Concentrations of riboflavin and related organic acids in children with protein-energy malnutrition[1–4]

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ABSTRACT

Background: Riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) concentrations have been little studied in cases of malnutrition.

Objective: Our objective was to investigate the effects of malnutrition on riboflavin status and riboflavin's relation with thyroid hormones and concentrations of urinary organic acids.

Design: Malnourished children from the savannah in Benin (group S, n = 30) and the coast in Togo (group C, n = 30), as well as 24 control subjects from both regions, were studied. Blood riboflavin, FMN, and FAD were analyzed by HPLC; urinary organic acids were analyzed by gas chromatography–mass spectrometry.

Results: Children in group S were more severely malnourished than children in group C. Triiodothyronine concentrations were lower in group S than in group C or the control group (1.12 ± 0.24 compared with 1.74 ± 0.18 and 2.92 ± 0.19 nmol/L, respectively; P < 0.0001). Plasma riboflavin concentrations in group S were higher than those in group C or the control group (66.90 ± 12.75 compared with 28.09 ± 9.12 and 20.08 ± 3.03 nmol/L, respectively; P < 0.001). Plasma FAD concentrations in group S were lower than those in group C or the control group (31.57 ± 10.19 compared with 59.02 ± 5.60 and 65.35 ± 5.23 nmol/L, respectively; P < 0.0001). Dicarboxylic aciduria was higher in group C than in group S or the control subjects.

Conclusions: Children in group S had low triiodothyronine concentrations and low conversion of plasma riboflavin into its cofactors, leading to a plasma FAD deficiency. Plasma FAD was not correlated with urinary dicarboxylic acid concentrations. *Am J Clin Nutr* 2000;71:978–86.

KEY WORDS Malnutrition, thyroid hormones, riboflavin, dicarboxylic acids, Benin, Togo, FAD, flavin adenine dinucleotide, FMN, flavin mononucleotide, organic aciduria, children

INTRODUCTION

Child malnutrition remains a major public health problem in underdeveloped countries. Vitamin deficiencies thought to result from protein-energy malnutrition (PEM) have been identified for vitamins A, C, D, E, thiamine, and biotin (1–6). In contrast, vitamin B-12 and folate concentrations have been found to be normal or even elevated in PEM (7–9). Riboflavin concentrations in children with PEM, however, have been poorly studied to date. Riboflavin is the precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are cofactors in intermediary metabolism. The conversion of riboflavin into FMN requires riboflavin kinase (EC 2.7.1.26), the activity of which is enhanced by triiodothyronine (T_3) (10–13). Previous studies showed that thyroid hormone concentrations are affected in PEM (14, 15) and that riboflavin deficiency is responsible for a specific organic aciduria (16, 17). The aim of the present work was to study the influence of PEM and thyroid hormone concentrations on riboflavin metabolism and concentrations of organic acid excreted in the urine.

SUBJECTS AND METHODS

Subjects

Sixty malnourished children from 2 different geographic regions in western Africa were examined in the present study. Thirty children came from the savannah region (group S) in the center of Benin and 30 children came from the gulf coast of Benin in the south of Togo (group C). The diagnosis of malnutrition type (kwashiorkor or marasmus) was made by using the Wellcome classification (18); additional criteria for the diagnosis of kwashiorkor were the existence of edema, thin and discol-

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ored hair, skin lesions, and low weight-for-height. Eighteen children with kwashiorkor and 12 with marasmus were identified in group S and 6 with kwashiorkor and 24 with marasmus were identified in group C. Diagnostic symptoms at hospitalization were fever, diarrhea, failure to thrive, vomiting, or infection. Parental, informed consent was obtained before the study. The social, clinical, and nutritional history of the child was noted, as were anthropometric data, which included weight, height, and arm and head circumferences. We calculated body mass index (BMI; in kg/m2) (19) and the ratio of arm to head circumference (AC:HC) (20). Weight and height data were compared with standards from the US National Center for Health Statistics (21). Overnight fasting blood and urine samples were collected the morning after admission of patients, just before commencement of the treatment program.

The control data provided in this study were obtained from 23 healthy, age-matched children from the same savannah (n = 15) and coastal (n = 9) regions. The study was conducted in accordance with guidelines of the Declaration of Helsinki in 1989.

Dietary investigation

The qualitative composition of the diet was determined for both malnourished groups. Breast milk was the principle food for children <24 mo of age, followed by porridges of rice, millet, and maize. After 24 mo of age, the diet was composed of rice or maize pudding, vegetables, and, occasionally, powdered cow milk. Riboflavin concentrations in maize (0.11–0.17 mg/100 g), millet (0.11–0.14 mg/100 g), and cow milk (0.15–0.20 mg/100 g) are higher than those measured in rice (0.01–0.03 mg/100 g) and human milk (0.03–0.04 mg/100 g) (22).

Collection and handling of samples

All samples were collected under low-intensity lighting. Venous blood from patients was collected in a 3-mL heparin-containing tube (wrapped in aluminum foil) and centrifuged at 2200 × g and 4°C for 5 min. Plasma was dispensed into aliquots and red cells were washed 3 times with a 0.9% sodium chloride solution. A 15–50-mL volume of urine voided after an overnight fast was collected from the subjects. Plasma, erythrocytes, and urine were immediately frozen at −20°C. All samples were transported to our laboratory on dry ice and were stored at −20°C until analyzed. Samples were protected from light during transport and storage.

Assay of biological variables

Albumin, transthyretin, transferrin, and α_1-acid glycoprotein were analyzed by Beckman immunonephelometry (Array 360 CE system; Beckman, Brea, CA), whereas C-reactive protein was analyzed by Behring immunonephelometry (Behring, Marburg, Germany).

HPLC procedure

Blood vitamin extraction

Vitamins were extracted from blood was performed according to Capo-chichi et al (23) with the use of galactoflavin as an internal standard. All experiments were carried out under low-intensity light. A 0.5-mL volume of hemolysate or a 1-mL sample of plasma was used for vitamin extraction, to which an equal volume of solution A (10 mmol dihydrogen potassium phosphate/L and 15 mmol magnesium acetate/L, adjusted to pH 3.4 with orthophosphoric acid) was added together with 100 μL galactoflavin (206 nmol/L). Samples were incubated at 65°C for 15 min to release FAD and FMN from apoenzymes present in the medium. Proteins were precipitated by adding a 0.5-mL aliquot of 10% trichloroacetic acid solution to the reaction mixture and centrifuging at 3200 × g for 10 min at room temperature; the supernate was kept in a separate tube and the original tube was then rinsed once with 1 mL solution A and centrifuged at 3200 × g for 5 min at room temperature. The supernate was added to the first one. Supernates were then loaded onto a C_{18} Sep-pak cartridge (Waters, Milford, MA) previously conditioned with 2 mL methanol (Prolabo, Paris) and 2 mL solution A. The Sep-pak cartridge was rinsed with 2 mL solution A; vitamins were then eluted with 2 mL solution B (a 1:1 mixture of methanol and solution A). A 100[hyphen]μL volume of the vitamin extract was then injected into the HPLC apparatus for analysis. Standard solutions of 265 nmol riboflavin/L (Merck, Darmstadt, Germany); 240 nmol FAD/L, 220 nmol FMN/L (Sigma Chemical Co, St Louis), and 206 nmol galactoflavin/L (Merck Sharp & Dohme, Gibstown, NJ) were used for calibration. The extraction recoveries from supplemented samples were 99.0 ± 3.81%, 99.0 ± 5.80%, and 97.0 ± 2.80% for FAD, FMN, and riboflavin, respectively. The extraction recovery of the internal standard (galactoflavin) was 97 ± 2.04%. The between-run CVs were 5.9%, 6.8%, 4.3%, and 2.1% for FAD, FMN, riboflavin, and galactoflavin, respectively.

HPLC separation and identification of riboflavin and riboflavin cofactors

Analyses for riboflavin and riboflavin cofactors were carried out with a C_{18} reversed-phase column (250 mm × 4 mm, 5 μm internal diameter; Interchim, Montluçon, France) with isocratic elution by using 15% acetonitrile in solution A at a flow rate of 1 mL/min. The HPLC system was composed of 2 Waters 501 pumps connected to a Shimadzu RF 535 fluorescence HPLC monitor and a Shimadzu CR6A Chromatopac integrator (Shidmadzu Corporation, Kyoto, Japan). The spectrofluorometer was set to 445 nm for the excitation wavelength and 530 nm for the emission wavelength.

Urinary organic acid analysis

Organic acids were extracted from volumes of urine containing 1 mg creatinine and analyzed by using a gas chromatograph–mass spectrometer (model 5971 A; Hewlett-Packard, Palo Alto, CA) according to Lefebvre et al (24). Only specific organic acids related to riboflavin concentrations (glutaric, succinic, methylsuccinic, ethylmalonic, suberic, adipic, methyladipic, and pimelic acids) were examined in this study.

Plasma thyroid hormone analysis

Plasma thyroid hormones were assessed by using a time-resolved fluoroimmunoassay (25) with a DELFIA kit and an LKB Wallac 1230 Arcus fluorometer (both from Wallac Oy, Turku, Finland).

Statistical analysis

STATVIEW software (version 4.02; Abacus Concepts, Inc, Berkeley, CA) was used for statistical analyses. Data are expressed as means ± SEs and ranges. Because of a skewed data distribution, logarithmic transformations were carried out to normalize distributions before the statistical analyses (Student's t test for unpaired data and Bonferroni adjustment). The following comparisons were made: *1*) group S with group C, *2*) each group of malnourished children with the control group, and *3*) the subgroups with kwashiorkor and marasmus with each other and with the control subjects. Differences were considered significant for P values < 0.05. Linear regression correlation coefficients (r) were used to assess the relations between nutritional proteins, riboflavin, FMN, FAD, thyroid hormones, and organic acids.

RESULTS

Anthropometric and biological data from age-matched malnourished children (groups S and C) and the control group are summarized in **Table 1**. Malnourished children in groups S and C had significantly lower average anthropometric measurements than did the control group. Weights and height expressed as percentages of normal weights- and heights-for-age (21), were significantly lower in group S than in group C. There were no significant differences in AC:HC or BMI values between groups S and C.

Except for transferrin concentrations of group C, plasma nutritional protein concentrations were lower in the malnourished children than in the control subjects. These nutritional measures were significantly more affected in group S than in group C. Plasma inflammatory protein concentrations were higher in both malnourished groups than in the control group.

As shown in **Table 2**, all malnourished children had lower thyroxine (T_4), T_3, free thyroxine (FT_4), and free T_3 (FT_3) concentrations than did the control subjects. T_3, T_4, and FT_3 concentrations were lower in group S than in group C. There were no significant differences in thyrotropin concentration between the groups. Although the mean of the ratio of T_3 to T_4 was low in group S (0.018 ± 0.003), it was not significantly different from that in group C (0.019 ± 0.001) or the control group (0.023 ± 0.001). In contrast, the mean FT_3-FT_4 ratio was significantly lower in group S (0.32 ± 0.04) than in group C (0.47 ± 0.04; $P < 0.01$) or the control group (0.48 ± 0.04; $P < 0.01$). No significant difference in this variable was observed between group C and the control group.

For the malnourished children, T_3 concentrations were positively correlated with albumin, transferrin, and transthyretin ($r = 0.49$, $P < 0.0001$) concentrations ($n = 41$). Graphs showing the correlation of T_3 concentration with that of albumin and transferrin are presented in **Figure 1** and show that all children with low T_3 concentrations had low albumin and transferrin concentrations. Correlation coefficients for T_4 concentrations with albumin, transferrin, and transthyretin concentrations were 0.82 ($P < 0.0001$), 0.81 ($P < 0.0001$), and 0.29 ($P < 0.05$), respectively. The T_3-T_4 ratio was positively correlated with albumin ($r = 0.34$), transferrin ($r = 0.25$), and transthyretin ($r = 0.29$) concentrations ($P < 0.05$, $n = 41$).

FT_3 concentrations were positively correlated with albumin ($r = 0.51$), transferrin ($r = 0.47$), and transthyretin ($r = 0.43$) concentrations ($P < 0.01$; $n = 36$); no significant correlation was observed with FT_4. In addition, the FT_3-FT_4 ratio was positively correlated with albumin ($r = 0.52$), transferrin ($r = 0.41$), and transthyretin ($r = 0.59$) concentrations ($P < 0.05$; $n = 36$).

Measured riboflavin, FMN, and FAD concentrations are summarized in Table 2, which shows that there were no significant differences in riboflavin concentrations between group C and the control group. The plasma riboflavin concentration was significantly higher in group S than in group C or the control group. FMN concentrations were not significantly different between groups, whereas plasma FAD concentrations were significantly lower in group S than in group C or the control group. Plasma riboflavin deficiency was observed in 3 children in group S and 1 child in group C. The plasma riboflavin and FAD concentrations in these patients were below the lowest values for the control group (riboflavin < 12.53 nmol/L and FAD < 33.53 nmol/L, respectively).

During HPLC analysis of riboflavin concentrations, 3 different chromatographic profiles were observed (**Figure 2**). *1*) In those in group C and the control group with normal riboflavin

TABLE 1
Anthropometric and biological data for malnourished children and a control group[1]

	Group S (n = 30)	Group C (n = 30)	Control group (n = 24)
Age (mo)	20.20 ± 1.37 (6–36)	18.40 ± 1.46 (6–36)	18.60 ± 2.76 (6–36)
Weight (% of NCHS standard)	60.53 ± 1.79[2]	67.22 ± 3.73[2]	91.51 ± 3.46
Height (% of NCHS standard)	88.13 ± 1.33[3,4]	93.7 ± 2.48	93.75 ± 2.46
AC:HC	0.24 ± 0.01[2]	0.26 ± 0.01[3]	0.32 ± 0.01
BMI (kg/m^2)	12.08 ± 0.35[2]	12.35 ± 0.38[2]	17.96 ± 0.58
Plasma measures			
Albumin (g/L)	19.77 ± 1.38 [26][2,5] (9.4–33.7)	31.47 ± 1.76 [29][2] (14.80–54.10)	39.97 ± 0.88 [24] (32.8–49.0)
Transthyretin (g/L)	0.10 ± 0.001 [26][2] (0.06–0.21)	0.13 ± 0.01 [29][2] (0.02–0.29)	0.17 ± 0.09 [24] (0.11–0.27)
Transferrin (g/L)	0.95 ± 0.14 [26][2,5] (0.13–2.98)	2.33 ± 0.24 [29] (0.53–5.44)	2.65 ± 0.12 [24] (1.56–3.49)
α-Acid glycoprotein (g/L)	2.43 ± 0.17 [26][6] (1.05–5.17)	2.38 ± 0.17 [29][6] (0.91–4.47)	1.49 ± 0.16 [24] (0.52–3.41)
C-reactive protein (mg/L)	48.16 ± 9.88 [26][3] (<3.00–200.0)	48.84 ± 10.91 [29][3] (<3.0–196.0)	15.66 ± 5.39 [24] (<3.0–121.80)

[1] $\bar{x}$ ± SE; range in parentheses; n in brackets. Group S was from the savannah in Benin; group C was from the coast of Togo. Weight and height are expressed as percentages of National Center for Health Statistics standard values for age (21).

[2,3,6] Significantly different from the control group (with Bonferroni adjustment): [2] $P < 0.0001$, [3] $P < 0.05$, [6] $P < 0.001$.

[4,5] Significantly different from group C (with Bonferroni adjustment): [4] $P < 0.05$, [5] $P < 0.0001$.

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TABLE 2
Plasma thyroid hormones and plasma and erythrocyte riboflavin and riboflavin cofactor concentrations in malnourished children and a control group[1]

	Group S	Group C	Control group
Thyrotropin (mU/L)	3.35 ± 0.55 [23] (0.38–9.54)	3.11 ± 0.48 [21] (0.49–10.28)	2.23 ± 0.26 [19] (0.73–4.62)
T_3 (nmol/L)	1.12 ± 0.24 [23][2,3] (0.01–4.84)	1.74 ± 0.18 [21][4] (0.40–3.12)	2.92 ± 0.19 [19] (1.50–4.25)
T_4 (nmol/L)	57.06 ± 5.87 [23][2,3] (9.13–111.40)	94.58 ± 8.59 [21][4] (21.76–169.80)	129.92 ± 6.11 [19] (83.54–185.60)
FT_3 (pmol/L)	4.71 ± 0.68 [19][4] (1.15–12.22)	5.25 ± 0.50 [17][5] (2.32–11.21)	7.19 ± 0.42 [19] (3.62–10.08)
FT_4 (pmol/L)	13.0 ± 0.97 [19][5] (8.01–20.22)	11.32 ± 0.65 [17][5] (7.94–17.08)	16.55 ± 1.77 [18] (10.35–38.69)
Plasma			
Riboflavin (nmol/L)	66.9 ± 12.75 [24][3,4] (3.0–277.98)	28.09 ± 9.12 [27] (3.0–227.83)	20.08 ± 3.03 [15] (12.53–44.49)
FMN (nmol/L)	13.70 ± 2.08 [24] (4.0–38.67)	16.40 ± 2.45 [27] (4.0–55.26)	13.02 ± 0.74 [15] (10.24–18.35)
FAD (nmol/L)	31.57 ± 10.19 [24][2,3] (4.0–217.0)	59.02 ± 5.60 [27] (4.15–108.50)	65.35 ± 5.23 [15] (33.34–94.20)
Erythrocyte			
Riboflavin (nmol/mol hemoglobin)	Trace [21]	Trace [22]	Trace [15]
FMN (nmol/mol hemoglobin)	23.05 ± 6.07 [21] (4.0–109.73)	15.61 ± 3.27 [22] (4.0–68.88)	17.39 ± 2.18 [15] (5.40–31.02)
FAD (nmol/mol hemoglobin)	79.35 ± 7.32 [21] (35.61–142.30)	67.86 ± 7.90 [22] (34.85–170.62)	83.85 ± 9.79 [15] (62.26–150.44)

[1] $\bar{x}$ ± SE; range in parentheses; *n* in brackets. Group S was from the savannah in Benin, group C was from the coast of Togo. T_3, triiodothyronine; T_4, thyroxine; FT_3, eg, free T_3; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide.

[2,4,5] Significantly different from the control group (with Bonferroni adjustment): [2] $P < 0.0001$, [4] $P < 0.001$, [5] $P < 0.05$.

[3] Significantly different from group C, $P < 0.0001$ (with Bonferroni adjustment).

status (Figure 2A), FAD concentrations were found to be higher than FMN and riboflavin concentrations. *2*) In cases of riboflavin deficiency (Figure 2B), FAD, FMN, and riboflavin concentrations were all lower than reference values. *3*) In cases of a decrease in riboflavin conversion into its cofactors (Figure 2C), riboflavin concentrations were higher than FMN and FAD concentrations in groups S and C, especially in the most malnourished children in group S.

The extent of riboflavin conversion into its cofactors was estimated by calculating the FMN-riboflavin, FAD-FMN, and FAD-riboflavin ratios for each subject. The mean values of these ratios in plasma were significantly lower ($P < 0.01$) in group S (0.44 ± 0.12, 2.48 ± 0.46, and 0.99 ± 0.31, respectively; $n = 24$) than in group C (2.05 ± 0.48, 5.33 ± 0.91, and 7.06 ± 1.96, respectively; $n = 27$) or the control group (0.78 ± 0.20, 5.68 ± 0.72, and 4.16 ± 0.64, respectively; $n = 15$). No significant difference in these flavin pair ratios was observed between group C and the control group.

There was no significant difference in erythrocyte FAD, FMN, or riboflavin concentrations, or erythrocyte flavin pair ratios between the groups. Erythrocyte riboflavin was measured in trace amounts in all groups (Table 2).

In group S, patients with low T_3 concentrations had plasma riboflavin concentrations that were higher than plasma FAD concentrations. However, in 2 patients with normal thyroid hormone values, higher plasma riboflavin concentrations than FAD concentrations were also observed. Only one patient in group C with low T_3 (0.69 nmol/L) had a plasma riboflavin concentration (227.8 nmol/L) that was higher than the plasma FAD and FMN concentrations (18.7 and 12.18 nmol/L, respectively).

Plasma riboflavin was negatively correlated with low T_3 concentrations ($r = -0.39$, $P < 0.05$; $n = 27$), whereas the FMN-riboflavin and FAD-riboflavin ratios were positively correlated with low T_3 concentrations ($r = 0.45$ and $r = 0.44$, respectively, $P < 0.05$; $n = 27$). No significant correlation was observed between low T_3 concentrations and the plasma FAD-FMN ratio. There was no significant correlation between riboflavin derivatives and T_3 concentrations in the control group or in the malnourished children with T_3 concentrations >1.50 nmol/L (lowest value for the control group).

FT_4 was positively correlated with FAD concentrations ($r = 0.39$) and the FAD-FMN ratio ($r = 0.38$, $P < 0.05$; $n = 27$). The T_3-T_4 ratio was positively correlated with FAD ($r = 0.57$) and FMN ($r = 0.26$) but negatively correlated with riboflavin ($r = -0.42$, $P < 0.05$; $n = 27$) concentration. In addition, the T_3-T_4 ratio was positively correlated with the FMN-riboflavin ($r = 0.46$), FAD-FMN ($r = 0.29$), and FAD-riboflavin ($r = 0.47$, $P < 0.05$; $n = 27$) ratios.

The relation between plasma riboflavin concentrations and plasma nutritional protein and T_3 concentrations (**Figure 3**) shows that children with riboflavin concentrations above the highest control riboflavin values (44.49 nmol/L) had very low concentrations of albumin (<33 g/L), transferrin (<1.56 g/L), and T_3 (1.50 nmol/L).

All urinary organic acids were analyzed and some specific organic acids related to fatty acid oxidation are presented in **Table 3**. Excretion of ethylmalonic, adipic, methyladipic, suberic, glutaric, and methylsuccinic acids was higher in groups S and C than in the control group. Dicarboxylic aciduria was higher in group C than in the most severely malnourished children in

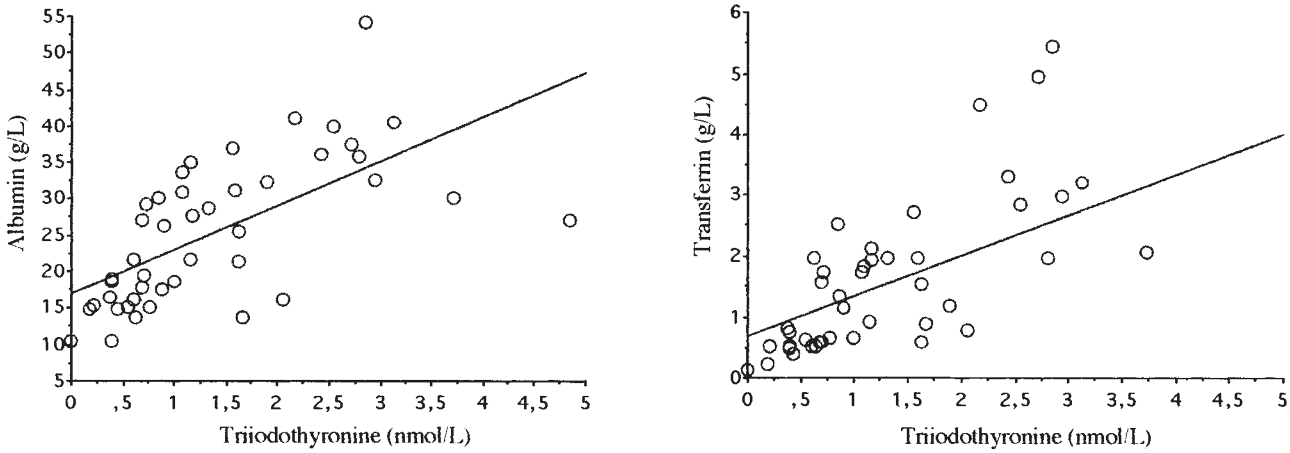

FIGURE 1. Correlations between albumin and triiodothyronine concentrations ($r = 0.78$, $P < 0.0001$) and between transferrin and triiodothyronine concentrations ($r = 0.72$, $P < 0.0001$) in malnourished children.

group S. No significant correlation was observed between urinary dicarboxylic acids and plasma or erythrocyte riboflavin, FMN, and FAD concentrations, nor between urinary dicarboxylic acids and plasma thyroid hormone concentrations.

Although children with kwashiorkor had albumin, transferrin, T_3, and T_4 concentrations that were significantly lower (18.25 ± 1.47 g/L, 0.95 ± 0.16 g/L, 1.10 ± 0.24 nmol/L, and 54.85 ± 6.97 nmol/L, respectively) than those of children with marasmus (31.89 ± 1.43 g/L, 2.24 ± 0.22 g/L, 1.68 ± 0.20 nmol/L, and 90.35 ± 7.44 nmol/L, respectively; $P < 0.0001$), no significant difference was observed between these groups in relation to transthyretin, α_1-acid glycoprotein, C-reactive protein, FT_3, FT_4, riboflavin, FMN, FAD, and urinary organic acid concentrations. Thyrotropin concentrations were significantly higher ($P < 0.05$) in the group with kwashiorkor (4.07 ± 0.66 mU/L) than in group with marasmus (2.57 ± 0.35 mU/L). No significant difference was observed for these variables in relation to the sex or age of the malnourished children.

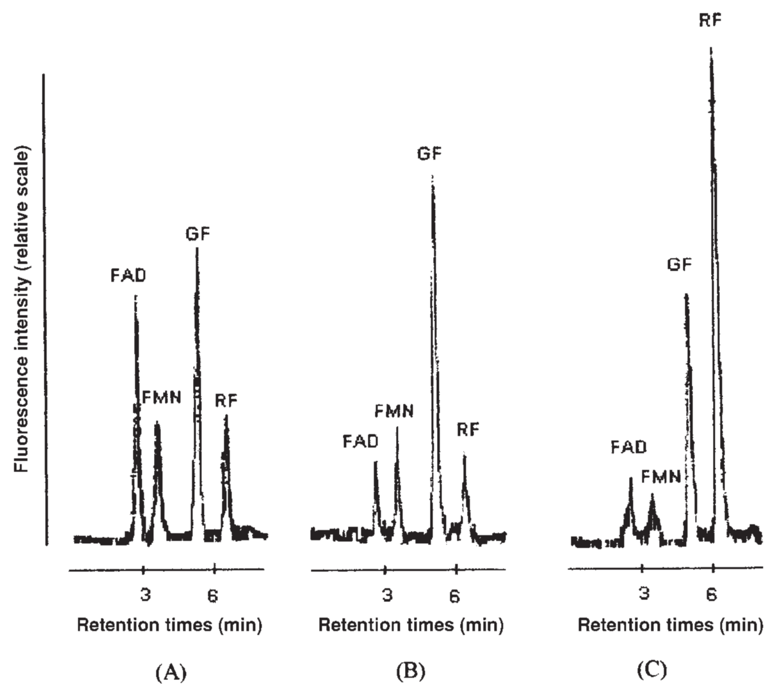

FIGURE 2. HPLC chromatogram of plasma flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), galactoflavin (GF), and riboflavin (RF) in children with normal plasma RF concentrations (A), plasma RF deficiency (B), and perturbations in plasma RF metabolism (C). The sample was injected onto a C_{18} reversed-phase column (250 mm × 4 mm, 5 μm) with isocratic elution using 15% acetonitrile and 85% of a solution of 10 mmol dihydrogen potassium phosphate/L and 15 mmol magnesium acetate/L, adjusted to pH 3.4 with orthophosphoric acid at a flow rate of 1 mL/min. FAD, FMN, GF, and RF were detected by spectrofluorometry at an excitation wavelength of 445 nm and an emission wavelength of 530 nm.

TABLE 3
Urinary organic acid concentrations in malnourished children and a control group[1]

	Group S (n = 22)	Group C (n = 26)	Control group (n = 21)
Ethylmalonic acid (μmol/mmol creatinine)	11.76 ± 4.21 (0.87–86.8)	14.98 ± 2.67[2] (1.73–64.19)	3.64 ± 0.80 (0.87–16.05)
Succinic acid (μmol/mmol creatinine)	43.35 ± 25.08 (2.87–610.30)	41.73 ± 7.99[2] (4.6–157.90)	14.48 ± 3.20 (2.98–60.64)
Methylsuccinic acid (μmol/mmol creatinine)	2.81 ± 0.57[2,3] (1.09–13.60)	5.06 ± 0.99[4] (1.36–22.58)	2.05 ± 0.22 (0.90–5.17)
Adipic acid (μmol/mmol creatinine)	26.35 ± 13.60[5] (3.66–187.30)	130.01 ± 60.78[4] (5.33–875.72)	12.23 ± 2.36 (1.0–45.7)
Methyladipic acid (μmol/mmol creatinine)	20.07 ± 5.54[3] (2.85–117.15)	92.32 ± 18.25[4] (5.7–380.0)	17.50 ± 2.96 (1.05–52.25)
Suberic acid (μmol/mmol creatinine)	34.08 ± 7.10[2,5] (6.02–122.0)	67.75 ± 14.48[4] (5.27–262.90)	16.85 ± 2.73 (1.36–39.17)
Glutaric acid (μmol/mmol creatinine)	5.46 ± 2.12[5] (0.77–46.96)	9.52 ± 1.33[4] (2.16–28.45)	3.58 ± 0.87 (0.23–17.61)
Pimelic acid (μmol/mmol creatinine)	22.73 ± 4.04 (4.91–65.52)	20.37 ± 4.54 (1.60–67.95)	15.81 ± 2.42 (2.05–40.94)

[1] $\bar{x}$ ± SE; range in parentheses. Group S was from the savannah in Benin, group C was from the coast of Togo.
[2,4] Significantly different from the control group (with Bonferroni adjustment): [2] $P < 0.01$, [4] $P < 0.001$.
[3,5] Significantly different from group C (with Bonferroni adjustment): [3] $P < 0.001$, [5] $P < 0.05$.

DISCUSSION

We studied 2 populations of malnourished children from 2 different geographic regions of West Africa. Clinical records, anthropometric data, and plasma nutritional protein concentrations observed in this and other studies (26, 27) indicate that the children in group S were more severely malnourished than were the children in group C. We observed low thyroid hormone concentrations in the malnourished children described here, as was reported previously reported (14, 15). The lower T_3 and T_4 concentrations observed in group S than in group C was more likely due to the severity of malnutrition in group S and to the lower concentrations of T_4-binding proteins (albumin and transthyretin) in group S than in group C or the control group (28) than to an iodine-deficient intake. The mean T_3-T_4 ratio and thyrotropin concentrations were indeed not higher than those measured in control subjects, in contrast with what was described in children with iodine intake deficiency (29). The positive correlation between T_3 and albumin and transferrin concentrations confirmed that thyroid hormone concentrations are affected by the severity of malnutrition.

The children in group C did not have significantly lower transferrin, FAD, FMN, or riboflavin concentrations than the control subjects (Table 1 and Figure 2A). In contrast, the children in group S had significantly lower plasma transferrin, albumin, and FAD and higher riboflavin concentrations than the control group(Figure 2C). This was probably because of a decrease in the conversion of riboflavin into its cofactors. As shown in Figure 2B, the impairment in riboflavin conversion was not manifested in cases of low plasma riboflavin concentrations.

Riboflavin is the precursor of FMN and FAD, which are implicated in energy metabolism and electron transfer pathways. The conversion of riboflavin into FMN and FAD is catalyzed by riboflavin kinase and FMN adenylyltransferase (EC 2.7.7.2) in the presence of ATP and Zn^{2+} (30). T_3 enhances riboflavin kinase activity (10, 13). The low T_3 concentrations observed in PEM might be responsible for a reduction in riboflavin kinase activity, which would give rise to an insufficient conversion of riboflavin into its cofactors. Zinc deficiency, which was described previously in severely malnourished children (31, 32), might also be implicated in the impairment of riboflavin conversion into its cofactors. Along with the thyroid hormone concentrations observed in groups S and C, estimation of energy and zinc intakes in severely malnourished children (group S) and moderately malnourished children (group C) might help explain the observed riboflavin concentrations in group S.

In cases of low T_3 concentrations, the positive correlation observed between T3 and plasma FMN:riboflavin and FAD:riboflavin, and the negative correlation between T_3 and riboflavin concentrations suggest that riboflavin kinase activity decreases with decreasing T_3 concentrations, leading to riboflavin accumulation and a reduction in plasma FAD and FMN concentrations. This observation is strengthened by the negative correlation of T_3-T_4 ratios with riboflavin concentrations and the positive correlation of T_3-T_4 ratios with FMN-riboflavin, FAD-FMN, and FAD-riboflavin ratios.

Although the fraction of bound riboflavin cofactors measured might not equal the total amount present, plasma FAD concentrations measured in this study were more affected by malnutrition than were FMN concentrations in cases of malnutrition accompanied by low T_3 concentrations. In group S children with low T_3 concentrations, there was a positive correlation between plasma FMN-riboflavin and FAD-riboflavin ratios ($r = 0.87$, $P < 0.01$), suggesting that a reduction in FAD synthesis in PEM is influenced by FMN availability. Two patients in group S with normal thyroid hormone concentrations and low albumin and transferrin concentrations also had plasma riboflavin concentrations that were higher than those of FAD, proving that factors other than a low T_3 concentration might be implicated in the impairment of riboflavin conversion into its cofactors. The higher plasma riboflavin than FAD concentrations observed in these malnourished children were also reported in some cases of severely malnourished anorexic girls

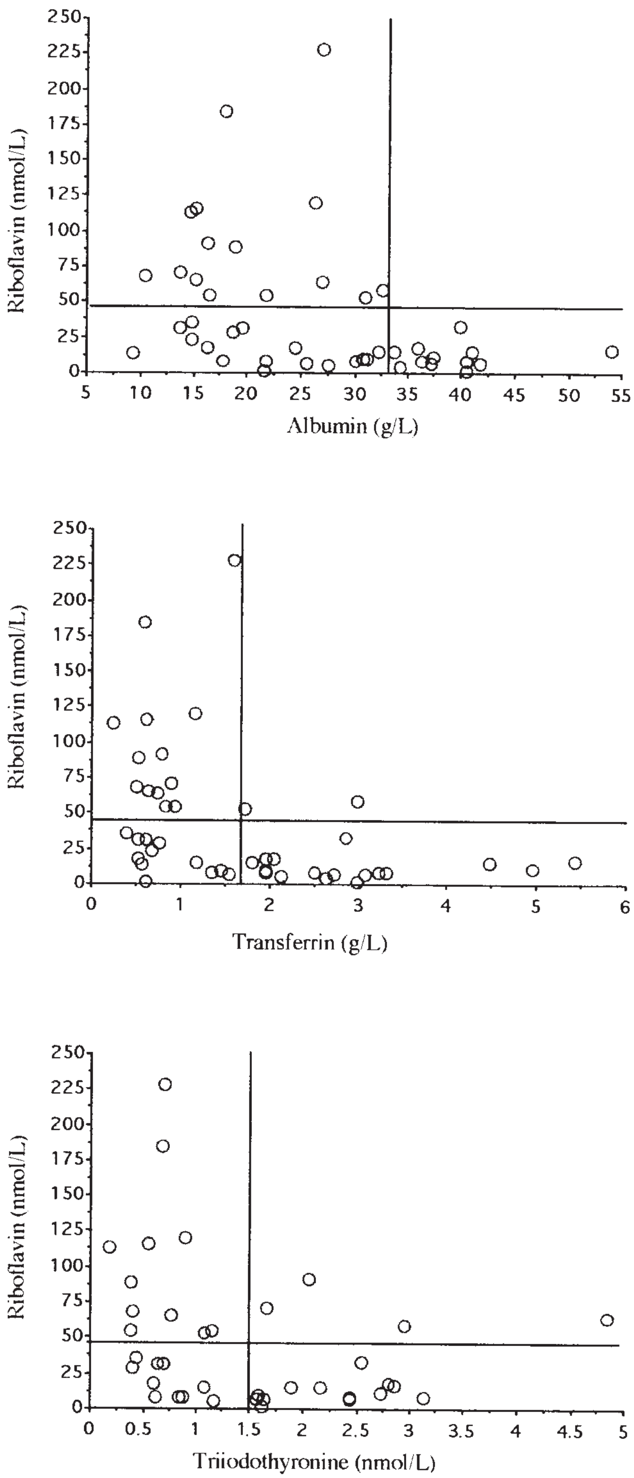

FIGURE 3. Relations between plasma riboflavin concentration and albumin (*n* = 48), transferrin (*n* = 48), and triiodothyronine (*n* = 41) concentrations in malnourished children from the savannah in Benin (S) and from the coast of Togo (C). All malnourished children with high plasma riboflavin concentrations (>44.5 nmol/L) had albumin concentrations <33 g/L, most of the malnourished children with high plasma riboflavin concentrations had transferrin concentrations <1.56 g/L, and most of the children with high plasma riboflavin concentrations had triiodothyronine concentrations <1.50 nmol/L

with low thyroid hormone concentrations (23). A previous study also reported an increase in riboflavin excretion during acute starvation and restriction of energy intake (33). Despite the anemia (34) and erythrocyte membrane perturbations (35) occurring in the malnourished children, erythrocyte riboflavin cofactor concentrations were not deficient in either of the groups of malnourished children in our study.

Some studies have implicated riboflavin and FAD deficiencies in a specific organic aciduria (16, 17, 36). The high urinary excretion of dicarboxylic acids (ethylmalonic, succinic, methylsuccinic, glutaric, adipic, methyladipic, and suberic acids) observed in PEM indicates a defect in fatty acid mitochondrial β-oxidation (37–41). In our study, adipic, methyladipic, and suberic aciduria were higher in group C children with normal plasma FAD concentrations than in group S children with low plasma FAD concentrations. This suggests that dicarboxylic aciduria was not correlated with plasma FAD concentrations. Organic aciduria does not always reflect vitamin deficiencies, but their analysis is useful in the assessment of the active forms of vitamins in cells. The lower concentrations of urinary organic acids observed in the most severely malnourished children (group S) leads to the hypothesis that fatty acids were less available for oxidation in cases of severe malnutrition.

Organic aciduria was described previously in cases of malabsorption (41), in malnourished children (42, 43), and in anorexia nervosa (23, 44). These latter studies also showed an increase in organic acid excretion during refeeding. A recent study of organic aciduria in infantile malnutrition (43) showed an increase in dicarboxylic aciduria during refeeding. These studies in anorexia nervosa patients and malnourished children (23, 43, 44) add weight to the hypothesis that the high organic aciduria observed in the group C children was due to more substrates being available to enter the mitochondria than in the group S children, in whom substrate availability might have been rate-limiting.

Although hepatic steatosis is observed in PEM (45), the pathogenesis of fatty liver in malnutrition remains unknown. Among the hypotheses proposed to explain it is an impairment of fatty acid β-oxidation (46). Previous studies have reported that riboflavin uptake by rat and human liver cells is energy dependent (47, 48) and that hepatic FAD is low in rats with hypothyroidism (10, 11). These findings might explain the fact that in cases of severe protein-energy restriction and hypothyroidism, the reduction of riboflavin uptake by liver cells is responsible for the decreased availability of riboflavin-derived cofactors for fatty acid β-oxidation via acyl-CoA dehydrogenase activities in mitochondria, leading to the shunting of fatty acids into the ω-oxidation pathway to form dicarboxylic acids. These findings also explain why malnourished children in both groups S and C had dicarboxylic aciduria despite the fact that most of them had normal plasma riboflavin concentrations.

Our data show that dicarboxylic aciduria occurs in patients with riboflavin, FMN, and FAD concentrations in plasma and erythrocytes that are not deficient. The observed organic aciduria is not the consequence of vitamin deficiency but, more likely, reflects a reduction of riboflavin uptake by hepatic cells and an imbalance between substrate amount and β-oxidation activity.

In conclusion, our work shows a relation between riboflavin concentrations, thyroid hormones, and plasma nutritional proteins. Severe malnutrition and low concentrations of T_4-binding proteins (albumin and transthyretin) result in low T_3 concentrations, which in turn impair the conversion of riboflavin into its cofactors. According to our results, thyroid hormone deficiencies might not be the only factor influencing riboflavin metabolism. In our study, severely malnourished children were not globally riboflavin deficient, but the lack of mitochondrial riboflavin cofactor biosynthesis might be implicated in the reduction of FAD-dependent enzyme activities, leading to the urinary excretion of dicarboxylic acids resulting from the ω-oxidation pathway. The fact that the severely malnourished children (group S) with insufficient riboflavin conversion into cofactors had lower excretions of dicarboxylic acids than the 2 other groups, suggests that the severity of the PEM might limit the availability of fatty acids to be oxidized in these children. This finding could explain the differences observed in the excretion of dicarboxylic acids in the groups of malnourished children studied here.

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