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Regional, Socioeconomic, and Dietary Risk Factors for Vitamin B-12 Deficiency Differ from Those for Folate Deficiency in Cameroonian Women and Children[1,2]

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Abstract

Background: Representative data on folate and vitamin B-12 dietary intake and status in low-income countries are rare, despite the widespread adoption of folic acid fortification.

Objective: The purpose of this study was to evaluate folate and vitamin B-12 intake, status, and risk factors for deficiency before implementation of a national fortification program in Cameroon.

Methods: A nationally representative cross-sectional cluster survey was conducted in 3 ecologic zones of Cameroon (South, North, and the 2 largest cities, Yaoundé/Douala), and information on dietary intake was collected from 10 households in each of 30 randomly selected clusters per zone. In a subset of women and their 12- to 59-mo-old children (n = 396 pairs), plasma folate and vitamin B-12, as well as breast milk vitamin B-12, were analyzed.

Results: Vitamin B-12 and folate dietary intake patterns and plasma concentrations were similar for women and children. In the subsample, 18% and 29% of women and 8% and 30% of children were vitamin B-12 (≤221 pmol/L) and folate (<10 nmol/L) deficient, respectively. Mean dietary folate ranged from 351 μg dietary folate equivalents/d in the North to 246 μg dietary folate equivalents/d in Yaoundé/Douala; plasma folate was negatively associated with socioeconomic status (P = 0.001). Plasma vitamin B-12 deficiency was similar in the South and North, 29% and 40%, respectively, but was only 11% in Yaoundé/Douala, and was positively associated with socioeconomic status. Mean breast milk vitamin B-12 was statistically significantly lower in the North (101 pmol/L) than in the South (296 pmol/L) or Yaoundé/Douala (349 pmol/L).

Conclusions: Folate intake and status are inadequate among women and young children in Yaoundé/Douala, whereas low vitamin B-12 intake and status are more common in poor and rural areas, especially in the North. Different strategies may be needed to control deficiency of these nutrients in different regions of Cameroon. *J Nutr* 2015;145:2587–95.

Keywords: vitamin B-12, folate, fortification, breast milk, Cameroon

Introduction

Folate and vitamin B-12 play important roles in maternal health during pregnancy and lactation, and deficiency of either can affect pregnancy outcome, as well as growth and neurologic development of young children (1–3). Poor folate status in genetically susceptible women is recognized as a preventable risk factor for neural tube defects, compelling many governments to mandate large-scale folic acid fortification of wheat flour (4), whereas vitamin B-12 deficiency is increasingly recognized as a public health concern in many regions of the world where animal source foods are consumed in low amounts (5).

In low- and middle-income countries where multiple micronutrient deficiencies often coexist, large-scale food fortification is considered a possible intervention strategy to prevent these deficiencies. The WHO Guidelines on Food Fortification with Micronutrients (6, 7) concluded that the design of fortification programs should be based on biochemical data on population micronutrient status, as well as information on dietary intake of nutrients and potentially fortifiable food vehicles, for targeted micronutrients in different population groups. However, there are very few examples of folate intake and/or status having been measured at baseline before folic acid fortification, either to justify the need for a fortification program or to guide the concentration of folic acid addition needed, even in the many populations in whom folic acid fortification of flour is in effect.

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Furthermore, given the global prevalence of vitamin B-12 deficiency, its association with an elevated risk of neural tube defects, and the limited availability of natural sources of the vitamin in low-income countries, the addition of vitamin B-12 to flour as a fortificant may be warranted (7, 8). Current WHO recommendations include the need for vitamin B-12 fortification of flour based on this evidence (7), and there is no concern about the safety of increasing intake (6). However, as for folate, few data on vitamin B-12 intake and/or status are available from representative samples of the population in low- and middle-income countries, especially in Africa.

In 2009, a nationally representative cross-sectional cluster survey was completed in Cameroon to assess the nutritional status of the population in preparation for a large-scale fortification program. The aim of the survey was to follow the WHO/FAO guidelines by assessing the micronutrient status of representative population groups and collecting data on dietary intake and patterns of consumption of specific foods and micronutrients before implementing a food fortification program. The results of the iron, zinc, and vitamin A status assessments, as well as data on the consumption of fortifiable foods by women and young children in Cameroon, have been published previously (9–12).

The present analysis examined the dietary intake of folate and vitamin B-12 and biochemical status of women of reproductive age and young children, before food fortification. The objectives were to measure the prevalence of inadequate intake and biochemical deficiency, assessed by measuring folate and vitamin B-12 concentrations in plasma and vitamin B-12 concentrations in breast milk, and to assess the relations of these indicators with each other and with other biomarkers. Finally, we aimed to identify the risk factors associated with deficiency of these vitamins among women and children in Cameroon.

Methods

Study design. The study was a nationally representative, cross-sectional, multistage cluster survey of women (aged 15–49 y) and preschool children (aged 12–59 mo) in the Republic of Cameroon. The study design and data collection methods have been reported in greater detail elsewhere (9–13).

Sampling. The study used a multistage cluster sampling design, with 3 strata based on ecologic regions: the North, consisting of 3 administrative regions representing the arid Sahel; the South, consisting of 7 administrative regions in the humid tropics with the exclusion of the 2 large cities; and the 2 major metropolitan areas of Douala and Yaoundé (the 2 largest cities in Cameroon). Thirty clusters (villages, or neighborhoods within cities) were selected from each region according to the probability-proportional-to-size method using 2005 census data from the Cameroon Central Office of Census and Population Studies. Approximately 10 households per cluster were sampled by using a random start point and systematic selection of adjacent households. Households were considered eligible for the full study (dietary intake and micronutrient status) if they included a child 12–59 mo of age and the child's primary female caregiver (aged 15–49 y). If the index woman was currently lactating, she was eligible to provide a breast milk sample if her breastfed child was at least 1 mo old. Households were excluded if the index woman or child had reported severe fever, diarrhea with dehydration, or other severe illness in the 72 h before data collection.

For the biochemical analyses of plasma folate and vitamin B-12, a ~50% subset of index woman and child dyads, from the larger survey for whom blood samples were available, was randomly selected using the RANDBETWEEN function in Excel (Microsoft Corp.). The children were then matched with the index caretaker/mother in the household for plasma folate and vitamin B-12 analyses. In addition, if a breast milk sample was available from the same household, it was analyzed for vitamin B-12 concentration. In a smaller number of households (n = 49), breast milk was collected from a woman who was breastfeeding the index child (from whom a blood sample was collected); however, in most cases, the mother was breastfeeding a younger child who was not part of the survey.

Informed oral consent was obtained from the index woman, and the study was approved by the Cameroon National Ethics Committee and the Institutional Review Board of the University of California, Davis.

Socioeconomic, demographic, and dietary data collection. Information on socioeconomic status (SES)[6], demographics, food consumption frequency, and 24-h dietary recalls was collected using interviewer-administered questionnaires in a language understood by the respondents. The data included the number of household members in various age groups, primary household language, occupation, employment status and educational attainment of the head of household and the index woman, and household possessions, including livestock, sources of energy for cooking and lighting, waste disposal facilities, and source of water. Pregnancy status was determined by self-report.

The 24-h dietary recall protocol was based on a method developed for use in populations with low literacy (14). Women provided information on all foods and beverages consumed on the previous day by them and the index child, with input from other household members where necessary. In one randomly selected household per cluster (~10% of the sample), the 24-h dietary recall interviews were repeated on a nonconsecutive day to enable estimation of intraindividual variation in nutrient intake. These data collection procedures have been described in greater detail elsewhere (12).

Anthropometric measures. The weight of each index caregiver and child was measured to the nearest 0.1 kg on a battery-powered electronic scale (Seca 899; Seca Weighing and Measuring Systems). For children aged <2 y, length was measured in duplicate to the nearest 0.1 cm using a portable length board (Seca 416; Seca Weighing and Measuring Systems). The standing height of caregivers and of children aged ≥2 y was measured in duplicate to the nearest 0.1 cm using a portable stadiometer (Seca Leicester Portable Height Measure; Seca Weighing and Measuring Systems). Anthropometric z scores were calculated based on the 2006 WHO standards (15).

Blood and breast milk collection and processing. Trained phlebotomists collected 5–7 mL blood by antecubital or metacarpal venipuncture into tubes containing lithium heparin as an anticoagulant (Sarstedt). Due to logistical constraints of the survey, it was not possible to require fasted samples, but the time of blood collection and the time of the meal before blood collection were recorded. In Yaoundé/Douala, plasma folate among women was positively associated with time elapsed since the previous meal, and plasma vitamin B-12 among children was negatively associated with the time of day of blood collection; otherwise, there were no associations between plasma folate or vitamin B-12 and time of day of blood collection or time of previous meal among women or children in any region. After collection, blood samples were immediately placed into insulated coolers containing cold packs. Within ~2 h of collection, samples were centrifuged for 10 min at 2500 × g at 4°C to separate plasma in the field (Hermle Z206A; Hermle LaborTechnik GmbH). Plasma was aliquoted under dim light into sterile polypropylene cryovials wrapped in aluminum foil, frozen on the day of collection, and stored at ≦−20°C until analysis.

Breast milk was collected by the casual sampling method, from the fuller breast. The mother first allowed her child to feed from the breast from which milk was to be collected. After exactly 30 s, the mother manually expressed 5–10 mL milk from the same breast. Milk was collected into sterile plastic containers covered in aluminum foil to

[6] Abbreviations used: AGP, α_1-acid glycoprotein; CRP, C-reactive protein; DFE, dietary folate equivalent; EAR, estimated average requirement; SES, socioeconomic status.

minimize exposure to light. In a few cases when the breastfeeding child was not available at the time of data collection, the woman manually expressed milk into a sterile plastic container for 30 s before providing the milk sample in a different container.

Laboratory analysis. Analysis of folate and vitamin B-12 concentrations in plasma samples was carried out at the Western Human Nutrition Research Center in Davis, California, using the SimulTRAC-SNB Radioassay Vitamin B-12 [^{57}Co]/Folate[^{125}I] Kit (MP Biomedicals). Breast milk (n = 118) was analyzed for vitamin B-12 by the IMMULITE 1000 solid-phase, competitive chemiluminescent enzyme immunoassay (Siemens). Validation of this method for breast milk vitamin B-12 analysis has been described elsewhere (16). Milk folate was not measured because concentrations are relatively unaffected by maternal folate status or intake (17). C-reactive protein (CRP) and α-$_1$-acid glycoprotein (AGP) in plasma were analyzed by a sandwich ELISA method (18). Hemoglobin was measured in the field immediately after the blood draw by using a portable Hemocue photometer (Hb201+; HemocueAB).

Data analysis. Data were analyzed with SAS statistical software version 9.3 (SAS Institute) by using the program's survey analysis procedures and appropriate weighting factors to account for the sampling design. Weighted descriptive statistics were calculated for participant characteristics and biochemical variables. All continuous variables were examined for normality and transformed if not normally distributed. Spearman correlations and survey linear regression analysis were used to compare continuous variables. The prevalence of micronutrient deficiencies was determined using established cutoffs for plasma folate (<10 nmol/L) and plasma vitamin B-12 (≤148 pmol/L for deficiency and ≤221 pmol/L for marginal status). Inflammation was diagnosed by elevated CRP (>5 mg/L) and AGP (>1 g/L). Potential risk factors were assessed for relations with plasma folate and vitamin B-12 concentrations using regression analysis (SAS proc surveyreg); the risk factors examined were the physiologic state of the women of reproductive age, as pregnant, lactating, or nonpregnant, nonlactating; quintiles of SES, based on a score created by factor analysis using information on household possessions, housing materials, and educational attainment of the caregiver and head of household; maternal and child dietary intake on the previous day; and household location by stratum (geographic region) as well as urban compared with rural location.

To calculate total folate and vitamin B-12 intakes, we constructed food composition tables by using values from the Nutrition Coordinating Center Nutrient Database for Standard Reference (19), supplemented with information from other sources (20, 21), including the manufacturer's information. To estimate total dietary folate equivalents (DFEs), all foods and beverages were considered unfortified and unenriched with the exception of specific processed foods that are commonly imported (e.g., biscuits and powdered beverages). Fractional absorption of vitamin B-12 is markedly lower from foods with high vitamin B-12 content (21). Thus, if the amount of vitamin B-12 contributed by an individual food portion was ≥3 μg, the vitamin B-12 content of that food was divided by 5 to adjust for lower absorption (22).

Ten days of data from women and 12 d of data from children in which energy intakes were greater than the group-specific mean + 3 SD (>6177 kcal/d for women and >3817 kcal/d for children) were excluded from analysis due to implausibility.

TABLE 1 Characteristics of the subset of women and children in a national survey in Cameroon who were selected for assessment of biochemical status of folate and vitamin B-12, nationally and by region[1]

	National[2]	South	North	Yaoundé/Douala	*P* value
Women					
n[3]	365–396	127–141	119–122	129–133	
Age, y	26.9 (26.0, 27.8)	27.6 (26.0, 29.2)	25.7 (24.3, 27.2)	27.4 (26.3, 28.6)	0.44
BMI, kg/m^2	24.1 (23.5, 24.6)	24.8 (23.8, 25.8)^b	21.4 (20.4, 22.0)^a	26.7 (25.8, 27.5)^c	<0.001
Hemoglobin, g/L	124 (122, 127)	129 (124, 133)^b	121 (117, 125)^a	119 (117, 122)^a	0.004
Anemic,[4] %	37.5 (31.7, 43.2)	32.1 (22.6, 41.6)^a	39.3 (28.0, 50.5)^{a,b}	46.5 (39.1, 53.9)^b	0.05
High CRP (>5 mg/L), %	15.6 (11.5, 20.0)	11.3 (5.1, 17.2)	19.0 (10.6, 27.6)	20.4 (12.7, 28.1)	0.11
High AGP (>1 g/L), %	4.7 (2.3, 7.1)	3.7 (0.2, 7.3)	7.3 (1.9, 12.7)	2.8 (0.0, 5.7)	0.32
High CRP and/or AGP, %	17.5 (13.2, 21.7)	12.7 (6.2, 19.2)	22.7 (14.4, 31.1)	20.4 (12.7, 28.1)	0.12
Pregnant, *n* (%)	9.0 (41)	6.1 (11)	13.3 (17)	8.6 (13)	0.22
Lactating, *n* (%)	31.4 (116)	38.7 (51)^b	26.5 (32)^{a,b}	23.2 (33)^a	0.02
Children					
n[3]	365–396	127–133	119–120	119–129	
Age, mo	31.9 (30.2, 33.6)	32.3 (29.4, 35.2)	30.7 (28.0, 33.3)	33.1 (29.6, 36.5)	0.68
Male, *n* (%)	178 (50.4)	71 (54.4)	59 (44.6)	63 (47.1)	0.26
Hemoglobin, g/L	106 (104, 108)	109 (106, 113)^b	98 (94, 102)^a	111 (109, 113)^b	0.001
Anemic,[4] %	57.4 (50.7, 64.1)	50.5 (37.5, 63.4)^a	74 (65.6, 82.3)^b	46.9 (38.1, 55.7)^a	0.001
High CRP (>5 mg/L), %	38.0 (31.3, 44.7)	38.9 (26.9, 50.8)	43.2 (31.9, 54.6)	27.9 (19.5, 36.3)	0.07
High AGP (>1 g/L), %	41.1 (33.9, 48.2)	40.2 (27.8, 52.7)^{a,b}	52.5 (40.3, 64.7)^b	25.3 (15.3, 35.3)^a	0.003
High CRP and/or AGP, %	49.6 (42.7, 56.7)	51.9 (37.9, 64.2)^{a,b}	56.0 (45.1, 67.0)^b	34.2 (23.9, 44.6)^a	0.01
Height-for-age *z* score	−1.4 (−1.6, 1.2)	−1.3 (−1.7, −0.9)^b	−2.0 (−2.3, −1.7)^a	−0.6 (−0.9, −0.4)^c	0.001
Stunted (HAZ <−2 SD), %	35.1 (28.4, 41.8)	34.6 (22.9, 46.3)^b	50.9 (38.8, 63.0)^b	10.1 (4.0, 16.3)^a	0.04
Weight-for-height *z* score	0.13 (0.1, 0.3)	0.1 (0.9, 0.3)^b	−0.4 (−0.6, −0.1)^a	0.3 (0.1, 0.5)^b	0.001
Wasted (WHZ <−2 SD), %	2.6 (0.8, 4.3)	0.9 (0.0, 2.7)	5.3 (1.0, 9.5)	1.9 (0.0, 5.7)	0.16
Weight-for-age *z* score	−0.7 (−0.9, −0.5)	−0.7 (−0.9, −0.5)^b	−1.4 (−1.6, −1.1)^a	−0.1 (−0.4, 0.1)^b	0.001
Underweight (WAZ <−2 SD),%	16.4 (11.6, 21.3)	16.4 (11.6, 21.3)^a	30.9 (19.1, 42.7)^b	3.4 (0.00, 7.8)^a	0.005

[1] Values are mean or % (95% CI) unless otherwise indicated. Labeled means in a row without a common letter differ, $P < 0.05$, using linear or logistic regression as appropriate (SAS PROC SURVEYREG or SURVEYLOGISTIC). AGP, α-$_1$-acid glycoprotein; CRP, C-reactive protein; HAZ, height-for-age *z* score; WAZ, weight-for-age *z* score; WHZ, weight-for-height *z* score.
[2] Represents a weighted mean of the South, North, and Yaoundé/Douala.
[3] The available sample size differed by indicator and is thus presented as a range.
[4] Anemia defined as hemoglobin <110 g/L for children and pregnant women and <120 g/L for nonpregnant women.

TABLE 2 Vitamin B-12 and folate status of Cameroonian women of reproductive age and children, as well as breast milk vitamin B-12 concentrations, nationally and by region[1]

	National[2]	South	North	Yaoundé/Douala	*P* value
Women					
n	390	137	122	132	
Plasma folate, nmol/L	18.0 [12, 26]	21.0 [14, 32][b]	16.0 [11, 24][a]	17.0 [15, 18][a]	0.01
Folate deficiency (<10 nmol/L), %	17.3 (11.4–23.1)	13.0 (3.0–23.1)	17.1 (7.7–26.6)	26.0 (16.4–37.2)	0.14
Plasma vitamin B-12, pmol/L	325 [199, 534]	358 [202, 556][a]	248 [157, 409][a]	466 [322, 601][b]	0.01
Severe vitamin B-12 deficiency (≤148 pmol/L), %	15.3 (10.2–20.0)	13.9 (6.2–21.6)[b]	24.5 (14.1–34.2)[b]	3.1 (0.0–6.4)[a]	0.04
Marginal vitamin B-12 deficiency (149–221 pmol/L), %	13.7 (9.3–18.1)	14.9 (7.2–22.7)	15.8 (8.7–23.0)	7.9 (2.6–13.3)	0.15
Any vitamin B-12 deficiency (≤221 pmol/L), %	28.8 (22.7–35.0)	28.8 (18.7–39.1)[b]	40.0 (28.3–52.0)[b]	11.1 (4.8–17.3)[a]	0.006
Breast milk vitamin B-12, pmol/L	180 [55, 291]	236 [159, 386][b]	47[20, 201][a]	287 [208, 422][b]	0.02
n	116	39	55	22	
Children					
n	393	136	122	132	
Plasma folate, nmol/L	21 [14, 31]	27 [19, 37][b]	17 [13, 27][a]	17 [13, 24][a]	0.01
Folate deficiency (<10 nmol/L), %	8.4 (0.5–11.7)	5.3 (0.8–10.0)	10.3 (2.9–17.7)	12.9 (6.5–19.2)	0.11
Plasma vitamin B-12, pmol/L	311 [198, 498]	309 [19, 505][a]	236 [151, 363][a]	474 [280, 634][b]	<0.001
Severe vitamin B-12 deficiency (≤148 pmol/L), %	15.0 (10.5–19.2)	12.1 (5.6–18.5)[a,b]	24.3 (14.2–34.6)[b]	6.6 (2.2–11.0)[a]	0.006
Marginal vitamin B-12 deficiency (149–221 pmol/L), %	17.8 (8.3–27.3)	18.3 (10.2–26.4)[a,b]	18.5 (10.2–26.9)[b]	5.7 (0.7–10.7)[a]	0.01
Any vitamin B-12 deficiency (≤221 pmol/L), %	30.4 (24.4–36.4)	29.9 (19.5–40.3)[b]	42.6 (32.1–53.1)[b]	12.3 (6.5–18.0)[a]	<0.001

[1] Values are median [25th percentile, 75th percentile] and mean or % (95% CI). Labeled means in a row without a common letter differ, $P < 0.05$, using linear or logistic regression as appropriate (SAS PROC SURVEYREG or SURVEYLOGISTIC).
[2] Represents a weighted mean of the South, North, and Yaoundé/Douala.

To present mean nutrient intakes and prevalence of inadequate intakes by region, we estimated usual intake distributions for nutrients by using the National Cancer Institute method (23, 24), which adjusts for intraindividual (day-to-day) variation in nutrient intake. To obtain appropriate estimates of SEs for the study design, we used a balanced repeated replication technique (25); further details on the adaptation of this method to the present data set are described in detail elsewhere (9).

Inadequate intake was defined as <320 and <120 μg DFE/d for women and children, respectively, and <2 and <0.7 μg/d vitamin B-12, the estimated average requirement (EAR) for nonpregnant, nonlactating women aged >19 y and children aged 1–3 y (22). Because breast milk intake was not quantified, dietary intakes from non–breast milk foods were assessed separately for breastfeeding (n = 108) and nonbreastfeeding children (n = 677), and prevalence of inadequate dietary intakes was assessed only among nonbreastfeeding children.

No women or children had excessive folic acid intake, defined as >1000 μg folic acid/d for women (upper limit for women aged >19 y) or >300 μg folic acid/d for children (upper limit for children aged 1–3 y), so these values are not presented. Excessive intakes were not calculated for vitamin B-12, because no upper limit has been defined.

For other analyses of dietary intake data (e.g., comparing nutrient intake among those in the folate/vitamin B-12 subset and the rest of the national survey participants, as well as relations between total vitamin B-12 intake and folate and animal source food intake), data from a single day of 24-h recall data were used, because the National Cancer Institute code used does not provide usual intake estimates for individuals and under the assumption that plasma concentrations of these vitamins are likely to reflect very recent intake. To illustrate the relative contributions of each food group to folate and vitamin B-12 intakes, we also did not apply the National Cancer Institute method to estimate the distribution of usual intakes of folate and vitamin B-12 from each food group separately, because theoretically, the unadjusted data yield the same estimate of population means (but not variation in the distribution) as adjusted data. Thus, we simply computed total intake of each nutrient from each food group using all available days of data. The contribution of each food group to nutrient intakes was expressed in μg DFE and μg vitamin B-12 to preserve the relative differences in the amount consumed by region.

Results

The characteristics of the total survey population have been described elsewhere (13). There were no significant differences between the folate/vitamin B-12 subset (n = 396) of mother and child pairs (**Table 1**) and the larger survey sample (n = 1002 households) (13) with regard to their geographic region, urban compared with rural residence, or household SES categories. In addition, the folate/vitamin B-12 subset did not differ from the remaining households in the full survey with regard to maternal BMI (in kg/m^2), child anthropometric z scores, maternal or infant CRP and AGP, or hemoglobin concentrations. However, the children selected for the folate/vitamin B-12 subset were slightly older, averaging 31.9 mo (n = 361) compared with 29.7 mo (n = 447) (P = 0.04), so child age was used as a covariate in all regression analyses. Children's age and plasma folate and vitamin B-12 were not associated in any of the zones, except that plasma folate in the North was negatively and significantly associated with child age (r^2 = 0.10, P = 0.0003).

As in the national sample, the mean BMI of women in Yaoundé/Douala (26.7) was 1.9 higher and significantly (P < 0.001) different from that of women in the South (24.8) and those in the North (21.4). Mean BMI values of the urban women would be classified in the overweight to preobese category of obesity (26). The prevalence of elevated plasma CRP and AGP did not differ regionally.

Among the children in the subset, age, sex distribution, and the prevalence of elevated CRP and wasting did not differ among regions, but all other characteristics differed significantly by region. Children in the North were generally more malnourished than in the other 2 regions, with a lower mean hemoglobin concentration (98 g/L); a higher prevalence of anemia (74%), stunting (50.9%), and underweight (30.9%); and a higher prevalence of elevated plasma CRP and/or AGP (22.7%) compared with children in the South and Yaoundé/Douala (Table 1).

Plasma folate and risk factors for deficiency

Plasma folate concentrations of women and children were strongly correlated nationally (Spearman's $\rho = 0.39$, $P < 0.0001$); this relation was consistent within the South and North ($\rho = 0.41$ and $\rho = 0.50$, respectively; both $P < 0.0001$) but not in Yaoundé/Douala ($\rho = 0.01$, $P = 0.91$).

Women. There were no significant relations between plasma folate concentrations and measures of inflammation (plasma CRP or AGP) among women in the national sample or within regions. Plasma folate concentrations indicated deficiency in 17% of women nationally, with 13% prevalence in the South, where the mean plasma folate was 21 nmol/L compared with 16 and 17 nmol/L in the North and Yaoundé/Douala, respectively ($P = 0.002$). In Yaoundé/Douala, 26% of women had deficiency, which was twice the prevalence in the South but not significantly different ($P = 0.14$) (**Table 2**).

Plasma folate was positively associated with hemoglobin ($r^2 = 0.04$, $P = 0.003$) and marginally associated with the prevalence of anemia ($P = 0.07$; data not shown). For the women in the South and North, plasma folate and vitamin B-12 were negatively correlated ($\rho = -0.32$, $P = 0.01$ and $\rho = -0.21$, $P = 0.06$, respectively), but in Yaoundé/Douala, this relation was not observed ($\rho = 0.02$, $P = 0.85$).

Plasma folate concentration was not associated with women's physiologic status (pregnant, lactating, or nonpregnant, nonlactating), nationally or within regions. Folate status differed significantly for women based on SES quintile at the national level, with the poorest and poor having higher plasma folate concentrations than the average and wealthy in the national sample, as well as in the South and Yaoundé/Douala. However, in the North, the poor had lower plasma folate concentrations than the wealthy ($P < 0.001$).

Children. Markers of inflammation (CRP and AGP) and age were also unrelated to plasma folate in children. Nationally, 8.5% of the children were folate deficient. The prevalence of deficiency did not differ among regions; however, as in the women, mean plasma folate concentration was significantly higher in the South and did not differ between the North and Yaoundé/Douala (Table 2). Plasma folate was significantly higher ($P = 0.04$) among children without anemia, and the positive relation between plasma folate and hemoglobin concentration was not significant ($r^2 = 0.01$, $P = 0.13$; data not shown).

Plasma folate was negatively associated with SES among the children, with the poor and poorest quintiles having higher concentrations than the average, wealthier, and wealthiest quintiles in the South and North but not in Yaoundé/Douala.

Plasma and breast milk vitamin B-12 concentrations and risk factors for deficiency

Plasma vitamin B-12 concentrations of women and children were strongly correlated nationally (Spearman's $\rho = 0.45$, $P < 0.0001$) and within each region (South, $\rho = 0.41$; North, $\rho = 0.47$; Yaoundé/Douala, $\rho = 0.25$; all $P < 0.0001$).

Women. Plasma vitamin B-12 concentrations and measures of inflammation were not associated among the women in the national sample. On the basis of plasma vitamin B-12 concentrations, 29% of the women were vitamin B-12 deficient, and of those, 15% were severely deficient (≤148 pmol/L) (Table 2). The prevalence of deficiency did not differ between the South and North (28.8% and 40%, respectively), but in Yaoundé/Douala, the prevalence of deficiency was 11.1%, which was significantly lower compared with the other 2 regions ($P < 0.01$); almost no severe deficiency was observed in Yaoundé/Douala. Plasma vitamin B-12 and BMI were negatively correlated among women in Yaoundé/Douala ($\rho = -0.19$, $P = 0.04$), but this was not the case for the other regions. Women's plasma vitamin B-12 was not associated with their hemoglobin concentration or risk of anemia in the national sample, but when stratified by region, women's plasma vitamin B-12 was positively associated with their hemoglobin concentration and negatively with risk of anemia in Yaoundé/Douala ($P = 0.03$ and $P = 0.05$, respectively).

In the national sample, plasma vitamin B-12 was lower in pregnant women than in lactating or nonpregnant, nonlactating women ($P = 0.0001$ and $P = 0.002$, respectively). Pregnant women in the North and in Yaoundé/Douala ($P = 0.0015$) had a significantly lower mean plasma vitamin B-12 concentration than pregnant women in the South. Plasma vitamin B-12 among women in the national sample and in the South was related to SES, with the women in the poorest and poor households having significantly lower vitamin B-12 concentrations than women in the average, wealthier, and wealthiest households.

Children. Plasma CRP and AGP were not associated with children's plasma vitamin B-12 once the children's age was

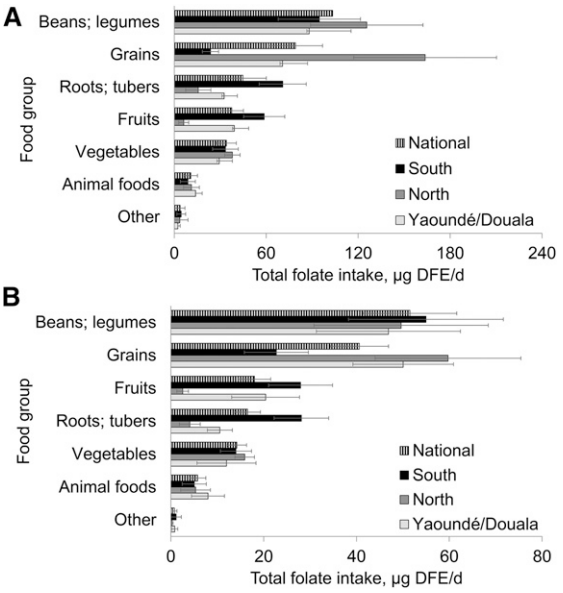

FIGURE 1 Contributions of food groups to total folate intake among Cameroonian women of reproductive age (A) and children 12–59 mo of age (B), nationally and by geographic region (South, North, and Yaoundé/Douala). Values are means and 95% CIs, without adjustment for intraindividual variation in intake; $n = 972$ person-days of data for women and $n = 937$ person-days of data for children. DFE, dietary folate equivalent.

TABLE 3 Usual intake distributions of energy, folate, and vitamin B-12 for women of reproductive age and breastfeeding and nonbreastfeeding children 12–59 mo of age in Cameroon, nationally and by region[1]

	National[2]	South	North	Yaoundé/Douala
Women				
n	902	305	300	297
Energy, kcal/d	2180 ± 20	2090 ± 20	2450 ± 30	1960 ± 20
Energy from ASF, %	9.3 ± 0.2	8.7 ± 0.3	8.9 ± 0.4	10.9 ± 0.4
Total food folate, μg/d	285 ± 5	281 ± 5	337 ± 10	214 ± 5
DFE, μg/d	300 ± 5	289 ± 6	351 ± 10	246 ± 7
Inadequate folate intake,[3] %	59 ± 2	65 ± 3	37 ± 7	79 ± 4
Total vitamin B-12, μg/d	4.50 ± 0.13	4.90 ± 0.21	3.09 ± 0.18	5.83 ± 0.26
Inadequate vitamin B-12 intake,[3] %	31 ± 4	26 ± 4	45 ± 4	22 ± 3
Total adjusted vitamin B-12,[4] μg/d	1.89 ± 0.04	1.92 ± 0.06	1.66 ± 0.07	2.17 ± 0.07
Inadequate adjusted vitamin B-12 intake,[3,4] %	62 ± 1	61 ± 2	69 ± 3	51 ± 2
Breastfeeding children				
n	108	25	72	11
Energy, kcal/d	530 ± 10	530 ± 20	510 ± 10	690 ± 30
Energy from ASF, %	7.5 ± 0.5	7.1 ± 0.6	7.5 ± 0.6	9.6 ± 0.8
Total food folate, μg/d	61 ± 3	57 ± 3	64 ± 3	60 ± 4
DFE, μg/d	69 ± 3	64 ± 3	70 ± 3	76 ± 4
Unadjusted vitamin B-12, μg/d	0.76 ± 0.06	1.02 ± 0.09	0.55 ± 0.05	1.60 ± 0.16
Adjusted vitamin B-12,[4] μg/d	0.54 ± 0.04	0.64 ± 0.05	0.45 ± 0.04	0.98 ± 0.08
Nonbreastfeeding children				
n	677	251	197	229
Energy, kcal/d	1130 ± 10	1130 ± 20	1180 ± 20	1080 ± 20
Energy from ASF, %	8.9 ± 0.3	8.6 ± 0.3	9.3 ± 0.5	9.0 ± 0.4
Total food folate, μg/d	136 ± 2	138 ± 3	152 ± 5	111 ± 3
DFE, μg/d	146 ± 3	145 ± 3	158 ± 5	132 ± 4
Inadequate folate intake,[3] %	39 ± 3	38 ± 3	35 ± 3	44 ± 3
Total vitamin B-12, μg/d	2.19 ± 0.07	2.41 ± 0.09	1.50 ± 0.12	2.57 ± 0.14
Inadequate vitamin B-12 intake,[3] %	29 ± 3	23 ± 4	41 ± 4	27 ± 3
Total adjusted vitamin B-12,[4] μg/d	1.23 ± 0.03	1.29 ± 0.03	1.02 ± 0.07	1.37 ± 0.05
Inadequate adjusted vitamin B-12 intake,[3,4] %	34 ± 4	29 ± 5	44 ± 4	31 ± 3

[1] Values are means ± SEs. Usual intake distributions were estimated using the National Cancer Institute method to adjust for intraindividual (day-to-day) variation in dietary intake. Usual intakes are presented for all national survey participants for whom valid 24-h dietary recall data were available, rather than just the subset selected for biochemical assessment. ASF, animal source food; DFE, dietary folate equivalent.
[2] Represents a weighted mean of the South, North, and Yaoundé/Douala.
[3] Inadequate intake was defined as <320 μg DFE/d and <2 μg/d vitamin B-12 for women (estimated average requirement for nonpregnant, nonlactating women aged >19 y) and as <120 μg DFE/d and <0.70 μg/d vitamin B-12 intake for children (estimated average requirement for children aged 1–3 y) (20). No women or children had excessive folic acid intakes, defined as >1000 μg folic acid/d for women (upper limit for women aged >19 y) or >300 μg folic acid/d for children (upper limit for children aged 1–3 y). The prevalence of excessive intakes was not calculated for vitamin B-12 because no upper limit has been defined for vitamin B-12. Breast milk consumption was not estimated; thus, the prevalence of inadequate nutrient intakes was not calculated for breastfeeding children.
[4] Total vitamin B-12 intake was adjusted to account for lower absorption from large doses of vitamin B-12, as described in the text.

taken into account in the regression model. Vitamin B-12 deficiency (based on plasma concentrations) was present in 30% of the children in the national sample, and 15% of children were severely deficient (≤148 pmol/L). The prevalence of deficiency was significantly higher in the North than in Yaoundé/Douala, with the South not differing from either. There were no significant differences in plasma vitamin B-12 between the rural and urban areas. Hemoglobin concentration and prevalence of anemia in children were significantly associated with plasma vitamin B-12 in the national sample ($P = 0.006$ and $P = 0.03$, respectively) but not within zones. In the North, being among the poorest quintile was a significant risk factor for low plasma vitamin B-12 concentrations ($P < 0.0001$; data not shown).

The regional patterns of breast milk vitamin B-12 concentrations were comparable to those for plasma; in the North, the vitamin concentration in milk was significantly and substantially lower than in the South or Yaoundé/Douala.

Folate and vitamin B-12 intake

Controlling for child age (in regression models of single-day intakes), there was no difference in consumption of energy, folate, or vitamin B-12 among women and children who were or were not selected for biochemical analysis of folate and vitamin B-12 status; thus, we present dietary intake data for the entire study population (n = ~900 households). Among women, folate intake was higher in the North than in the South and Yaoundé/Douala. Dietary vitamin B-12 and folate were positively associated; however, this relation became negative once energy intake was included as a covariate in the model.

Folate intake is reported as food folate from nonfortified foods or DFEs, which include folic acid from the few available fortified foods (**Figure 1** and **Table 3**). The prevalence of inadequate usual folate intake among women was 59% nationally and 79% in Yaoundé/Douala. This was different in the North, where fewer women had intakes below the EAR compared with the South and Yaoundé/Douala. Nonbreastfeeding children's

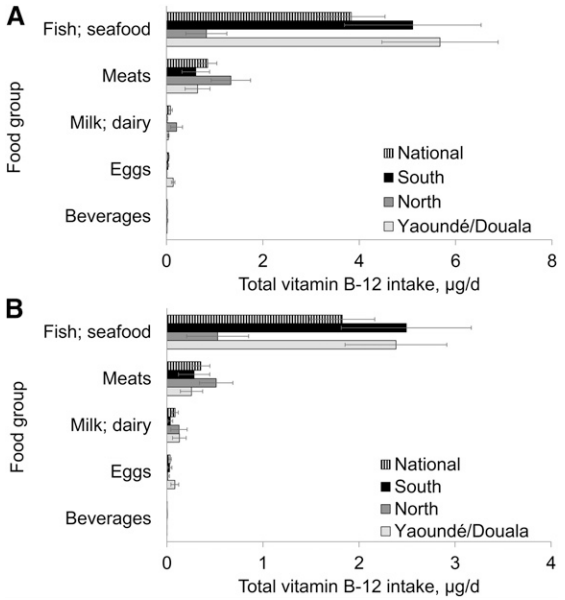

FIGURE 2 Contributions of food groups to adjusted total vitamin B-12 intake among Cameroonian women of reproductive age (A) and children 12–59 mo of age (B), nationally and by geographic region (South, North, and Yaoundé/Douala). Values are means and 95% CIs, without adjustment for intraindividual variation in intake; n = 972 person-days of data for women and n = 937 person-days of data for children.

intake was slightly more adequate, with 39% below the EAR nationally and 44% in Yaoundé/Douala. The children's mean intake and prevalence of inadequate intakes were similar across regions.

Beans and legumes, grains, fruit, roots and tubers, and vegetables contributed 98% of total folate intake of women and children, and the rest came from animal source foods (Figure 1). The folate sources were different among regions: in the North, grains provided ~50% of total folate, whereas grains contributed only 8% in the South and 26% in Yaoundé/Douala. Consumption of folate from beans, other legumes, and vegetables was similar among regions; however, only small amounts of folate were derived from fruit and root vegetables in the North (Figure 1).

After adjustment for absorption, mean usual vitamin B-12 intake among women was 1.66 μg/d in the North compared with 2.17 μg/d in Yaoundé/Douala, consistent with the mean plasma concentrations of vitamin B-12 in the 2 regions (Table 3). The usual total vitamin B-12 intake amounts were 20–270% higher than the corresponding amounts after adjustment for absorption, depending on the region and group (women compared with children). As expected, vitamin B-12 intake was strongly and positively associated with total energy intake from animal source foods (r^2 = 0.72, P < 0.001). At the national level as well as by region, the dietary intake data revealed that a high proportion of women and children had vitamin B-12 intakes below the EAR (Table 3). Fish and seafood were the main sources of vitamin B-12 in the national sample, the South, and Yaoundé/Douala (**Figures 2** and **3**). In the North, where cattle, sheep, and goat herding is common, meat provides 50% of the total vitamin B-12 in the diet, whereas for the other regions, <11% of vitamin B-12 intake was from this source. Eggs, milk, and some insects and fortified foods provided smaller amounts of vitamin B-12. In the South, infant's plasma vitamin B-12 was predicted by breastfeeding status rather than by dietary vitamin B-12 intake; status was better in nonbreastfeeding infants.

Discussion

We report national and regional estimates of dietary intake and biochemical status of folate and vitamin B-12 for women of reproductive age and young children in Cameroon. Nationally, low folate status occurred in about 17% of the women but was less common (9%) among children. Vitamin B-12 deficiency was more prevalent than folate deficiency, being present in around one-third of both women and children. The study suggests that higher intakes of both nutrients are needed, potentially through a fortification program.

In general, for each nutrient, the same risk factors identified population groups with inadequate dietary intakes and biochemical evidence of deficiency. The prevalence of both deficiencies varied by region, SES, and residence in urban or rural areas; however, the risk factors for low folate and low vitamin B-12 status were different. Folate status was better among women with poor SES households and in the North, where folate intake was adequate or high with proportion of inadequate intakes, because of the larger amount of grains consumed. In Yaoundé/Douala, three-fourths of the women had inadequate folate intake and the prevalence of deficiency significantly higher than in the 2 other regions.

In contrast to the picture for folate, vitamin B-12 status was substantially poorer for women and children living in the North. Two-thirds of the women and 40% of nonbreastfeeding children consumed less than the EAR in this region, although the proportion of energy from animal source foods was similar to the other regions. In addition, vitamin B-12 status was worse in low SES households and for children whose mothers had low plasma concentrations.

Maternal and child vitamin B-12 status were strongly correlated, as we have observed in other populations (27). This is likely explained in part by the strong contribution of maternal pregnancy vitamin B-12 status to infant stores of the vitamin at birth

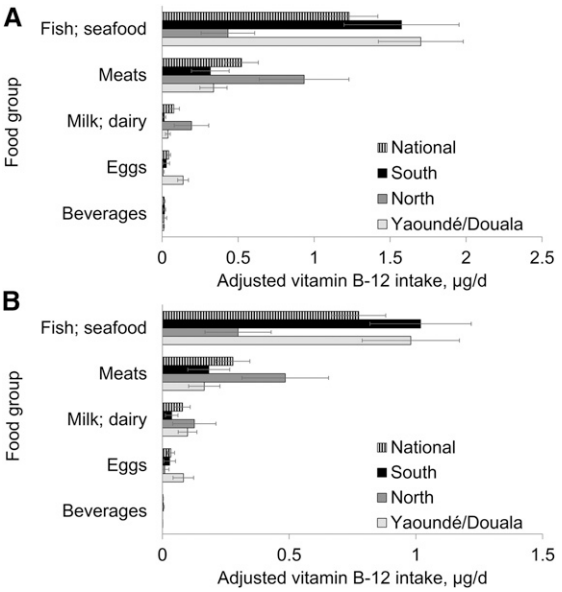

FIGURE 3 Contributions of food groups to unadjusted total vitamin B-12 intake among Cameroonian women of reproductive age (A) and children 12–59 mo of age (B), nationally and by geographic region (South, North, and Yaoundé/Douala). Values are means and 95% CIs, without adjustment for intraindividual variation in intake; n = 972 person-days of data for women and n = 937 person-days of data for children.

and, as demonstrated in Cameroon, by the relations between maternal vitamin B-12 intake from animal source foods, maternal plasma B-12, breast milk vitamin B-12, and infant vitamin B-12 status. Breast milk vitamin B-12 concentration could be a valuable indicator of maternal vitamin B-12 intake and status and is a predictor of infant status. It might also serve as a useful indicator of the effectiveness of the Cameroon fortification program, which could be tested during program evaluation.

Although it is clear that deficiencies of both vitamins were common in Cameroon (except folate among children), estimates of the absolute prevalence of folate deficiency were quite different based on prevalence of inadequate intakes compared with plasma concentrations, whereas for vitamin B-12, the prevalence estimates were similar. We do recognize that the 1–2 days of intake data collected on each individual may not represent usual intake. However, because duplicate 24-h recalls were conducted in a subset of participants, we were able to adjust statistically for intraindividual variation in nutrient intakes to produce better estimates at the regional level.

One study estimated that the birth prevalence of neural tube defects at 3 main hospitals in Yaoundé was 2 per 1000 (28), much greater than that observed in the United States before folic acid fortification but similar to that observed in Jordan prefortification (29). Although the findings represent a selected population, they are consistent with the results of the current study in suggesting that addressing vitamin B-12 and folate deficiencies among women may have a beneficial impact on the incidence of neural tube defects.

In 2011, the government of Cameroon instituted mandatory fortification of wheat flour (most of which is imported and milled domestically) with iron, zinc, folic acid, and vitamin B-12. The important differences in risk factors for deficiency of folate compared with vitamin B-12 have implications for the extent to which fortification of a single food vehicle will effectively reach individuals with biochemical nutrient deficiency. Data from this study also showed that wheat flour intake was greatest in Yaoundé/Douala (12); thus, fortification of wheat flour would be useful for targeting individuals with low folate status. Although wheat flour fortification would not specifically target those in the North with lower vitamin B-12 status, wheat flour consumption was still common in the North, where ~90% of women reported consuming wheat flour products in the past week, with a mean frequency of ~7 times/wk. Thus, flour fortification would be expected to improve status of both folate and vitamin B-12. However, if the amount of each nutrient in wheat flour is not sufficient to reduce deficiency, targeted approaches may be needed for each region. Efforts in the North may then need to focus on folate and particularly vitamin B-12, whereas the focus in Yaoundé/Douala may need to be on folate only.

Dietary and biochemical data suggest that folate deficiency is relatively uncommon among children in Cameroon; thus, after folic acid fortification of wheat flour, it would be important to monitor their folate intake and status to ensure that they do not consume excessive amounts of folic acid (30). Cofortification with vitamin B-12 could reduce the potential risk of excess folic acid exacerbating vitamin B-12 deficiency (29).

This is one of the first surveys to our knowledge to collect detailed information on micronutrient status and dietary intake from a nationally representative sample to inform the development of a national food fortification program. Among countries that have conducted national micronutrient surveys, few have assessed folate and vitamin B-12 status, despite the widespread inclusion of folic acid in food fortification programs globally. As illustrated by these data from Cameroon, such information is critical to justify the need for fortification, assess whether the deficient population subgroups will benefit from the program, and provide a baseline against which to monitor program impact.

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