3 SD to define severe wasting, which may independently cause anemia [26].

Biochemical and survey data were analyzed with SPSS Version 10.0 (SPSS, Chicago, IL, USA). To assess the magnitude of the association between iron deficiency and several risk factors, the odds ratio (OR) and correlation were calculated. The OR is defined as the prevalence of iron deficiency in the exposed group divided by the prevalence of iron deficiency in the nonexposed group [27].

Backwards regression models were used to evaluate the effect of socioeconomic factors on iron status while controlling for individual-level factors (age and sex). Economic status was, in this analysis, dichotomized into poor vs. sufficient using a scale previously described [24]. In order to correctly specify the models and more closely model real processes impinging on iron status, interactions between independent variables were also carefully evaluated. The final models include variables that remained after a stepwise backwards elimination process with $p \leq .05$.

Twenty-four hour dietary recall data were analyzed by using two programs: Nutritionist IV Software Program (First Data Bank, 1995, Indianapolis, IN, USA), which computes macronutrients and micronutrients, including total dietary iron; and the WorldFood 2 Dietary Assessment System, version 2.0 [28], which calculates bioavailable iron. Food-composition data for Kenyan foods were obtained from the database of WorldFood 2, and values for northern Kenyan foods not included in this database (e.g., blood and camel's milk) were obtained from published food-composition tables [29–31].

Recipes of combined foods were entered into Nutritionist IV and used to estimate the composition of specified portions. WorldFood 2 calculates the bioavailability of iron using an algorithm developed by Murphy et al. [32]. It assumes that heme iron constitutes 40% of the iron in meat, poultry, and fish, and that heme iron is 25% available. The availability of nonheme iron ranges from 5% to 15%, depending on the enhancing and inhibiting factors consumed in the same meal. Since iron absorption is also influenced by individual iron status [33], it is assumed that each individual has a basal iron status in which iron stores are depleted, but that iron status is high enough to prevent anemia. Although iron status may be better for many individuals, this provides an estimate of bioavailability to maintain at least this low level of iron status. In WorldFood 2, the weights of foods and ingredients were entered in grams. For foods measured by volume using local cups, conversion to grams was based on test weights of foods per measured volume.

Content analysis was used to analyze qualitative data on child-feeding practices [34]. Emergent themes identified common food prescriptions and restrictions in each community.

Results

Characteristics of study subjects

Complete demographic and health data were collected from 300 children aged 5 to 10 years and their mothers or primary caretakers. The descriptive data show a mean household size of 5.5, with an average of 3.5 dependents (children under 15 years of age) per household. Seventy percent of children were currently attending primary school, and 30% were currently not in school. Most households participated in subsistence agriculture, milk marketing, or bartering of items such as firewood, while less than 12% of households had wage-earning adults. In general, living conditions in these communities are considered poor by Kenyan standards. Using our socioeconomic status index, 42% of households were categorized as poor.

Prevalence of anemia and iron deficiency

Descriptive statistics for biochemical indices are given in **table 1**. Only 8.0% of children had subnormal hemoglobin, whereas 32% had elevated ZPP:H. Using the multiple-criterion model, we found that the overall prevalence of iron deficiency was 31.2% (**fig. 1**). Additionally, we found that preanemic iron deficiency

TABLE 1. Descriptive statistics for biochemical indices

Biochemical index	Median (min, max)	% beyond cutoff[a]
Hb (g/L)	133.0 (30.0, 163.0)	8.0
ZPP:H (μmol/mol heme)	69 (34, 458)	32.0
TfR (g/L)	4.5 (2.3, 232)	18.5
CRP (mg/L)	0.04 (0, 21.2)	15.6

ZPP:H, Ratio of zinc protoporphyrin to heme; TfR, transferrin receptor; CRP, C-reactive protein.

a. Cutoff values: hemoglobin in highland Karare < 110 g/L for age 5 or < 115 g/L for ages 6–10; hemoglobin in lowland Korr < 100 g/L for age 5 or < 105 g/L for ages 6–10; ZPP:H > 80 μmol/mol; TfR > 6.7 mg/L; CRP > 1.5 mg/L.

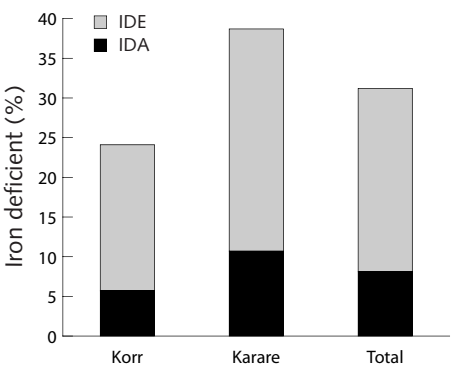

FIG. 1. Prevalence of iron deficiency in Korr and Karare

(23.1%) was significantly more common than iron-deficiency anemia (8.1%), and that the overall levels of iron deficiency (iron-deficiency erythropoiesis and iron-deficiency anemia combined) were significantly higher in Karare (39%) than in Korr (24%).

Health risk factors associated with iron deficiency

Several health and nutritional risk factors were examined in relation to their association with iron deficiency (**table 2**). Hematuria, which is often used to screen for urinary schistosomiasis (with 69% sensitivity and 89% specificity, according to Savioli et al. [20]), was found in 4.2% of subjects. All but two cases were in children from the town of Karare. Malaria, which may contribute to the etiology and severity of anemia through several mechanisms, including destruction of parasitized red blood cells, immune mechanisms, and dyserythropoiesis [35], was confirmed in only 1.3% of subjects, and was not significantly correlated with iron deficiency. Severe wasting, assessed as WHZ < –3 SD, was found in only 1.3% of subjects, and was not significantly correlated with iron deficiency. The results exclude wasting as an important preventable risk factor for iron deficiency, but the importance of other parasitic infections, such as hookworm, remains unclear.

TABLE 2. Health risk factors and their association with iron deficiency

Risk factor	Prevalence of risk factor (%)	Odds Ratio	Correlation with iron deficiency (p)
Hematuria	4.2	1.24	ns
Malaria	1.3	2.06	ns
Severe wasting (WHZ < –3 SD)	1.3	0.79	ns

ns, Not significant; WHZ, weight-for-height Z score.

Nutritional risk factors for iron deficiency

The nutritional nature of iron deficiency in this study population was investigated by analysis of dietary intake data and corresponding biomarkers. As shown in **table 3**, the median daily dietary intake of iron in Karare approached the recommended levels, whereas the median intake in Korr was only 65% of recommended dietary allowances. The levels of iron deficiency were, however, higher in Karare than Korr. Therefore, it is essential to examine bioavailability of iron and dietary constituents that enhance or inhibit iron absorption. The bioavailability of iron in children's diets was low in both locations: 4.3% of total iron intake in Karare and 7.7% in Korr. The median bioavailable iron intakes were well below the median absorbed iron requirements for growth and maintenance, especially for 7- to 10-year-old children [36].

Vitamins that have been reported to influence hemopoiesis and iron absorption include ascorbic acid, vitamin A, vitamin B_{12}, and folate [37–39]. Folate intakes were high in both communities (median, 216.6 and 235.4 μg in Korr and Karare, respectively), whereas the median daily intakes of ascorbic acid and vitamin A were low. Because the RDAs are set high to maximize sensitivity, it has been suggested that a value of ¾ of the RDA value be used to determine inadequate nutritional intake [40]. By this standard, the intakes of both vitamin A and ascorbic acid are inadequate among children in both communities.

The main dietary staples among Rendille children were maize meal (cooked as a stiff porridge, *ugali*, or a thin porridge, *uji*), tea with milk and sugar, and *githeri,* a dish made from red beans and maize. These foods contain nonheme iron, as well as several inhibitors or iron absorption (tannins in tea, phytates in maize, polyphenols in legumes, and calcium in milk). Heme iron, which enhances the absorption of nonheme iron, was consumed in the form of meat and blood by only 13% of the children. Additionally, 89.9% of children were reported to consume tea with at least one meal.

TABLE 3. Median 24-hour intakes of energy and select micronutrients according to location

Nutrient	Korr	Karare	Recommended intake[a]
Total kilocalories	1,164 (60)	1,496 (79)	1,800[b]–2,000[c]
Iron (mg)	6.5 (65)	9.3 (93)	10
Bioavailable iron (mg)[d]	0.5 (55)	0.4 (52)	0.5[b]–0.71[c]
Ascorbic acid (mg)	28.1 (62)	23.1 (51)	45
Vitamin A (μg RE)	104.6 (17)	239.3 (41)	500[b]–700[c]
Vitamin B_{12} (μg)	0.68 (57)	1.14 (102)	1.0[b]–1.4[c]
Folate (μg)	216.6 (256)	235.4 (276)	75[b]–100[c]

RDA, Recommended dietary allowance; RE, retinol equivalent. The percentages of age-specific RDAs supplied by the nutrients are given in parentheses.

a. RDAs, 1989 [54] for energy and all micronutrients except bioavailable iron. Median absorbed iron requirements [36] for daily required bioavailable iron.

b. Recommended daily intake for 5- to 6-year-old children.

c. Recommended daily intake for 7- to 10-year-old children.

d. Calculated from the WorldFood 2 Dietary Assessment Program [28].

The leading food sources of iron, as well as of ascorbic acid and vitamin A, which have been reported to improve iron absorption [38, 39, 41, 42], are shown in **table 4**. Potatoes and milk were the main sources of ascorbic acid. Camel's milk, which was consumed most often in Korr, contains three times the level of ascorbic acid as cow's milk, and contributes to the higher ascorbic acid intake among Korr children. Fruits were consumed by only 4.1% of the children and were not a significant source of ascorbic acid. Although vegetables were consumed by only 10% of the children, sukuma, a dark-green leafy vegetable, contributed to ascorbic acid and vitamin A intake among children in Karare. Milk was the leading source of vitamin A, and maize meal and legumes were the sources of approximately 75% of the iron consumed.

Overall, this diet is low in bioavailable iron. In Korr iron deficiency appears to be caused by low dietary iron intake, whereas in Karare iron deficiency appears to be attributable more to poor iron bioavailability than to low iron intake per se.

Socioeconomic and cultural context of iron deficiency

In a backward stepwise regression controlling for the effects of community- and individual-level factors, a number of socioeconomic variables were evaluated as predictors of iron deficiency (iron-deficiency anemia and iron-deficiency erythropoiesis), including age and school attendance of the child, birth order, mother's age and level of education, whether there is a male or a female head of the household, prolonged absence of the husband (more than six months during the past year), household size, number of dependents under 15 years old, and economic status of the household.

Community was found to have a highly significant effect, and in order to more clearly evaluate socioeconomic factors, separate analyses were performed for subjects from each town, Korr and Karare. In Karare, all socioeconomic factors and interaction terms failed to significantly predict iron deficiency. The only significant predictor was age, with the prevalence of iron deficiency declining with age in 5- to 10-year-olds. Qualitative data analysis did not, however, reveal age-related food proscriptions or preferences.

In Korr, by contrast, age was not found to be a significant predictor of iron deficiency. A backwards regression revealed that sex, economic status and the interaction between sex and economic status are significant predictors of iron deficiency. As shown in **Table 5**, children in poor households have a higher prevalence of iron deficiency. The interaction with sex reveals that in poor households, all children have an elevated prevalence of iron deficiency. However, in economically sufficient households, girls are 2.4 times as likely to have iron deficiency as boys.

Qualitative information on food practices and beliefs sheds light on the cultural factors contributing to the observed pattern of iron deficiency. Caretakers were asked to describe their beliefs about good and harmful foods for children, and asked whether and how often they followed food proscriptions. In Korr, food proscriptions are highly gender-specific. Many informants reported a preference for feeding girls "soft foods." This category includes rice, milk, and *uji* (a maize meal porridge), and these foods are described as soft because they are easily digested. Soft foods are believed to be adequate for girls because they perform lighter household tasks such as cooking and caring for young children. Boys, by contrast, are thought to benefit from "hard foods," including iron-rich blood and meat, as well as *ugali* and *githeri* (a bean and maize dish). Blood in particular is singled out as good for boys and harmful for girls. Boys are believed to benefit from "hard foods" because these foods give boys strength and energy for performing labor-intensive tasks such as herding and watering animals.

The responses to the question how often caretakers follow described food proscriptions were sharply divided according to economic status. Respondents from poor households often indicated that they could

TABLE 4. Leading food sources of iron, ascorbic acid, and vitamin A in the two locations

	Median consumption (per 24 hrs)	
Nutrient	Korr	Karare
Iron (mg)		
Total	6.5	9.3
Maize meal	3.8	4.0
Legumes	1.6	3.1
Meat	0.4	0.5
Blood	0.2	0.3
Ascorbic acid (mg)		
Total	28.1	23.1
Potatoes	11.3	8.1
Dairy	6.9	2.3
Legumes	1.8	2.3
Vitamin A (μg RE)		
Total	104.6	239.3
Dairy	52	44
Meat	12	25
Sukuma	11	132

RE, Retinol equivalent.

TABLE 5. Prevalence (%) and odds ratios of iron deficiency by economic status and sex in Korr

	Male	Female	OR
Poor	32.4	27.0	0.833
Not Poor	11.5	28.1	2.44

not afford "hard foods" and were forced to feed their children inexpensive foods, largely maize meal and tea. Consequently, in poor households, boys as well as girls are provided with diets low in bioavailable iron. In economically sufficient households that can afford iron-rich foods such as blood and meat, these foods are preferentially allocated to boys, resulting in a much lower prevalence of iron deficiency.

Discussion

The results of this study confirm that iron deficiency is a significant nutritional disorder among Rendille children, with a prevalence of 31.2% among 5- to 10-year-olds. This finding contributes to a growing body of research documenting poor iron nutrition among African school-age children [1, 4, 21, 43], and it underscores the importance of examining this age group when assessing the need for intervention.

Several disease conditions were examined in relation to iron deficiency. During the study period, parasitic infections from malaria and *Schistosoma* had a very low prevalence and were not significantly associated with iron deficiency. Malaria, which is known to be a very serious health problem in this region [10], does not influence iron deficiency, but it may influence levels of anemia in a seasonal fashion. Hookworm infection has been reported to be significantly correlated with iron-deficiency anemia in Zanzabari schoolchildren [1], but it was not investigated in this study. Wasting, which may independently contribute to the development of anemia [25, 43], was also not significantly associated with iron deficiency.

The role of dietary intake in the etiology of iron deficiency was investigated through the analysis of 24-hour dietary recall data. These data suffer from a number of limitations. Single 24-hour dietary recalls, as obtained in this study, are not as reliable in estimating usual nutrient intake as multiple dietary recalls. Accurate estimation of food portions is difficult, particularly for children, and it is possible that the mother or caretaker may not have observed all child-feeding events. Hence, estimation of portions, even with the aid of visual aids and appropriate references, is only an approximation of the true amounts consumed [44]. Additionally, the computation of nutrient values from 24-hour-recall data assumes that recipes were similar for all informants, that food-composition data are accurate for local Rendille foods [45], and that foods are free from contamination with iron [43]. Nonetheless, it has been shown that 24-hour dietary recall data can provide unbiased estimates of community or group means [23]. Finally, the algorithm used to estimate the bioavailability of iron assumes that iron stores are depleted but that clinical signs of iron deficiency are absent [28]. This may lead to an under- or overestimation of iron absorption in individuals with poorer or better iron status, respectively.

Iron deficiency was found among Rendille children within a dietary context constrained by economic, cultural, and environmental factors that limit food availability. Meat and blood, which have high bioavailable heme iron, are consumed infrequently by children. The staple foods were mainly maize cooked as a porridge, *ugali* or *uji*, and tea with milk and sugar, which contain nonheme iron that is often poorly absorbed because it is accompanied by compounds that inhibit iron absorption. Although the overall dietary iron intake was very low in Korr (median, 6.5 mg), it was approaching recommended levels in Karare (median, 9.3 mg). However, bioavailable iron intakes were below metabolic requirements in both locations when enhancers and inhibitors of absorption were taken into account. The estimated bioavailable iron was 4.3% to 7.7% of the total iron intake after adjustment for both enhancers and inhibitors.

The diets were also found to be alarmingly low in vitamin A. Several studies have documented an association between retinol and biochemical indices of iron status [46, 47], and supplementation studies document that vitamin A enhances recovery from iron deficiency [47–52]. Additionally, Garcia-Casal et al. [38] have shown an enhancing effect of vitamin A and β-carotene on the absorption of nonheme iron from cereal-based diets. Consequently, the effect of low dietary vitamin A on iron status among northern Kenyan children merits further investigation.

One approach to preventing iron deficiency may be to improve bioavailability by increasing intakes of enhancers, such as meat, or—more likely because of economic constraints—vitamin A and ascorbic acid. Both experimental and population-based studies report substantial increases in iron bioavailability when ascorbic acid is added to a maize-based diet [41, 42]. Fresh fruits and vegetables rich in vitamin C and vitamin A are available at the Marsabit market, where many Karare women sell milk. Therefore, intervention efforts could promote modified food purchasing and consumption. Tatala and colleagues also recommend dietary modifications that alter traditional food-processing techniques, such as soaking, germinating, or lactic acid fermentation of cereals [43].

In the town of Korr, cultural beliefs regarding child feeding act as a further barrier to iron intake. Although the prevalence of iron deficiency, at 24%, was lower than in the town of Karare, the average daily iron intake was estimated at only 65% of the daily recommended allowance. Iron deficiency is therefore the outcome not merely of low bioavailability, but also of overall inadequate iron intake. A regression analysis of sociodemographic factors reveals a significant interaction between sex and economic status as a predictor of iron status. A bivariate analysis showed a similar

prevalence of iron deficiency among boys and girls in poor households. Girls in economically sufficient households were 2.4 times as likely to have iron deficiency as boys. Although poverty is a barrier to accessing iron-rich foods, cultural factors also influence the distribution of iron-rich foods along gender lines. Key iron-rich foods are classified as "hard foods" and are prescribed to be fed to boys, whereas "soft foods" such as *uji*, rice, and tea are believed to be beneficial for girls. Therefore, in households economically able to purchase foods high in bioavailable iron, these foods are often preferentially fed to boys.

Development efforts are currently aimed at overcoming economic barriers; marketing and income-generating projects, particularly channeled through women's organizations, are intended to increase the ability of women to purchase food and medicine [53]. It is believed that an outcome of improved economic conditions will be better health and nutrition [9]. Although improved energy intake is often correlated with reduced iron deficiency [5], cultural practices surrounding food distribution in Korr pose an additional barrier to improving the iron intake of girls. The findings of this research indicate that economic development may improve iron status in boys but is unlikely to benefit girls in the absence of dietary modification intervention. Gender-based food prescriptions defining "soft foods" as culturally acceptable for girls must be modified to include iron-rich foods, and such modification may provide a sustainable approach to controlling and preventing iron deficiency.

The finding of different constraints on dietary iron intake in two Rendille communities underscores the fact that local answers are needed to questions of the causes of vulnerability and acceptable avenues for dietary modification. Natural food-based interventions may be sustainable approaches to preventing iron deficiency, but for these approaches to be effective, it is necessary to identify cultural and environmental barriers to adequate iron intake.

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Prevalence of aflatoxins in blood and urine of Egyptian infants with protein–energy malnutrition

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Abstract

The aim of the present work was to study the presence of aflatoxins in blood and urine of infants with protein–energy malnutrition (PEM). The study was conducted on 60 infants, 30 with kwashiorkor and 30 with marasmus, with 10 age-matched healthy infants studied as a control group. Complete blood count, liver function tests, and determination of the level of aflatoxins (B_1, B_2, G_1, G_2, M_1, M_2, G_{2a}, B_3, GM_1, P, and aflatoxicol R_0) in blood and urine were carried out in all studied infants. Serum aflatoxins were detected in more infants with kwashiorkor (80%) than in those with marasmus (46.7%). The mean serum levels of total aflatoxins, AFB1, AFG1, and AFB2a, were significantly higher in infants with kwashiorkor ($p < .001$). Aflatoxin B_1 (AFB_1) was the most commonly detected type. The prevalence of aflatoxin excretion in the urine of infants with kwashiorkor was 80%, a higher value than that in infants with marasmus (46.7%). The mean urinary concentration of total aflatoxins followed the same pattern of distribution ($p < .052$). There were no significant differences between groups in the mean urinary concentrations of AFB_1, AFG_1, AFB_{2a}, AFM_1, and AFG_{2a}. Aflatoxins were not detected in any of the serum or urine samples of the control group. Aflatoxins are highly prevalent in this study population and show a high degree of correlation with severe PEM.

Key words: Aflatoxins, Aspergillus flavus, Aspergillus parasiticus, kwashiorkor, marasmus

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Introduction

Protein–energy malnutrition (PEM) is a range of pathological conditions arising from reduced protein and energy intake, occurring most frequently in infants and young children and commonly associated with infection [1].

There is a wide variation in the pattern of incidence of PEM in different countries. Over two-thirds (70%) of the world's malnourished children live in Asia (especially southern Asia), while 26% are found in Africa and 4% in Latin America [2]. Developing countries have optimal conditions for fungal growth and toxin production, with a mean temperature of about 28° to 38°C and a relative humidity of more than 70% [3–5].

The earlier concept that kwashiorkor was caused by severe protein deficiency in the presence of relative carbohydrate energy excess has been discredited [6].

Aflatoxins are a group of bis-furano-iso-coumarin secondary metabolites produced by some strains of *Aspergillus flavus* and most, if not all, strains of the *Aspergillus parasiticus* group of fungi [7, 8]. Four major types of aflatoxins have been known for a long time: B_1, B_2, G_1, and G_2, as well as a number of derivatives, e.g., M_1, M_2, and aflatoxicol. At least 17 compounds designated as aflatoxins have been discovered [9].

Exposure to aflatoxins occurs mostly by ingestion, but also by dermal and inhalation routes [10–12]. Aflatoxins may enter the food supply by direct contamination resulting from mold growth on food, or by indirect contamination through the use of contaminated ingredients in processed food or through use of animal products such as milk, milk products, eggs, or meat [13].

After ingestion, aflatoxins are concentrated in the liver. In the liver cells, aflatoxin B_1 (AFB_1) is converted by cytoplasmic reductase to form aflatoxicol (R_0) and by the microsomal mixed function oxidase system to form aflatoxins M_1, P_1, B_{2a}, Q_1, and $B_{1,2,3}$ epoxide. Aflatoxins are excreted either unchanged or as metabolites in urine, stool, bile, and breastmilk [14].

The biologic effects of aflatoxins in humans include

acute toxicity in the form of liver cell necrosis, nephritis, bleeding, and lung congestion [15–17]. Reye's syndrome has also been reported. In addition, chronic effects include cell damage, carcinogenicity, mutagenicity, and teratogenicity [7, 18].

The presence of aflatoxins in foods in Egypt has been reported. AFB levels in some popular foods have reached and in some cases exceeded the maximum permissible levels, according to Egyptian standardization of quality control [19].

The similar geographic and climatic predilections of kwashiorkor and aflatoxins and the remarkable similarities in the biochemical, metabolic, immunologic, and pathologic derangement observed in kwashiorkor and that recorded in controlled studies on animals exposed to aflatoxins [20] promoted exploration of possible associations between kwashiorkor and aflatoxins in young children.

The aim of the present work was to study the presence of aflatoxins in the blood and urine of infants with PEM, in particular those with kwashiorkor.

Subjects and methods

The study was conducted on 60 infants with PEM selected from children presenting at the outpatient clinic of Alexandria University Children's Hospital at El-Shatby. The subjects were 30 infants with kwashiorkor aged 7 to 20 months, including 19 boys (63.3%) and 11 girls (36.7%); 30 infants with marasmus aged 6 to 13 months, including 16 boys (53.3%) and 14 girls (46.7%); and a control group of 10 healthy infants of matched age and sex aged 6 to 24 months, including 6 boys and 4 girls. Patients with secondary malnutrition were excluded from the study.

Laboratory investigations performed on blood samples from all studied infants included complete blood count, plasma proteins, and liver function tests consisting of measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total serum bilirubin (TSB).

Determination of total and individual aflatoxins AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2, AFB_{2a}, AFG_{2a}, AFB_3, $AFGM_1$, and AFP, in addition to aflatoxicol R_0, the metabolite of AFB1 and AFB2), in blood and urine was performed [21]. Aflatoxin concentrations in serum and urine were determined by two-dimensional thin-layer chromatography after extraction of the toxins by the method described by Stubblefield and Shotwell [21].

The study and parental consent forms were approved by the ethical committee at the Faculty of Medicine, Alexandria University.

Results

All studied children with kwashiorkor and marasmus fulfilled the World Health Organization (WHO) criteria for diagnosis of PEM. According to the Wellcome classification [22], infants were diagnosed as having

TABLE 1. Age, sex, and anthropometric measurements of studied cases

Variable	Kwashiorkor ($n = 30$)	Marasmus ($n = 30$)	Control ($n = 10$)	F	p value
Age (mo)				11.895	< .001
Min–max	7–20	6–13	6–24		
Mean ± SD	11.90 ± 2.975[b,c]	9.80 ± 1.859[c]	15.90 ± 0.03		
Sex					
Males (%)	19 (63.3%)	16 (53.3%)	6 (60%)		
Females (%)	11 (36.7%)	14 (46.7%)	4 (40%)		
Weight (% of standard)[a]				300.55	< .001
Min–max	60–75	43–58	89–126		
Mean ± SD	66.20 ± 4.614[b,c]	50.56 ± 3.402[c]	101.39 ± 11.657		
Length (% of standard)[a]				210.2095	< .001
Min–max	58.5–87.9	51.6–64.1	88.2–101.5		
Mean ± SD	66.60 ± 4.614[b,c]	57.68 ± 2.591[c]	97.53 ± 4.168[b]		
Head circumference (% of standard)[a]					< .001
Min–max	88–100	89–100	95–101.6		
Mean ± SD	94.69 ± 2.598[c]	95.36 ± 2.198[c]	98.84 ± 1.611	12.2657	

a. % of standard at 50th percentile [1].
b. Significantly different from marasmus at $p \leq .05$.
c. Significantly different from control at $p \leq .05$.

kwashiorkor if the body weight was 60% to 80% of the standard taken as the 50th percentile of normal values for weight and edema was present. This corresponds to the "edematous malnutrition" described in the recent WHO classification [23]. Although infants were diagnosed as having marasmus if the body weight was less than 60% of the standard taken as the 50th percentile of normal values for weight and edema was absent, this corresponds to "severe wasting" in the recent classification [23]. The clinical data for the studied children are shown in **tables 1 and 2**.

Aflatoxins were not detected in any of the serum or urine samples from the control group. In infants with kwashiorkor, total and individual aflatoxins were detected at a significantly higher prevalence (80%) than in infants with marasmus (46.7%) ($\chi^2 = 7.177$, $p = .007$) (**fig. 1**).

The prevalence of aflatoxins was significantly higher in the urine of infants with kwashiorkor (80%) than in the urine of infants with marasmus (46.7%), ($\chi^2 = 7.177$, $p = .007$) (**fig. 2**).

The mean serum and urine concentrations of total and individual aflatoxins in infants with kwashiorkor and marasmus are shown in **tables 3 and 4**. The mean serum level of aflatoxins was significantly higher in boys than in girls ($t = 2.72$, $p = .019$) among infants with marasmus, but not among infants with kwashiorkor ($t = 0.78$, $p = 0.446$). However, there were no significant differences between the sexes in the mean urine levels of total aflatoxins in either group of subjects.

The children consumed various diets, which included breastmilk, formula, and other foods, such as rice water, cereals, and canned foods. Milk (breast or formula) was consumed from birth by all infants, and mixed foods were consumed from the age of six months until the time of the study. Diets were assessed by taking dietary histories from the mothers. There was no significant difference between the studied groups in the effect of the type of the diet consumed on the presence of aflatoxins in the serum and urine of the studied infants with malnutrition (**table 5**).

Diarrhea was present in 14 infants with kwashiorkor (46.6%) and in 11 infants with marasmus (36.6%). In both PEM groups, there were no significant differences between infants with and without diarrhea in the prevalence of aflatoxins in the serum or urine ($\chi^2 = 0.205$, $p = .651$).

The mean serum and urinary concentrations of total and individual aflatoxins were higher in infants with diarrhea than in those without diarrhea; however, this difference was statistically nonsignificant ($z = 1.359$; $p = 0.174$) and ($z = 1.425$, $p = .154$) respectively.

Chest infection (bronchopneumonia) was present in six infants with kwashiorkor (20%) and eight infants with marasmus (26%). However, there was no statisti-

TABLE 2. Clinical findings from infants with kwashiorkor and marasmus

Variable	Kwashiorkor ($n = 30$)	Marasmus ($n = 30$)
Delayed milestones	24 (80%)	24 (80%)
Anorexia	15 (50%)	19 (63.33%)
Edema	30 (100%)	0
Skin changes	4 (13.33%)	0
Hair changes	18 (60%)	0
Muscle wasting	30 (100%)	30 (100%)
Loss of subcutaneous fat	0	30 (100%)
Diarrhea	14 (46.6%)	11 (36.6%)
Chest infection	6 (20%)	8 (26.7%)
Hepatomegaly	10 (33.33%)	0

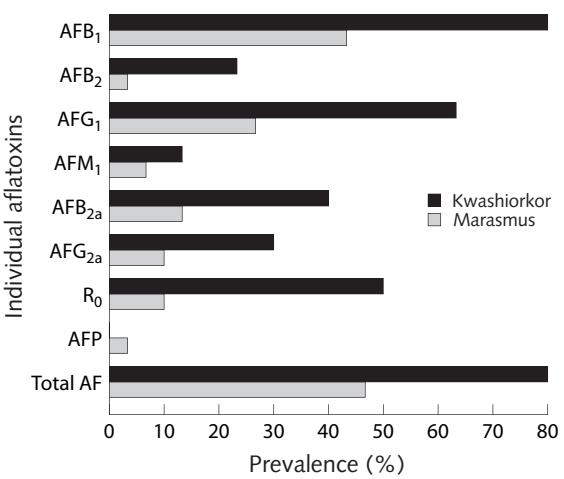

FIG. 1. Prevalence of individual aflatoxins in the serum of infants with kwashiorkor and marasmus

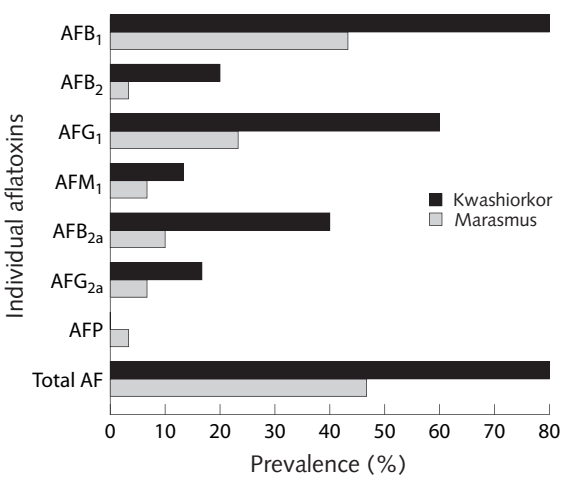

FIG. 2. Prevalence of individual aflatoxins in the urine of infants with kwashiorkor and marasmus

TABLE 3. Mean serum concentration (ng/100 ml) of individual aflatoxins in infants with kwashiorkor and marasmus

	Kwashiorkor (n = 30)				Marasmus (n = 30)					
Aflatoxin[a]	No.	Min–max	Mean	SD	No.	Min–max	Mean	SD	Z	p value
AFB_1	24	4–69	32.38	13.39	13	10–18	13.62	2.75	4.203	< .001*
AFB_2	7	4–16	12.00	4.12	1	5	5.00	0	1.104	.269
AFG_1	19	2–38	21.50	9.29	8	1–12	7.75	3.11	3.325	.001*
AFG_2	—	—	—	—	—	—	—	—	—	—
AFM_1	4	2–12	8.25	4.35	2	10–15	12.50	3.54	1.174	.240
AFM_2	—	—	—	—	—	—	—	—	—	—
AFB_{2a}	12	4–35	15.58	9.07	4	3–9	6.00	2.58	2.127	.033*
AFG_{2a}	9	2–17	8.22	5.36	3	2–28	11.33	14.47	0.187	.852
R_0	15	2–20	9.27	5.64	3	2–14	6.33	6.66	0.893	.372
AFB_3	—	—	—	—	—	—	—	—	—	—
$AFGM_1$	—	—	—	—	—	—	—	—	—	—
AFP	—	—	—	—	1	7	7.00	0	—	—
Total	24	37–111	70.58	20.89	14	15–38	25.21	6.60	5.040	< .001*

a. More than one aflatoxin may be present in the same patient.
*Statistically significant ($p \leq .05$).

TABLE 4. Mean urinary concentration (ng/100 ml) of individual aflatoxins in infants with kwashiorkor and marasmus

	Kwashiorkor (n = 30)				Marasmus (n = 30)					
Aflatoxin[a]	No.	Min–max	Mean	SD	No.	Min–max	Mean	SD	Z	p value
AFB_1	24	1–15	8.29	3.98	13	5–9	6.92	1.32	1.635	.102
AFB_2	6	2–4	2.67	1.03	1	2	2.00	0.00	0.633	.527
AFG_1	18	1–11	4.78	2.69	7	2–8	3.57	2.07	1.103	.270
AFG_2	—	—	—	—	—	—	—	—	—	—
AFM_1	4	1–3	2.25	0.96	2	4–7	5.50	2.12	1.879	.060
AFM_2	—	—	—	—	—	—	—	—	—	—
AFB_{2a}	12	1–9	3.58	2.68	3	2–4	3.00	1.00	0.222	.825
AFG_{2a}	5	1–3	1.60	0.89	2	1–16	8.50	10.61	0.641	.522
R_0	—	—	—	—	—	—	—	—	—	—
AFB_3	—	—	—	—	—	—	—	—	—	—
$AFGM_1$	—	—	—	—	—	—	—	—	—	—
AFP	—	—	—	—	1	2	2.00	0.00	—	—
Total	24	5.0–28	14.96	6.24	14	7.0–20	11.14	3.66	1.946	.052

a. More than one aflatoxin may be excreted by the same patient.
*Statistically significant ($p \leq .05$).

TABLE 5. Relation of aflatoxins to dietary patterns of infants with kwashiorkor and marasmus (n = 38)

Variable	Group I[a]	Group II[b]	T	p
Aflatoxins in blood				
No. of aflatoxin positive infants	16	22		
Min–max concentrations (ng/100 ml)	17–111	15–111		
Mean concentration (ng/100 ml)	51.44	55.63	0.448	.657
SD (ng/100 ml)	29.257	27.933		
Aflatoxins in urine				
No. of aflatoxin positive infants.	16	22		
Min–max concentrations (ng/100 ml)	6–28	5–28		
Mean concentration (ng/100 ml)	13.44	13.64	0.105	.917
SD (ng/100 ml)	6.033	5.643		

a. Infants receiving breastmilk or formula only.
b. Infants receiving a mixed diet.

TABLE 6. Liver function test results

Variable	Kwashiorkor ($n = 30$)	Marasmus ($n = 30$)	Control ($n = 30$)	F	p value
AST (IU)					
Min–max	15–222	10–40	15–23	10.32	
Mean ±SD	62.90 ± 52.318[a,b]	26.73 ± 6.539[a]	20.10 ± 2.234	3	< .001*
ALT (IU)					
Min–max	20–215	15–42[a]	20–25	12.72	
Mean ± SD	75.53 ± 59.204[a,b]	30.20 ± 5.365	22.10 ± 1.663	6	< .001*
TSB (mg/dl)					
Min–max	0.8–2.0	0.7–1.0	0.7–0.9	16.43	
Mean ± SD	1.03 ± 0.235[a,b]	0.81 ± 0.052[a]	0.80 ± 0.067	8	< .001*

AST, Aspartate transaminase; ALT, alanine transaminase; TSB, total serum bilirubin.
*Statistically significant difference among the three groups ($p \leq .05$).
a. Significantly different from marasmus ($p \leq .05$).
b. Significant different from control ($p \leq .05$).

cally significant difference between infected and noninfected infants regarding the prevalence ($\chi^2 = 0.301$; $p = .583$), the mean serum ($\chi^2 = 0.018$; p = .986), and urinary ($\chi^2 = 0.198$; p = 0.843) concentrations of total and individual aflatoxins.

Ten (33.3%) of the infants with kwashiorkor had hepatomegaly. However, there were no significant differences in the prevalence of aflatoxins in the serum and urine of hepatomegalic and nonhepatomegalic infants with kwashiorkor ($\chi^2 = 0.230$; $p = .623$).

The mean serum concentration of total aflatoxins was higher in infants with hepatomegaly (66.14 ± 23.97 ng/dl) than in those without hepatomegaly (51.10 ± 28.32 ng/dl). This difference was statistically nonsignificant (z = 1.676; $p = .0937$).

The mean urinary concentration of total aflatoxins was slightly higher in infants with hepatomegaly (14.57 ± 6.65 ng/dl) than in those without hepatomegaly (13.32 ± 5.55 ng/dl). This difference was statistically nonsignificant (z = 0.624; $p = .532$).

Only aflatoxin AFB_2, with its mean urinary value of 4.0 ± 0.00 ng/100 ml, was significantly higher in hepatomegalic malnourished infants than in nonhepatomegalic infants (2.0 ± 0.00 ng/100 ml; z = 2.450; $p = .014$).

All liver function test results were significantly higher in infants with kwashiorkor than in the marasmus and control groups (**table 6**).

No significant correlation was found between the total levels of aflatoxins and the studied liver function tests in either group of infants with PEM (**table 7**).

Discussion

During the past 10 years or more, controlled investigations have been undertaken on children with PEM and normally nourished controls to detect evidence of exposure to aflatoxins, including their potential role in the etiology of kwashiorkor [24].

In the present study, aflatoxins were more prevalent and had significantly higher mean serum concentrations in kwashiorkor patients than in marasmic patients; no aflatoxins were detected in the control group. On the other hand, Household and Hundt in South Africa did not isolate aflatoxins from the serum of any of their patients. They concluded that aflatoxins do not play a primary role in the pathogenesis of kwashiorkor [14].

However, our study confirmed most of the early reports. Coulter et al. detected aflatoxins in the serum samples of 37.5% of Sudanese kwashiorkor patients and in no marasmic patients, but they did not study controls [25]. Hendrickse detected aflatoxins in 36.4% of the serum samples of kwashiorkor patients and in only 19.3% of the serum samples of marasmic patients, and unlike the results of our study, he detected aflatoxins in the serum of 15.9% of the control patients [24]. Therefore, our data confirm Hendrickse's postulate that the toxic effects of aflatoxins are directed primarily towards the liver and can account for many of the clinical features of kwashiorkor [24]. Furthermore, Adhikari et al. in South Africa detected aflatoxin in the

TABLE 7. Correlation between the total level of aflatoxins and liver function tests

	Kwashiorkor ($n = 30$)		Marasmus ($n = 30$)	
Variable	Blood	Urine	Blood	Urine
ALT	0.0579 $p = .788$	0.0609 $p = .777$	–0.1881 $p = .520$	0.0020 $p = .995$
AST	0.0819 $p = .704$	0.1192 $p = .579$	–0.3931 $p = .164$	–0.23369 $p = .415$
TSB	–0.0737 $p = .732$	0.1506 $p = .483$	–0.0504 $p = .864$	–0.0745 $p = .800$

AST, Aspartate transaminase; ALT, alanine transaminase; TSB, total serum bilirubin.

serum of 58% of his studied kwashiorkor patients, and they did not examine other nutritional groups [26]. The same observation was true for individual aflatoxins. AFB_1 and AFG_1 were the most common types detected in the serum, with prevalences as well as mean serum concentrations significantly higher in kwashiorkor than in marasmic infants. However, the mean concentration of aflatoxicol R_0 was not significantly different in the two groups of infants. In similar observations, Ramjee et al. found in 1992 that the serum concentrations of aflatoxins AFB_1 and AFM_1 were considerably higher in the kwashiorkor group than in the marasmus group, but there was no statistically significant difference between the groups [9]. Moreover, Hendrickse found that the mean serum concentrations of individual aflatoxins AFB_1, AFB_2, AFG_1, AFG_2, AFM_1, and AFM_2 were highest in the kwashiorkor group and lowest in the control group [24].

There are no reports in the available literature of measurements of the recently discovered types of aflatoxin (AFB_{2a}, AFG_{2a}, AFB_3, AFM_1, and AFP) in patients with malnutrition. However, in the present study, AFB_{2a} and AFG_{2a} were detected in both groups of children with PEM, with a higher mean serum concentration in the kwashiorkor group. Furthermore, in the present work, aflatoxicol, a metabolite of AFB_1 and AFB_2, was detected in significantly more kwashiorkor patients than marasmic patients. In an attempt to explain such results, Hendrickse stated that detection of aflatoxicol in children with kwashiorkor but not in the control or marasmic children indicates some fundamental differences in the metabolism of aflatoxins in the two categories of PEM [24], or that their levels in kwashiorkor build up because of the inability of malnourished liver to metabolize them.

In the current study, excreted aflatoxins were detected at statistically significant levels in the urine of most of the infants with kwashiorkor (80%), at less than significant levels in infants with marasmus (46.7%), and in none of the control group infants.

Although Hendrickse screened 250 urine specimens for aflatoxins and detected aflatoxins in the urine of control subjects (19.8%), he reported the same pattern of a significant difference between children with kwashiorkor and those with marasmus (33.3% vs. 25.7%, respectively)[24]. Similar results were reported by Ramjee et al., who detected aflatoxins in 16% of urine samples of South African infants with kwashiorkor, 10% of those with marasmus, and 25% of the control group. However, these differences were not statistically significant [9].

The study by de Vries et al. detected aflatoxins in the urine samples from patients of all nutritional groups (i.e., infants with marasmus or kwashiorkor, and controls) [27]. On the other hand, Househam and Hundt did not isolate aflatoxins from the urine of any nutritional groups [14]. These findings suggest either that children with kwashiorkor have a greater exposure to aflatoxins, or that their ability to transport and excrete aflatoxins is impaired.

On the other hand, de Vries et al. isolated aflatoxins in higher concentrations in marasmic patients than in those with kwashiorkor and controls. Similar results were obtained by Hendrickse et al., who thought that excretion of aflatoxins in patients with kwashiorkor is impaired by metabolic derangement [24, 27], whereas de Vries et al. concluded that aflatoxins accumulate in the body fluids and tissues in kwashiorkor patients, with slow elimination [27].

The discrepancies between the mean urinary concentrations of total aflatoxins in the present study and those found in other studies could be explained by differences in the numbers of cases studied and/or hepatic affection or derangement in the aflatoxin metabolism.

Aflatoxicol R_0, the end metabolite of aflatoxins in microsomes, was measured in studied cases, and it was not detected in the urine of any of the examined groups. This result was similar to those obtained by many authors [24, 27].

In this study, AFB_1 and AFG_1 were the most common types of aflatoxins detected. AFP was isolated from the urine specimen of only one subject with marasmus and was not isolated from any subject with kwashiorkor.

Ramjee et al. found that urinary levels of aflatoxins were highest in the control group, less in patients with kwashiorkor, and lowest in those with marasmus. AFB_1 was detected in the urine of 54.2% of the control group, 28.8% of those with kwashiorkor, and in none of those with marasmus. AFB_2, AFG_1, and AFG_2 were also not detected in any urine samples from patients with marasmus [9].

In the present study, the serum level of aflatoxins in infants with marasmus was significantly higher in boys than in girls; however, this is not the case in infants with kwashiorkor. No significant difference between boys and girls was found in the urinary levels of aflatoxins in infants with marasmus or in those with kwashiorkor. However, Hendrickse stated that aflatoxins were detected more frequently in the serum of boys than in girls among both kwashiorkor and marasmic patients, while urinary aflatoxins were higher in girls than in boys [24]. Furthermore, Jonsyn-Ellis found a significantly higher level of AFB_1 in healthy boys than in girls [28]. The effect of sex was also reported by other authors, for example, in Sierra Leone [28].

Foods were blamed by many authors as a source of aflatoxins that may cause malnutrition [19, 24, 29]. In this work, the studied groups were receiving foods other than breastmilk or formula milk. The relation between aflatoxins and diet pattern was statistically nonsignificant for both the mean serum and the mean urinary levels of aflatoxins. Both human milk and cow's milk can contain varying quantities of afla-

toxins, depending on the degree of fungal infection and the amounts of such foods consumed [25, 30]. Analyses of breastmilk in Sudan, Ghana, Nigeria, and Kenya have found aflatoxins in 30% of samples; these are usually small amounts of relatively nontoxic AFM_1, but occasionally there are large amounts of the very toxic parent compound AFB_1 [20, 26]. This finding may explain the occurrence of kwashiorkor in some breastfed babies [24].

Hendrickse concluded that foods in tropical developing countries are often contaminated by mycotoxins, either continually or seasonally. Warmth and humidity provide optimum conditions for the growth of many molds. The author also stated that even human breastmilk may contain aflatoxins [20]. Several authors have suggested that foods bought in markets or stored at home frequently contain aflatoxins [31, 32]. Neel et al. in Cairo, Egypt, isolated aflatoxins from many foods, including cow and buffalo milk, canned juices, some jams, and other foods consumed by children [19].

It is believed that infections such as gastroenteritis, chest infections, and others are commonly associated with malnutrition. Although aflatoxins have inhibitory effects on the immune system of humans, the present study found no significant relation between aflatoxins and diarrhea or chest infections. This may be due to the small number of studied cases.

Oyelami et al. examined autopsy lung specimens from 20 children with kwashiorkor for the presence of aflatoxins. He detected aflatoxins in 18 children who died from kwashiorkor. Of the 5 children who died with pneumonia, all had detectable levels of aflatoxins in their lungs. He concluded that Nigerian children are exposed to aflatoxins and that high levels can accumulate in lung tissue [33].

Adhikari et al. found that in kwashiorkor patients, the aflatoxin-positive group had an increased number of infections as a result of metabolic hazards of aflatoxins [26].

In the present study, higher prevalences as well as higher mean serum and urinary concentrations of aflatoxins were detected in hepatomegalic malnourished infants than in nonhepatomegalic infants, but these differences were statistically nonsignificant. We found that only the urinary concentration of AFB_2 was significantly higher in hepatomegalic than in nonhepatomegalic infants. In a study of autopsied livers from children with PEM, aflatoxins were detected in those with kwashiorkor and marasmic kwashiorkor, but not in those with marasmus. The aflatoxins detected were AFB_1 and aflatoxicol, apart from a small concentration of AFM_1 detected in one case [25]. Hendrickse suggested that the hepatotoxicity of aflatoxins results from the formation of epoxides of AFB_1 when the normal enzymatic capacity of the liver to metabolize and excrete aflatoxins is exceeded. Aflatoxin epoxides are thought to initiate the biologic and metabolic derangement attributable to aflatoxins by binding nucleic acids [24]. Ramjee et al. stated that it is unlikely that the basic cause of kwashiorkor is a function of aflatoxin consumption. However, impairment of hepatic detoxification of aflatoxin seems to be a distinct feature of kwashiorkor [9].

In the present study, all liver function test results were significantly higher in infants with kwashiorkor than in the marasmus and control groups. Similar results were reported by Etukudo et al. [34] and Guler et al. [35]. Moreover, Akinyinka et al. evaluated prothrombin time, serum albumin, aminotransferases, and liver size in 40 patients with kwashiorkor and compared these parameters in patients who died and survivors. The results indicated a predictive mortality value of prothrombin time in kwashiorkor [36]. However, in the present study, there was no significant correlation between aflatoxins and liver function test results. In general, the liver in kwashiorkor seems to suffer little pathological damage and responds to dietary therapy with a rapid return to normal appearance and function [3]. On the other hand, aflatoxin ingestion is invariably associated with severe necrotic and sometimes progressive cirrhotic lesions, which respond to treatment very slowly if at all. Whether aflatoxins are the initial cause of impaired liver function remains unclear, but there is support for this theory in the remarkable similarities in biochemical derangement in aflatoxin poisoning and kwashiorkor [20].

Thus, we can conclude that aflatoxins are closely associated with PEM, especially kwashiorkor. Whether this association is a cause or result remains unclear and needs further study.

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Special Section

Recent trends in malnutrition in developing regions: Vitamin A deficiency, anemia, iodine deficiency, and child underweight

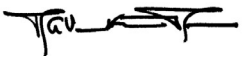
John Mason, Jonathan Rivers, and Carol Helwig, guest editors

The research reported here, commissioned by the Micronutrient Initiative, was carried out in the Department of International Health and Development at the School of Public Health and Tropical Medicine, Tulane University. Its aim is to describe progress made in controlling micronutrient deficiencies, and to provide a benchmark on current prevalences of vitamin A deficiency, anemia, iodine-deficiency disorders, and child underweight as a measure of general malnutrition. The results and extensive supporting database—which can be made available to researchers for further analysis—provided the basic information for the recent publications of the Micronutrient Initiative, with UNICEF, of Vitamin and Mineral Deficiency: A Global Progress Report [1], and for national damage assessment reports for some 80 countries. This special section of the *Food and Nutrition Bulletin* gives full details of data sources, analytical methods, and results in terms of prevalences and trends by regional groupings, and "best guess" estimates for individual countries for 2000.

The results published here, and in the Vitamin and Mineral Deficiency Global Progress Report, can be used for tracking progress toward controlling these deficiencies over the coming years. They should thus contribute to monitoring progress toward the Millennium Development Goals and the specific micronutrient goals set by the 2002 UN General Assembly Special Session on Children.

In the Vitamin and Mineral Deficiency Global Progress Report and the 80 national reports, the Micronutrient Initiative and UNICEF highlight the disadvantages brought by deficiencies, ranging from lowering the intellectual capacity of people in many countries by an estimated 10 to 15 IQ points; impairing mental development in 40% to 60% of young children through iron deficiency; lowering productivity by an estimated two percentage points of gross domestic product through anemia; compromising immune systems through vitamin A deficiency; and contributing to increased infant and child mortality. These reports also outline the broad strategies that can be used for addressing vitamin and mineral deficiencies, including fortification, supplementation, education, and disease control, and some of the challenges that lie ahead.

Controlling vitamin and mineral deficiencies is considered one of the best and most cost-effective strategies for improving human welfare. By supporting this research and its publication, we aim to provide some of the knowledge needed to facilitate global action to control vitamin and mineral deficiencies.



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