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

Rafael Monge-Rojas, Mauro Barrantes, Ileana Holst, Hilda Nuñez-Rivas, Thelma Alfaro, Sara Rodríguez, Lowella Cunningham, Priscilla Cambronero, Lisbeth Salazar, and F.H. Herrmann

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

Rafael Monge-Rojas, Hilda Nuñez-Rivas, Thelma Alfaro, Sara Rodríguez, and Lowella Cunningham are affiliated with the Costa Rican Institute for Research and Education on Nutrition and Health. Mauro Barrantes is affiliated with the Health Office, University of Costa Rica. Ileana Holst and Priscilla Cambronero are affiliated with the Faculty of Microbiology, University of Costa Rica. Lisbeth Salazar is affiliated with the Unit of Hemostasis and Thrombosis, CIHATA, University of Costa Rica. F.H. Herrmann is affiliated with the Institute of Human Genetics, Ernst-Moritz-Arndt-University, Greifswald, Germany.

Please direct queries to the corresponding author: Ileana Holst, Faculty of Microbiology, University of Costa Rica, Postal Code 2060, San Pedro Montes de Oca, San José, Costa Rica; e-mail: iholst@cariari.ucr.ac.cr

References

1. Scrimshaw NS, Morales J, Salazar BA, Loomis CP. Health aspects of the community development project, rural area, Turrialba, Costa Rica, 1948–51. Am J Trop Med Hyg 1953;2(4):583–92.
2. Scrimshaw NS, Tejada C. Pathology of living Indians as seen in Guatemala. In: Stewart TD, ed. Handbook of Middle American Indians. Vol 9. Austin, Tex, USA: University of Texas Press, 1970:203–25.
3. Muñoz C, Scrimshaw NS, eds. The nutrition and health transition of democratic Costa Rica. Boston, Mass, USA: International Foundation for Developing Countries, 1995. Alma-Ata 1978: Primary health care. (Health for All Series No. 1). Geneva: World Health Organization, 1978.

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].

Acknowledgments

The authors are grateful to Minor Soto, Gerardo Jiménez, Rafael Contreras, Raquel Castillo, and Laura Gómez for their help in data collection. We also thank Rosario Cordero, José Miguel González from Pineapple Development Corporation (PINDECO) and the Regional Indigenous Association of the Dikes (ARADIKES) for their help in introducing the researchers to the Indian reservation. This work was supported by the Office of the Vice-Chancellor of Research of the University of Costa Rica (Project No. 807-A2-309) and by the Costa Rican Institute for Research and Education on Nutrition and Health (INCIENSA).

References

1. Mata L, Salas P, Ulate E, Barrantes R. Estado nutricional de Amerindios en Costa Rica 1979-1984. Rev Med Hosp Nal Niños CR 1985; 20:211–32.
2. Pan American Health Organization. Health in the Americas. Vol II. Washington, DC: PAHO, 2002.
3. Instituto Nacional de Estadística y Censos (INEC). IX Censo Nacional de Población: Características Sociales y Demográficas. San José, Costa Rica: INEC, 2002.
4. Kuhnlein HV. Benefits and risks of traditional food for indigenous peoples: focus on dietary intake of Arctic men. Can J Physiol Pharmacol 1995;73:765–71.
5. Gracey M. Historical, cultural, political and social influences on dietary patterns and nutrition in Australian aboriginal children. Am J Clin Nutr 2000; 72: 1361S–1367S.
6. Moffatt ME. Current status of nutritional deficiencies in Canadian aboriginal people. Can J Physiol Pharmacol 1995; 73:745–8.
7. Diez-Ewald M, Torres-Guerra E, Leets I, Layrisee M, Vizcaino G, Artega M. Anemia en población indígena del occidente de Venezuela. Invest Clin 1999;40:191–202.
8. Daniel M, Gamble D. Diabetes and Canada's aboriginal peoples: the need for primary prevention. Int J Nurs Stud 1995;32:243–59.
9. Alpert JS, Goldberg R, Ockene IS, Taylor P. Heart disease in native Americans. Cardiology 1991;78:3–12.
10. WHO. Physical status: the use and interpretation of anthropometry. WHO Technical Report Series No. 854. Geneva: World Health Organization, 1995.
11. Chinnock A, Sedó-Masís P. Porciones de alimentos y preparaciones comunes en Costa Rica y equivalencias del sistema de listas de intercambio. San José, Costa Rica: Escuela de Nutrición Humana, Universidad de Costa Rica, 2000.
12. Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, Erdman JW Jr, Kris-Etherton P, Goldberg IJ, Kotchen TA, Lichtenstein AH, Mitch WE, Mullis R, Robinson K, Wylie-Rosett J, St Jeor S, Suttie J, Tribble DL, Bazzarre TL. AHA Dietary guidelines. Revision 2000. A statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102:2284–99.
13. Castillo R, Gómez L. Evaluación del consumo de alimentos de adolescentes Kabekwas de la reserva de Ujarrás. Thesis, Escuela de Nutrición, Universidad de San José, 2000.
14. Institute of Medicine. Dietary Reference Intakes: Applications in Dietary Assessment. National Academy Press: Washington DC, USA, 2000.
15. Connor SL, Gustafson JR,Artaud-Wilds SM, Classick-Kohn CJ, Connor WE. The cholesterol-saturated index for coronary prevention: Background, use, and a comprehensive table of foods. J Am Diet Assoc 1989;86: 807–16.
16. Hampl JS, Betts NM, Benes BA. The "age + 5" rule: comparisons of dietary fiber intake among 4- to 10-year-old children. J Am Diet Assoc 1998;98:1418–23.
17. National Committee for Clinical Laboratory Standards (NCCLS). Procedures for the collection of diagnostic blood specimens by venipuncture; approved Standard, 5th ed. Wayne, Pa, USA: NCCLS, 1991.
18. Dary O, Arroyave G. Determinación del retinol

sanguíneo por cromatografía líquida de alta presión (HPLC). In: Manual para la fortificación de azúcar con vitamina A. Parte 3. Metodologías analíticas para el control y la evaluación de la fortificación de azúcar con vitamina A, 2nd ed. Guatemala City: Institute of Nutrition of Central American and Panama (INCAP) and United States Agency for International Development (USAID)1996:39–47.
19. Landers GM, Olson JA. Absence of isomerization of retinyl palmitate, retinol, and retinal in chlorinated and nonchlorinated solvents under gold light. J Assoc Off Anal Chem 1986;69:50–5.
20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
21. National Cholesterol Education Program (NCEP). Highlights of the report of the expert panel on blood cholesterol levels in children and adolescents. Pediatrics 1992;89:495–501.
22. Beiri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. Am J Clin Nutr 1979;32:2143–9.
23. Horwitt MK, Harvey CC, Dahm CH Jr, Searcy MT. Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. Ann NY Acad Sci 1972;203:203–36
24. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx analyzer. Clin Chem 1995;41:991–4.
25. Sáenz G, ed. Hematología analítica: teoría, técnicas, interpretaciones, 3rd ed. San José, Costa Rica: Editorial Nacional de Salud y Seguridad Social-Caja Costarricense del Seguro Social (EDNASSS-CCSS), 1995.
26. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
27. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Mathews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–3.
28. Lynch MJ, Raphael SS, Mellor LD, Spare PD, Inwood MJH. Métodos de laboratorio, 2nd ed. Mexico City: Editorial Interamericana, 1972.
29. Nicklas TA, Webber LS, Koschak M, Berenson GS. Nutrient adequacy of low fat intakes for children: the Bogalusa Heart Study. Pediatrics 1992;89:221–8.
30. Willett WC. Nutritional epidemiology, 2nd ed. New York: Oxford University Press, 1998.
31. Bozzolli de Wille ME. Indígenas Actuales en Costa Rica. San José, Costa Rica: Editorial EDUCA, 1987.
32. Majia LA, Hodges RE, Arroyave G, Viteri F, Torun B. Vitamin A deficiency and anemia in Central American children. Am J Clin Nutr 1977;30:1175–84.
33. Muller R, Baker J. Medical parasitology. Philadelphia, Pa, USA: Lippincott Williams and Wilkins, 1998.
34. Herbert V. Biology of disease. Megaloblastic anemias. Lab Invest 1985;52:3–19.
35. Knittingen EA, Spangen S, Lindemans J, Fowler B. Methionine synthase deficiency without megaloblastic anemia. Eur J Pediatr 1997;156:925–30.
36. Herrmann FH, Salazar-Sánchez L, Schröder W, Grimm R, Schuster G, Jimenez-Arce G, Cháves M, Sing J. Prevalence of molecular risk factors FV Leiden, FV HR2, FII 20210G→A and MTHFR 677C→T in different populations and ethnic groups of Germany, Costa Rica and India. IJHG 2001;1:33–9.
37. Arruda VR, Siqueira LH, Goncalves MS, von Zuben PM, Soares MC, Menezes R, Annichino-Bizzacchi JM, Costa FF. Prevalence of the mutation C677→T in the methylene tetrahydrofolate reductase gene among distinct ethnic groups in Brazil. Am J Med Genet 1998;78:332–5.
38. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–57.
39. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. Annu Rev Med 1998;49:31–62.
40. Shaw JT, McWhinney B, Tate JR, Kesting JB, Marczak M, Purdie D, Gibbs H, Cameron DP, Hickman PE. Plasma homocysteine levels in indigenous Australians. Med J Aust 1999;170:19–22.
41. Mudd SH, Levy HL, Skovby F. Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. The metabolic and molecular bases of inherited disease, 7th ed. Vol 1. New York: McGraw-Hill, 1995:1279–327.
42. Kang SS, Wong PW, Norusis M. Homocysteinemia due to folate deficiency. Metabolism 1987;36:458–62.
43. Webber LS, Srinivasan SR, Wattigney WA, Berenson GS. Tracking of serum lipids and lipoproteins from childhood to adulthood: the Bogalusa Heart Study. Am J Epidemiol 1991;133:884–99.
44. Howard BV, Davis MP, Pettitt DJ, Knowler WC, Bennett PH. Plasma and lipoprotein cholesterol and triglyceride concentrations in the Pima Indians: distributions differing from those of Caucasians. Circulation 1983;68:714–24.
45. Connor WE, Cerqueira MT, Connor RW, Wallace RB, Malinow FR, Casdorph HR. The plasma lipids, lipoproteins and diet of the Tarahumara Indians of Mexico. Am J Clin Nutr 1978;31:1131–42.
46. Sacks FM, Katan M. Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease. Am J Med 2002;113:13S–24S.
47. Tso TK, Park S, Tsai YH, Williams G, Snook JT. Effect of apolipoprotein E polymorphism on serum lipoprotein response to saturated fatty acids. Lipids 1998;33:139–48.
48. Berenson GS, Srinivasan S, Webber LS, Nicklas TA, Hunter SM, Harsha DW, Johnson C, Arbeit M, Dalferes E, Wattigney W, Lawrence M. Cardiovascular risk in early life: the Bogalusa Heart Study. Kalamazoo, Mich., USA: The Upjohn Company, 1991.
49. Blackwell N, Hayllar J. Aboriginal health. Lancet 1998;351:1363.
50. World Health Organization. Toronto declaration on equity in health. Pan Am J Public Health 2002;12:465–7.