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Dietary intake of sulfur amino acids and risk of kwashiorkor malnutrition in eastern Democratic Republic of the Congo

Merry C Fitzpatrick,[1,2] Anura V Kurpad,[3] Christopher P Duggan,[4] Shibani Ghosh,[1] and Daniel G Maxwell[1,2]

[1]Friedman School of Nutrition Science and Policy, Tufts University, Boston, MA, USA; [2]Feinstein International Center, Tufts University, Boston, MA, USA; [3]Division of Nutrition, St John's Research Institute and St John's Medical College, Bengaluru, Karnataka, India; and [4]Center for Nutrition, Division of Gastroenterology, Hepatology and Nutrition, Boston Children's Hospital, Boston, MA, USA

ABSTRACT

Background: Kwashiorkor is an often-fatal type of severe acute malnutrition affecting hundreds of thousands of children annually, but whose etiology is still unknown. Evidence suggests inadequate sulfur amino acid (SAA) status may explain many signs of the condition but studies evaluating dietary protein intake in relation to the genesis of kwashiorkor have been conflicting. We know of no studies of kwashiorkor that have measured dietary SAAs.

Objectives: We aimed to determine whether children in a population previously determined to have high prevalence of kwashiorkor [high-prevalence population (HPP)] have lower dietary intakes of SAAs than children in a low-prevalence population (LPP).

Methods: A cross-sectional census survey design of 358 children compared 2 previously identified adjacent populations of children 36–59 mo old in North Kivu Province of the Democratic Republic of the Congo. Data collected included urinary thiocyanate (SCN), cyanogens in cassava-based food products, recent history of illness, and a 24-h quantitative diet recall for the child.

Results: The HPP and LPP had kwashiorkor prevalence of 4.5% and 1.7%, respectively. A total of 170 children from 141 households in the LPP and 169 children from 138 households in the HPP completed the study. A higher proportion of HPP children had measurable urinary SCN (44.8% compared with 29.4%, $P < 0.01$). LPP children were less likely to have been ill recently (26.8% compared with 13.6%, $P < 0.01$). Median [IQR] intake of SAAs was 32.4 [22.9–49.3] mg/kg for the LPP and 29.6 [18.1–44.3] mg/kg for the HPP ($P < 0.05$). Methionine was the first limiting amino acid in both populations, with the highest risk of inadequate intake found among HPP children (35.1% compared with 23.6%, $P < 0.05$).

Conclusions: Children in a population with a higher prevalence of kwashiorkor have lower dietary intake of SAAs than children in a population with a lower prevalence. Trial interventions to reduce incidence of kwashiorkor should consider increasing SAA intake, paying particular attention to methionine. *Am J Clin Nutr* 2021;114:925–933.

Keywords: kwashiorkor, edematous malnutrition, methionine, cysteine, protein, diet, Congo, 24-h recall

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Supplemental Figure 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

Address correspondence to MCF (e-mail: merry.fitzpatrick@tufts.edu).

Abbreviations used: AA, amino acid; DRC, Democratic Republic of the Congo; EAR, estimated average requirement; GAG, glycosaminoglycan; GSH, glutathione; HAZ, height-for-age *z* score; HPP, high-prevalence population; LPP, low-prevalence population; MoH, Ministry of Health; MUAC, midupper arm circumference; SAA, sulfur amino acid; SAM, severe acute malnutrition; SCN, thiocyanate; WAZ, weight-for-age *z* score; WHZ, weight-for-height *z* score.

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Introduction

Kwashiorkor, also known as edematous malnutrition, is 1 of 2 major classifications of severe acute malnutrition (SAM) and is estimated to affect hundreds of thousands of children each year (1, 2). In eastern Democratic Republic of the Congo (DRC), the majority of SAM is kwashiorkor, with >10,000 cases of kwashiorkor treated annually in North Kivu Province alone (3, 4).

Although formally described >80 y ago, kwashiorkor's etiology remains uncertain and there are no effective preventive interventions other than a well-balanced diet (1, 2). Protein deficiency is the longest-standing proposed causal factor because kwashiorkor exists only in areas where protein intake is low, but the role of protein intake is debated and evidence has been inconclusive (5–13). Gopalan (8) found that 30 children developing kwashiorkor were not eating "qualitatively different diets" than those not developing kwashiorkor, but were at the "lower limit of the range of intakes" recorded by the study (p. 53). Subsequent studies using food-frequency measures did not find differences in protein intake (14–16). Two studies using quantitative 24-h recalls found households with a case of kwashiorkor had lower protein intakes than those without a case (17, 18).

Roediger and Waterlow (19) suggested kwashiorkor may be due to "a patterned deficiency of amino acids" rather than a "global deficiency of amino acids" (p. 130). They proposed a deficiency of sulfur amino acid (SAA) intake, methionine and cysteine, as central to the etiology of kwashiorkor. Children with kwashiorkor have low circulating SAAs and altered SAA metabolism (20–24). The richest sources of SAAs are animal source proteins whereas common vegetable protein sources such as beans and soy are particularly low in SAAs (25). In regions where very-low-protein starchy tubers or plantains are the primary complement to beans rather than a grain, and where very little animal protein is eaten, the amino acid (AA) profile of beans will dominate the profile of the diet. SAAs are then likely to be the first limiting AAs. Cassava, a major staple often associated with kwashiorkor, naturally contains linamarin, a cyanogenic glucoside (9). When the leaves or tubers are ground, linamarin rapidly decomposes through enzymatic hydrolysis to cyanohydrin and then hydrogen cyanide. Cyanide then requires sulfur derived substantially from cysteine to detoxify it, further increasing the SAA intake requirement (9, 26).

To our knowledge, no studies on the relation between diet and kwashiorkor, other than the 2 aforementioned studies, have used quantitative 24-h recalls and none have considered the adequacy of individual AAs in those diets. The aim of this study was to test the hypothesis that SAA intake is inadequate for more children in populations previously determined to have a high prevalence of kwashiorkor than in those with a low prevalence.

Methods

This study was conducted in the Malehe/Murambi Health Area, Kirotshe Health Zone, North Kivu Province, DRC, comparing 2 adjacent populations of children 36–59 mo old, using a cross-sectional census survey design. Data for this study were collected during July and August, the period when the least rain falls and when beans are being harvested.

The primary outcome of interest was the difference in intake of SAAs between a high-prevalence population (HPP) and a low-prevalence population (LPP). All other outcomes noted in this article were secondary outcomes. One secondary outcome was exposure to cyanide derived from cyanogenic glucosides found in cassava. The cyanogenic glucosides, cyanohydrin and hydrogen cyanide, will be generally referred to as cyanogens except where 1 of these compounds is specifically indicated.

Ethical reviews and consent

Formal review and approval of this study were provided by the Tufts University Social, Behavioral, and Educational Research Internal Review Board in Boston, Massachusetts and the *Université Catholique de Bukavu (UCB) Commission Institutionnelle d'Ethique* in Bukavu, DRC. Caregivers provided verbal informed consent and permission for all registered children.

Recruitment and sample selection

A separate anthropometric census survey conducted in June 2016 identified 2 neighboring villages in the Murambi Health Area, 1 with a kwashiorkor prevalence of 0% and another with a prevalence of 9.6% among children 12–59 mo old (27). This study compares these 2 populations, with the addition of 1 small area contiguous with the higher-prevalence population to ensure a large enough sample size. The 2 populations shared the same climate, market, water table, Health Center, transportation, language, school system, and often even the same churches, reducing these as potential factors affecting the outcomes of interest. To minimize within-group variation, the age range for this study was narrowed to 36–59 mo old (4). Age was the only inclusion criterion. The only exclusion criterion was if the caregiver reported the child was being treated for an illness that had lasted for >6 mo, for example, tuberculosis, but no children fit this criterion. If a household contained >1 eligible child, all were included.

Of all outcome variables, urinary thiocyanate (SCN) required the largest estimated sample size. A sample size of 145/comparison group ($n = 290$) was required to detect a difference in urinary SCN of 26 mMol/L, with an SD of 80 mmole/L using 2-sided $\alpha = 0.05$ and power = 0.8, similar to those found in other studies on cassava-consuming populations (28–31). To account for potential missing samples, the target sample size was increased by 25% to 180 children/comparison group (LPP compared with HPP).

Household data collection

Enumerator training.

Enumerators were trained in taking anthropometric measurements and evaluating the signs of kwashiorkor. They were shown numerous live examples of children during training to ensure a common understanding. An experienced health staff from the Ministry of Health (MoH) supported the training and followed up on the enumerators during data collection to ensure they were correctly measuring the children and assessing the signs of kwashiorkor.

Enrollment and anthropometry.

Enumerators passed from door to door in the targeted areas, seeking all eligible children and their caregivers. If a caregiver was not present, the enumerator passed again at a later time.

Upon receiving verbal consent from the caregiver, the enumerator enrolled the child, recording the caregiver's name and the child's name, age, and sex.

The enumerators also measured each child's height to the nearest 1 mm using standard UNICEF stadiometers. Birth date, as reported by the caregiver, was recorded to the nearest month. Children were weighed without shoes or heavy clothing to the nearest 100 g using digital scales (model Tian Shan—2003B). Midupper arm circumference (MUAC) was measured to the nearest millimeter. Weight was measured 3 times and MUAC twice with the average of each respective measurements used for analysis. If there was a difference of >200 g or >2 mm between measurements, the surveyors were instructed to repeat the measurements.

Pitting edema was measured by pressing a thumb firmly against the skin and holding it for 5 s. If a visible dimple was left, the child was categorized for edema. Changes to hair were evaluated visually, sometimes wiping the hair clean with water if the child was especially dusty. Light-colored unkinked hair, facial edema, and bilateral pitting edema in the feet were recorded as potential signs of kwashiorkor (32). Diagnosis of kwashiorkor used only bilateral pitting edema in the feet in accordance with the criteria for admission to nutrition treatment (33). At the end of the registration process, the enumerator scheduled an appointment to return for a longer interview.

Twenty-four-hour diet recall and caregiver interview.

Enumerators received extensive training in conducting the dietary recall and full interview, with practice sessions. We used a single quantitative 24-h diet recall of the child. The diet recall used the USDA Multi-Pass 24-h recall method with minor modifications to accommodate local meal preparation and eating habits (34). One recall per child was used. Recalls were timed to ensure weekdays and weekends were proportionally represented. Caregivers were asked to use their own pots and plates along with models. They used these to demonstrate volumes of each ingredient used in the preparation of each dish, the prepared food for the family, and the cooked food that went to the child's plate as well as amounts left uneaten on the child's plate. If multiple people ate from the child's plate, adult male equivalents were used to estimate the child's portion of that serving, calculated based on equivalents of energy by age and sex, following the procedures used by Smith and Subandoro (35). Enumerators also asked specifically about food the child ate outside of meals.

Other data collected from the caregiver included sociodemographic data of the household (the household being defined as those who eat and sleep regularly in the house), as well as the child's recent health history, defecation habits, and attendance at vaccination and growth monitoring days (36).

Urine samples

An evening urine sample was requested from each child within 7 d of the diet recall. During focus group discussions and key informant interviews, caregivers reported that most cyanogenic foods (i.e., cassava products) were eaten at the evening meal. Cyanogens are quickly metabolized and excreted in the urine as SCN; therefore, a urine sample was collected from the child between the main evening meal and going to bed (37).

On the evening of the urine collection, coolers with ice were prepositioned near the children's homes. That same evening, local MoH staff delivered a receptacle with the child's unique identifier on it to each home and instructed mothers on how to collect the children's urine samples. Caregivers were instructed to take each sample to a collection point as soon as it was collected. The urine samples were collected from the villages in the morning and kept on ice until they could be frozen later in the morning.

Food sample collection

To assess cyanide exposure, enumerators collected samples of uncooked cassava flour from 28 randomly selected households in the HPP and 27 households in the LPP. Ten households from each population provided samples of cooked cassava leaves.

Samples of staples, greens, and primary protein sources were purchased in the market where both study populations bought and sold produce. Samples of fresh, immature beans were purchased in the study area because they were not available in the market. Portions of the immature beans, taro, and sweet potatoes were cooked in an open pot of boiling water, recording volumes before and after cooking. Greens were air dried to prevent spoilage before analysis, with fresh and dried weights recorded.

Nutrient calculations

Samples of cassava flour, taro, sweet potatoes, potatoes, rice, ground nuts, dried beans (2 varieties), sorghum flour, maize flour, immature beans, dried cassava leaves, dried amaranth leaves, dried fish (*ndagala*), and smoked fish (*mbuta*) were analyzed by the University of Missouri-Columbia Agricultural Experiment Station Chemical Laboratories using AOAC Official Method 994.2 for the essential AA profile (plus cysteine) and AOAC Official Method 990.03 for crude protein. For all other foods, the AA composition and digestibility scores were taken from the USDA Food Composition Database and other literature (25, 26, 38–43). Although pig ileal digestibility scores are preferable, they are available for a limited range of foods, whereas rat fecal digestibility scores are widely available. For the sake of consistency and comparability, rat fecal digestibility scores were used for all foods except cassava leaves and fish-meal for which only pig ileal digestibility scores were available (39, 44).

Cassava roots in the study area are normally processed over several weeks through soaking, fermenting, drying, and pounding into a flour which is then stored. Samples of uncooked cassava flour from participating households were analyzed for total cyanide using Picrate Kit B2, sourced from Australia University, following Protocol B2 version 1.3. Picrate kit A in combination with Protocol E version 1.1 were designed for uncooked leaves, but cooked leaves would be more indicative of true cyanide intake (45). On the personal recommendation of Dr. Howard J Bradbury, the designer of the kit, the protocol was modified and cooked leaves were squeezed of moisture, then 100 mg of the squeezed leaves were added to the vial in place of the uncooked, unprocessed leaves normally used with the kit. These protocols converted the cyanogenic glucosides present in the plant material into cyanide (HCN) and the results are given in HCN equivalents, or "total cyanide."

Urine analysis

The evening urine sample was analyzed for SCN using a picrate kit (Kit D) sourced from Australia University following Protocol D1 version 1.3 (37, 45).

TABLE 1 Description of the population[1]

	Low-prevalence population		High-prevalence population	
Registered households (n = 301)	149		152	
Households interviewed[2]	141		138	
Household size		7.1 ± 0.20[b]		6.3 ± 0.17[b]
Registered children (n = 358)	181		177	
Children with interviews	170		169	
Female (n = 172)		89 (49)		83 (47)
Age, mo		47.7 ± 0.63[a]		45.7 ± 0.56[a]
Measures of the child				
WAZ	174	− 1.14 ± 0.09[a]	161	− 1.42 ± 0.08[a]
WAZ <−2		40 (23.0)		43 (26.7)
HAZ score	164	− 2.24 ± 0.13[a]	172	− 2.61 ± 0.10[a]
HAZ <−2		100 (60.9)		119 (69.2)
WHZ score	160	0.393 ± 0.07	160	0.237 ± 0.08
WHZ <−2		1 (0.6)		3 (1.7)
MUAC, mm	179	148.3 ± 0.9[a]	176	145.4 ± 0.9[a]
MUAC <125 mm		6 (3.3)		9 (5.1)
Edema in feet	181	3 (1.7)	177	8 (4.5)
Edema in face	181	26 (14.4)[a]	177	44 (24.9)[a]
Hair changes	181	17 (9.4)[c]	177	43 (24.3)[c]
Any signs	181	31 (17.1)[b]	177	58 (32.8)[b]
Health and environment characteristics				
Household had case in past 5 y	141	20 (14.5)[c]	138	48 (34.0)[c]
Child had no illness in past 30 d	178	45 (26.8)[b]	169	23 (13.6)[b]
Child attends health days at clinic	169	83 (49.1)[a]	166	103 (62.0)[a]
Child uses a latrine	170	63 (37.3)	169	52 (30.8)
Child has thiocyanate in urine	163	48 (29.4)[b]	146	65 (44.8)[b]

[1] Values are n, n (%), or mean ± SEM. HAZ, height-for-age z score; MUAC, midupper arm circumference; WAZ, weight-for-age z score; WHZ, weight-for-height z score.

[2] Some households had >1 malnourished member in the past 5 y, not all households answered all questions.

[a–c] Significantly different between populations: [a] $P \leq 0.05$, [b] $P \leq 0.01$, [c] $P \leq 0.001$, using Student's t test for differences in means, Wilcoxon's rank-sum (Mann–Whitney U) test for differences in medians, and chi-square test for differences in proportions.

Data analysis

z Scores for weight-for-age (WAZ), weight-for-height (WHZ), and height-for-age (HAZ) were calculated using the WHO Anthro module of commands with Stata 13 SE (StataCorp LLC) (46, 47). z Scores were calculated based on the WHO Child Growth Standards (46). Children were considered underweight if their WAZ was <-2, stunted if their HAZ was <-2, and wasted if their WHZ was <-2 or if they had a MUAC measurement <125 mm. Measurements were dropped by the software as implausible if the z score was <-6 or $>+6$ for height-for-age, <-6 or $>+5$ for weight-for-age, or <-5 or $>+5$ for weight-for-height.

Percentages of children at risk of inadequacy were calculated as the total number of children whose total estimated intake of a nutrient was below the estimated average requirement (EAR) divided by the total number of children with diet recalls. The protein energy ratio was calculated using the following formula: (crude protein grams × 4)/total kilocalorie intake. The requirements for energy intake used the FAO guidelines, accounting for age, weight, and sex (48).

The reference protein quality (mg AA/g protein) used for cysteine and methionine, and the intake requirements for AAs (mg · kg body weight^{-1} · d^{-1}), were extrapolated from the ratio of cysteine and methionine recommended for adults by the 2007 FAO/WHO/UNU Expert Consultation (49). This Consultation recommended 14.5 mg · kg^{-1} · d^{-1} for SAAs for adults, within which ≥10.4 mg · kg^{-1} · d^{-1} should come from methionine and the remaining 4.1 mg · kg^{-1} · d^{-1} from cysteine. We used this methionine:cysteine ratio, extrapolated to the 2013 FAO Expert Consultation's requirement of 17 mg · kg^{-1} · d^{-1} for children 3–10 y old, to arrive at 12.19 mg · kg^{-1} · d^{-1} for methionine and 4.81 mg · kg^{-1} · d^{-1} for cysteine (50). Bioavailable protein was calculated according to the method outlined by the FAO and WHO (49, 50).

Stata 12 IC (StataCorp LLC) was used for all statistical analysis other than the Anthro calculations when Stata 13 SE (StataCorp LLC) was used. As is commonly observed, dietary intake data were skewed with a long right tail. Where data were not normally distributed, medians with their IQRs were reported and nonparametric tests for differences (Wilcoxon's rank-sum test/Mann–Whitney U test) used. For parameters that were normally distributed, means ± SEMs were reported, and Student's t test for differences in means was used. The chi-square test for differences was used for proportions when all cells had ≥5 observations, otherwise Fisher's exact test was used.

Results

Table 1 presents the characteristics of the study participants and their households. A total of 358 children 36–59 mo old within 301 households were registered in July 2016 and all data gathered in July and August 2016 (**Supplemental Figure 1**). From these, caregivers representing 279 households and 347 children completed interviews, although dietary recall sections

TABLE 2 Macronutrient intake comparison of the 2 populations[1]

	Low-prevalence population ($n = 165$)	High-prevalence population ($n = 168$)	EAR
Energy intake, kcal/d	1171 [777–1484][b]	1015 [703–1396][b]	
Children below requirement for energy	72 (43.6)	90 (53.6)	
Protein, $g \cdot kg^{-1} \cdot d^{-1}$	1.78 [1.24–2.59][bd]	1.47 [0.81–2.22][bd]	0.73
Digestibility-corrected protein, $g \cdot kg^{-1} \cdot d^{-1}$	1.36 [0.98–2.0][bd]	1.17 [0.64–1.80][bd]	0.73
Bioavailable protein,[2] $g \cdot kg^{-1} \cdot d^{-1}$	1.02 [0.75–1.57][ad]	0.93 [0.53–1.50][ad]	0.73
Children with bioavailable protein intake below requirement ($0.73\ g \cdot kg^{-1} \cdot d^{-1}$)	38 (23.0)[c]	66 (39.3)[c]	
Protein:energy ratio, %	7.4 [4.9–9.3][bd]	6.3 [4.2–8.1][bd]	5.0

[1] Values are median [IQR] or n (%) unless otherwise indicated. EAR, estimated average requirement.
[2] Using separate requirements for cysteine and methionine.
[a–c] Significantly different between populations: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, chi-square test for differences in proportions and Mann–Whitney U test for differences in medians. [d] Significantly different from the EAR using 1-sample Wilcoxon's signed rank test, $P < 0.001$.

of the survey were completed and accepted for only 333 children. Mean household size was significantly larger in the LPP than in the HPP. Mean age of children in the LPP was ~2 mo older than in the HPP ($P < 0.05$). Registered households who did not complete the interviews did not have significantly different anthropometric measurements from those who did complete the interviews and were approximately equally divided between the 2 populations. Reasons for not completing the interview were time burdens on the mother and travel out of the village.

A previously reported survey for this population showed the HPP and LPP had significant differences in prevalence of kwashiorkor (27). The HPP sample for this study had a prevalence of kwashiorkor cases of 4.5% compared with 1.7% for the LPP, but this sample size was too small to detect a statistical difference. Nevertheless, other signs of kwashiorkor (hair changes and facial edema), as well as a family history of kwashiorkor, were significantly different and followed the same trend. Anthropometric measurements showed that although children in the HPP had a lower mean WAZ, this population did not have significantly more children below the −2SD threshold for low WAZ. The lower mean WAZ is primarily attributable to a significantly lower mean HAZ in the HPP. Although both populations clearly exceeded the WHO "critical" benchmark of 40% stunting, and the mean HAZ for both populations was <-2, few enrolled children in either study population were wasted (36). Households in the HPP were twice as likely to have had a case of kwashiorkor in the previous 5 y. This indicates that the differences in the nutritional status of the populations were long-term. More children in the LPP had not been ill during the previous 30 d, as reported by the caregiver, but children in the HPP were more likely to have attended growth monitoring and vaccination days at the local health clinic or post. Although the difference was not significant, latrine use among the children sampled was higher in the LPP.

Only 30% of the HPP and 9% of the LPP cassava flour samples had detectable concentrations of cyanide. All of the samples with detectable concentrations had cyanide measurements ≤5 ppm (well below the recommended limit of 10 ppm), indicating the flour had been well processed and was not a significant potential source of cyanide exposure (51). Cyanide concentrations from the cooked cassava leaves were much higher. Those from the HPP had almost 3 times the mean concentration of cyanide as those from the LPP (107.5 ± 97.7 ppm compared with 37.0 ± 58.6 ppm, $P = 0.0159$). Whereas all samples from the HPP had detectable cyanide, only 60% of the LPP samples had detectable concentrations. Not only did the leaf samples in the HPP have higher mean concentrations of cyanide, children in the HPP ate cassava leaves on average 31.4% more frequently than those in the LPP ($P < 0.01$). Children in the HPP were more likely to have measurable SCN (the urinary metabolite of cyanide) in their urine and had a higher mean urinary concentration (0.88 ± 1.46 ppm compared with 0.53 ± 1.11 ppm, $P < 0.05$).

Table 2 shows a comparison of dietary protein and energy intakes. The children from the LPP consumed significantly more energy than those in the HPP, although the difference in the proportion of children below their individual requirements was not significant. Median bioavailable protein intake was significantly lower in the HPP although well above the requirement of 0.73 g protein · kg body weight^{-1} · d^{-1} (50). Significantly more children in the HPP were below this protein intake requirement and their protein:energy ratio was lower than for children in the LPP, although still just above the requirement.

Table 3 examines the quality of the protein in the diets, comparing it against the scoring pattern of AA requirements, a type of reference protein (49, 50). SAAs are the limiting AAs in the diets of both populations, specifically methionine. The guiding hypothesis for this study addressed SAAs as a combined requirement rather than individual AAs. The differences between the population intakes of methionine and cysteine individually, rather than as the combined intake of SAAs, and the implications of this distinction, were discovered during post hoc analysis.

Table 4 compares the AA intake of the diets, and their sufficiency. The median intake for all AAs (presented as mg · kg^{-1} · d^{-1}) exceeded the WHO minimum requirements. Intakes for all AAs were significantly higher in the LPP. A higher proportion of children in the HPP had intakes below the EAR for all AAs except isoleucine and the aromatic AAs. Although the profile in Table 3 shows methionine to be less limiting for the HPP, lower total protein intake in combination with protein that is very low in methionine results in lower total methionine intake in the HPP and there was a significantly higher proportion of children in the HPP with methionine intake below the EAR.

TABLE 3 Comparison of median protein quality by population[1]

	Mg/g protein			Ratio of AA:reference	
	LPP ($n = 165$)	HPP ($n = 168$)	Reference	LPP	HPP
Tryptophan	11.5 [11.0–12.9]	11.5 [11.0–12.7]	6.6	1.75	1.74
Histidine	26.4 [24.8–27.8]	25.3 [22.8–27.9]	16	1.65	1.58
Threonine	39.1 [37.1–40.5]	37.1 [33.9–40.2]	25	1.56	1.48
Isoleucine	46.1 [44.3–47.9]	44.6 [40.6–47.3]	30	1.53	1.49
Leucine	75.8 [70.1–81.7]	72.4 [62.1–80.5]	61	1.24	1.19
Lysine	66.0 [60.4–70.2]	65.3 [58.0–69.9]	48	1.37	1.36
Sulfur AAs	23.1 [21.5–27.0]	25.5 [22.2–28.9]	23	1.00	1.11
Cysteine	10.2 [9.7–11.3]	10.4 [9.7–11.8]	6.50[2]	1.57	1.60
Methionine	12.7 [11.1–14.8][a]	14.0 [11.6–18.1][a]	16.50[2]	0.77	0.85
Aromatic AAs[3]	82.5 [74.2–94.5]	77.8 [65.5–88.0]	41	2.01	1.90
Valine	52.0 [48.2–55.9]	48.5 [41.8–55.0]	40	1.30	1.21

[1]Values are median [IQR] unless otherwise indicated. AA, amino acid; HPP, high-prevalence population; LPP, low-prevalence population.
[2]The references (mg/g protein) used for cysteine and methionine were extrapolated from a ratio of methionine to cysteine of 2.54:1 (50).
[3]Tyrosine and phenylalanine.
[a]Significantly different from the requirement, using 1-sample Wilcoxon's signed rank test, $P < 0.001$.

Discussion

The results of this study support the hypothesis that children in a population with a high prevalence of kwashiorkor have lower SAA intake than children with a low prevalence of kwashiorkor. Methionine, in particular, was the first limiting AA. The HPP had a higher proportion of children with low bioavailable protein intake, but the proportions with low energy intake in the 2 populations were not significantly different.

Although children in the HPP were more likely to have inadequate SAA intake, the situation is probably worse than it appears using current requirements. WHO EARs are based largely on studies of replete infants and adults living in clean environments. The WHO gives the caveat that children who have parasitic loads, are stunted, have recently been ill, or live in an environment with poor sanitation will have higher requirements (49). Children in the HPP were more likely to defecate in the open, had more illness, and were more stunted than those in the LPP, hence they likely had higher requirements than either the WHO reference population or the LPP. Children in the HPP were also exposed to more cyanide in their food. This is important because detoxification of cyanide requires sulfur predominantly from the SAAs (28, 52). Children in the HPP therefore likely had higher demand for SAAs, but lower intakes of SAAs, methionine in particular.

Children in the HPP were more likely to have consumed cassava leaves, and those cooked leaves had, on average, higher concentrations of cyanogens. The SCN in the children's urine supported this pattern. Mothers in both populations explained that cassava leaves take a long time to prepare and are generally the least preferred of the leaves. They also explained that when they were overburdened they had less time to prepare food in the evenings—often this was the only meal cooked during the day. At these times, they skipped steps in the preparation of

TABLE 4 Comparison of intake of individual AAs[1]

	Intake, mg · kg^{-1} · d^{-1}			Population below EAR	
	LPP ($n = 165$)	HPP ($n = 168$)	EAR	LPP ($n = 165$)	HPP ($n = 168$)
Tryptophan	16.4 [11.5–23.6][b]	13.8 [7.3–20.8][b]	4.8	6 (3.64)[a]	15 (8.93)[a]
Histidine	36.0 [24.2–55.4][b]	29.2 [15.4–48.4][b]	12	17 (10.3)[a]	30 (17.86)[a]
Threonine	53.7 [37.4–83.1][b]	42.8 [22.6–70.7][b]	18	12 (7.27)[a]	25 (14.88)[a]
Isoleucine	61.8 [43.1–92.2][b]	51.6 [26.7–83.3][b]	22	16 (9.7)	26 (15.48)
Leucine	106.6 [72.3–159.2][c]	82.5 [43.3–147.2][c]	44	20 (12.12)[b]	43 (25.6)[b]
Lysine	88.2 [62.6–132.8][b]	73.1 [41.4–118.8][b]	35	18 (10.91)[b]	36 (21.43)[b]
Sulfur AAs	32.4 [22.9–49.3][a]	29.6 [18.1–44.3][a]	17	22 (13.33)[a]	39 (23.21)[a]
Cysteine	14.4 [10.5–22.3][b]	13.1 [7.0–20.0][b]	4.81[2]	10 (6.06)[a]	21 (12.5)[a]
Methionine	17.3 [14.8][a]	16.6 [15.6][a]	12.19[2]	39 (23.64)[a]	59 (35.12)[a]
Aromatic AAs[3]	119.2 [79.5–185.2][b]	92.5 [49.2–13.7][b]	30	10 (6.06)	19 (11.31)
Valine	68.6 [49.5–107.9][b]	56.1 [28.8–97.4][b]	29	16 (9.7)[c]	42 (25.0)[c]

[1]Values are median [IQR] or n (%) unless indicated otherwise. Differences between mean intakes used the Mann–Whitney U test, differences of proportions below the EAR used the chi-square test. AA, amino acid; EAR, estimated average requirement; HPP, high-prevalence population; LPP, low-prevalence population.
[2]The references (mg/g protein) used for cysteine and methionine were extrapolated from a ratio of methionine to cysteine of 2.54:1 (50).
[3]Tyrosine and phenylalanine.
[a–c]Significantly different between populations: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

the cassava leaves that would normally eliminate some of the cyanogens. Owing to historical reasons, the LPP had better access to productive land to cultivate and pasture for animals. The HPP often had to rent marginal fields suitable only for cassava. They depended on low-wage labor that left little time or money for nutritious, well-cooked meals. It is likely that other factors, such as less supervision and more infrequent meals for young children, would also be associated with this dynamic and could also have affected the children's nutritional status.

This study focused on low intake of SAAs as 1 factor potentially unique to populations with a high prevalence of kwashiorkor. As early as 1952, Brock and Autret (32) estimated that methionine was more limiting in Nigerian populations with higher prevalence of kwashiorkor. Researchers in the 1960s found that children with kwashiorkor consistently have low circulating concentrations of SAAs (21, 22, 53). Roediger and Waterlow (19) first proposed plausible metabolic pathways linking SAA deficiency with signs characteristic of kwashiorkor >20 y ago, and others have added extensively to this body of knowledge.

One of the riddles of kwashiorkor is the wide variety of symptoms commonly seen together and the lack of a common biological mechanism to link them all. Methionine and cysteine are both necessary for all human proteins, with hair and skin proteins generally considered the lowest-priority proteins and among the first tissues to show depletion when SAAs are inadequate (54). Epithelial cells in the mucosa have very high turnover and require relatively large amounts of both SAAs, a shortage of which compromises the integrity of the mucosa, such as seen in kwashiorkor (55, 56).

Cysteine can be derived metabolically from methionine, but methionine cannot be derived from cysteine. Methionine is therefore an essential nutrient that must be obtained through the diet. Cysteine is conditionally essential, depending in part on the amount of methionine available to be converted to cysteine. Although cysteine and methionine are usually combined into a single requirement, each serves very different metabolic functions. S-adenosylmethionine, a metabolite of methionine, is a primary methyl donor. Methionine is involved in the choline, betaine, folate, and vitamin B-12 cycles and forms a key substrate for carnitine (56, 57). Choline and betaine can both be obtained independently from the diet as a source of labile methyl groups, potentially reducing the demand for methionine, but all must link with methionine to maintain their cycles. Supplementing these other metabolites may serve some of the need for methyl groups, but insufficient methionine affects them all (58). A shortage of methionine interrupts the production of proteins, fatty acid oxidation, and mitochondrial function, among other effects (59, 60). Some of the signs associated with these interrupted processes are low protein turnover, increased oxidative stress, lethargy, and a fatty liver, all signs associated with kwashiorkor (32, 61, 62).

The unique properties of cysteine derive from the position of its sulfur atom, donating the sulfur-containing moiety during synthesis reactions or stabilizing the secondary structure of proteins through sulfur–sulfur bonds (63). Each of these can also be linked to many of the signs of kwashiorkor (63). Glutathione (GSH) is produced from cysteine but is converted back to cysteine when concentrations of cysteine are insufficient for other priority uses like de novo protein synthesis during an immune response or the detoxification of cyanide (64). Studies on GSH and cysteine in kwashiorkor are not conclusive. But they indicate that low concentrations of GSH observed in kwashiorkor may be due to a combination of increased demand for GSH due to heightened oxidative stress, and the limited availability of cysteine for its production (20, 65). Children with kwashiorkor showed marked increases in GSH production during recovery after 5 d of supplementation with *N*-acetylcysteine (20). The glycocalyx, of which sulfated glycosaminoglycans (GAGs) constitute an important component, forms a coating on endothelial cells, providing a continuous layer lining vessels, to include capillaries. The integrity of the glycocalyx is important to regulate the flow of fluid into the interstitium (66). Sulfated GAGs also form a gel within the interstitium to support the regulation of the flow of molecules within that space and the volume of interstitial fluid (67). Clinical observations indicate a reduction in sulfated GAGs in kwashiorkor (68). Amadi et al. (68) have proposed that degradation of the GAGs through the loss of sulfur releases water from this gel matrix, resulting in the pitting edema characteristic of kwashiorkor.

This study was able to show an association between high prevalence of kwashiorkor and low intakes of SAAs, in a diet where methionine was the first limiting AA, and requirement was likely heightened owing to increased incidence of illness, exposure to an environment with poor hygiene, and exposure to cyanide. We have described the multiple potential metabolic pathways by which inadequate SAAs might contribute to signs characteristic of kwashiorkor. This was an observational study and does not provide evidence on causation. Although a trial that supplemented cysteine without methionine did not reduce incidence of kwashiorkor, trials providing increased SAAs containing a balance of methionine with cysteine are still necessary to explore the causative role of SAAs in the etiology of kwashiorkor (69).

The dietary limitations presented here may not be generalizable to other kwashiorkor-endemic populations. Owing to logistical constraints, a single diet recall was used to calculate population-level intake of nutrients. This may overestimate the proportion of the population at risk. On the other hand, the data were also collected during a bean harvest, with possibly higher than usual intakes of beans, increasing intake estimates. Urine sample collection rates were not identical at all HPP sites because of local sensibilities. However, similar patterns of cassava leaf consumption and measured cyanide content across HPP sites suggest this did not alter the study's findings.

One barrier to studying factors both leading to kwashiorkor and preventing it is knowing where these HPPs and LPPs are found. Prevalence surveys generally aggregate large populations, hiding populations such as the HPP and therefore the factors unique to these communities (27). Prevalence surveys are also inappropriate for accurately demonstrating the scale of the problem of kwashiorkor because children develop, die, or spontaneously recover from kwashiorkor in a very short time (2). Instead, incidence would be a more appropriate measure, requiring surveillance. Local health care staff supporting this study were able to easily identify those communities within their zones that generated the most cases, suggesting potential qualitative methods to reduce the resource burden of surveillance.

This unique study compares, in detail, the diets of a high-kwashiorkor-prevalence population with those of a neighboring low-kwashiorkor-prevalence population. We found that methionine was the limiting AA in both diets, but the HPP had lower intakes of methionine. These results support an emerging hypothesis that methionine deficiency is a significant factor in risk of kwashiorkor.

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Data Availability

De-identified data described in the article, code book, and analytic code will be made available upon request pending application to the corresponding author.

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