Suboptimal zinc status in pregnant Malawian women: its association with low intakes of poorly available zinc, frequent reproductive cycling, and malaria[1–3]

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ABSTRACT A study of 152 rural Malawian women aged 23.2 ± 5.5 y ($\bar{x}$ ± SD) at 24 wk gestation included measurements of biochemical indexes of zinc (plasma and hair), protein (serum albumin), and infection (serum C-reactive protein, white blood cell count, and malaria), and dietary intakes (via three interactive 24-h dietary recalls). Data on health, demographic and socioeconomic status, family characteristics, reproductive history, and anthropometry were also collected. The study revealed a high prevalence of suboptimal zinc status: 36% of the women had low plasma and 46% had low hair zinc values. Median daily intake of zinc (9.0 mg) was low and poorly available: 61% was provided by cereals and 20% by flesh foods. Median intake of animal protein was only 5.6 g/d, and phytate intakes were high (1.4 g/d). Women consuming diets with phytate-zinc ratios > 17 (the median) had lower hair zinc concentrations (1.6 compared with 1.8 μmol/g, $P < 0.03$), were older (24 compared with 20 y, $P < 0.02$), and had a higher number of pregnancies (3 compared with 2, $P < 0.02$) than those consuming diets with a phytate-zinc ratio < 17. Frequent reproductive cycling was related to zinc status; hair zinc was higher for a prima- than for a multigravida (2.0 compared with 1.6 μmol/g, $P < 0.01$). Malaria prevalence was also associated with hair zinc ($P < 0.05$) but not with plasma zinc, after the number of pregnancies was controlled for. We conclude that low intakes of poorly available dietary zinc, frequent reproductive cycling, and malaria prevalence are three major factors in the etiology of suboptimal zinc status in these rural, pregnant Malawian women. *Am J Clin Nutr* 1998;67:702–9.

KEY WORDS Zinc, pregnancy, malaria, hair, plasma, diet, phytate, nutrient intake, gravida, reproductive cycling

INTRODUCTION

Investigations of the zinc status of pregnant women in Africa are limited, despite increasing evidence that they may be especially vulnerable to zinc deficiency. Studies of pregnant women in Nigeria, Egypt, Zaire, and more recently Malawi have all reported lower plasma, and in some cases hair, zinc concentrations (1–6) than in pregnant women from developed countries (7–11). These findings are significant because zinc deficiency during pregnancy may have far-reaching consequences for maternal and fetal outcomes and subsequent child survival. Results of several zinc supplementation studies have confirmed that zinc deficiency during pregnancy may cause poor fetal growth and complications during parturition, resulting in increased morbidity and mortality in mothers and neonates (12).

To date, the etiologic factors associated with zinc deficiency during pregnancy in less industrialized countries have not been established, in part because of the coexistence of many other health problems, which confound the diagnosis of zinc deficiency. Therefore, in this cross-sectional study of 152 rural, preliterate Malawian women at 24 wk gestation, we investigated factors potentially associated with zinc deficiency during pregnancy. Identification of these factors is essential for designing effective intervention strategies to alleviate zinc deficiency in rural Malawi.

SUBJECTS AND METHODS

Subjects

Women in this study were recruited from prenatal clinics in a rural health center in Mangochi District, Southern Malawi. Approximately 53% of eligible women agreed to participate. The study group consisted of a convenience sample of 152 women aged 14–45 y ($\bar{x}$ ± SD: 23.2 ± 5.5 y) at 23.5 ± 2.5 wk gestation (based on the last date of the menstrual period, fundal height, or both) with no history of cesarean section and an initial hemoglobin concentration > 80 g/L. Verbal consent was obtained from the traditional authorities in the villages surrounding the health center and from the participants after the nature of the study had been fully explained to them. The study protocol was approved

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by both the Human Ethics Committee of the University of Guelph, Canada, and the Health Sciences Research Committee, University of Malawi.

After recruitment, the women were interviewed in their homes by trained research assistants using a pretested questionnaire and observations to obtain data on general health, demographics, socioeconomic status (SES), reproductive history, previous lactation behavior, and family characteristics, as described previously (6).Women were treated with antihelminthic medication at the start of the study.

Most of the women were subsistence farmers with little or no formal education. They lived in houses made of sun-dried bricks and thatched roofs with a shared latrine. Of the women, 75% (n = 109) fell into the two lowest SES categories (ie, equivalent to SES scores of 1–3 and 4–7), 19.4% (n = 28) into the next higher category (ie, SES scores of 8–10), and only 4.2% (n = 6) in the highest SES category (ie, SES scores of 11–14). The ratio of adult females to adult males per household was 1.4, with the mean (±SD) number of people per household equal to 3.5 ± 1.8.

The number of pregnancies of the women ranged from 1 to 11. There were relatively more primagravidas in the study group than in the entire group of women (n = 973) who presented at the health center between October 1993 and April 1994 (ie, 30% in the study group compared with 23% of all presenting women) and a lower proportion of women with a gravida ≥ V (ie, 26% compared with 34%). The women with ≥2 pregnancies averaged 3.5 live births and 1.0 dead children, almost the same as the national average in Malawi for women aged 15–49 y: 3.48 births and 0.93 dead children (13). Of the multiparous women, 93% had given birth in the previous 5 y, 65% in the previous 3 y, and 3% in the last year. According to a national survey conducted in 1992, < 12% of rural women in Malawi use any form of birth control (13).

Methods

At entry to the study, selected anthropometric measurements were made and a nonfasting peripheral blood sample was obtained by venipuncture in the morning by health center nurses while participants were sitting. Women were educated about the modified interactive 24-h dietary recall method (14) and arrangements were made for home visits in the following week to conduct three interactive recalls on Sunday, Tuesday, and Thursday or on Monday, Wednesday, and Friday.

Dietary assessment

Details of the modified interactive 24-h recall procedure were described previously (14). Briefly, women were provided with plates and picture charts of commonly consumed food items on the day before the recall to enhance the estimation of both portion size and recall of all food items consumed. To facilitate quantification of portion sizes, women were asked to use their own plates and utensils for serving portions of actual foods consumed on the recall day, which were then weighed by a research assistant. Recipe data were used for composite dishes. The procedure used for calculating intakes of major food sources, energy, selected nutrients and antinutrients, as well as molar ratios of phytate to zinc were described previously (14). Mean zinc availability was estimated from the mean molar ratios of phytate to zinc of the diets for each subject; for phytate-zinc molar ratios between 15 and 30, absorption was assumed to be 15% and 30% for phytate-zinc ratios < 15 (15). The calcium and animal protein contents of the diets were too low to have an effect on zinc absorption and hence were not taken into account in the calculation of available dietary zinc. Daily energy and protein intakes were adjusted for digestibility by multiplying by 0.95 and by 0.85, respectively. The adequacy of energy intakes was evaluated as the percentage of women with intakes below the FAO/WHO/UNU recommendations (16) for energy. The predicted prevalence of inadequate intakes of protein and zinc were calculated by using probability analysis (17), based on the FAO/WHO/UNU recommendations (16) for protein and the WHO's (18) estimates for normative and basal zinc requirements for the second trimester of pregnancy.

Anthropometric assessment

The following anthropometric measurements were taken with women wearing light clothing and no shoes: standing height, weight, elbow breadth, midupper arm circumference, and skinfold thickness at five sites (biceps, triceps, subscapular, suprailiac, and medial thigh). Details were given in a study by Huddle et al (6). Only the results for standing height, body weight, and elbow breadth are reported here. Each measurement was taken by using standardized equipment and techniques (19) and the same anthropometrist (except for body weight) to eliminate interexaminer error. Height-for-age z scores were calculated by using reference data from the first and second National Health and Nutrition Examination Survey (NHANES I and II) for nonpregnant African Americans (20).

Biochemical assessment

Rigorous precautions were taken to avoid all sources of adventitious iron and zinc contamination during the collection, separation, and analyses of the blood and hair samples. The accuracy and precision of all analytic methods was checked by analyzing appropriate certified reference materials and pooled samples with each assay, and were reported previously (6).

Initial hemoglobin measurements were performed by using a finger-prick blood sample and a portable hemoglobinometer (Hemocue Inc, Mission Viejo, CA). For those women with hemoglobin concentrations ≥8g/L, nonfasting blood samples (15 mL) were drawn by venipuncture early in the morning into trace element–free, heparin-containing evacuated tubes with or without lithium (Monovette; Sarstedt, St Laurent, Canada) while subjects were sitting. Plasma was used for zinc analyses by flame atomic-absorption spectrophotometry using standard procedures (21) after reducing the freeze-dried serum samples to ash. Concentrations of C-reactive protein and albumin in serum were also analyzed via turbidimetric methods (Behring Turbitimer System, Somerville, NJ) (6).

Aliquots of freshly collected blood to which lithium and heparin were added were used for the white blood cell count (WBCC), and thick blood smears for malarial screening. The WBCC was performed manually by one examiner using the Unopette collection system (Becton Dickinson, Rutherford, NJ) and a Neubauer counting chamber (Propper Manufacturing Co Inc, Long Island, NY). Thick blood smears were stained with 4% Giemsa stain and examined for malaria by trained technicians in Mangochi District Hospital. Parasites and leukocytes were counted in the same fields until 300 leukocytes or 500 parasites were recorded. Parasite densities were determined as the ratio of parasites to WBCs. The limits of detection were < 10 000 parasites/L blood.

Hair samples (20–50 mg) were collected from close to the occipital portion of the scalp with stainless steel scissors. Only the proximal 1.0–1.5 cm of the hair strands were retained, washed according to a standard procedure, and then analyzed for zinc by instrumental neutron-activation analyses (22).

Statistical analyses

All data were tested for normality by using the Kolmogorov-Smirnov Lilliefors test, which showed that some of the dietary variables had strongly skewed distributions. Initially, Spearman correlation coefficients were computed to examine relations among demographic, socioeconomic, anthropometric, dietary, biochemical, and infection variables. Subsequently, medians (first and third quartiles) for selected demographic, anthropometric, dietary, and biochemical indexes for women with dietary phytate-zinc molar ratios above and below the median were compared by using the Kruskal-Wallis test.

To examine the effects of the number of pregnancies, the pregnant women were classified by gravida I, II–IV, or > IV. Within each gravida class, medians and quartiles for demographic, dietary, anthropometric, biochemical, and infection variables were computed and then compared across the gravida classes, again by using the Kruskal-Wallis test.

To assess the effect of malaria and the number of pregnancies on the zinc status of the pregnant women, two-way analysis of variance using general-linear-model procedures was performed by using the number of pregnancies and malaria prevalence as classificatory independent variables and hair and plasma zinc concentrations as continuous dependent variables. Statistical analyses were performed with the statistical package SYSTAT (23) and with software (version 6.09 and PC SAS for Windows version 6.11) from the Statistical Analysis System, Cary, NC.

RESULTS

Median (first and third quartiles) daily dietary intakes (per day and per MJ) of energy and selected nutrients and antinutrients are presented in **Table 1**. Major food sources of energy, protein, and zinc are shown in **Table 2**. Cereal grains (mainly maize) provided the major source of energy, protein, and zinc, followed by legumes for energy, and flesh foods (mainly fish) for protein and zinc. Less than 5% of the energy intake was provided by flesh foods. Of these, fresh and dried fish were consumed by the women on 6% and 23% of the recall days, respectively, whereas chicken and goat combined were eaten only on 3% of days. Moreover, the mean (± SD) weights of fresh and dried fish consumed were 6.9 ± 4.5 and 13.3 ± 7.4 g/d compared with only 3.1 ± 1.6 g meat/d. Hence it is not surprising that fish provided > 85% of the protein from animal sources.

Significant correlations were noted between intakes of zinc and the following dietary variables: energy (r = 0.65, P = 0.0001), protein (r = 0.83, P = 0.0001), animal protein (r = 0.52, P < 0.0001), iron (r = 0.65, P = 0.0001), and dietary fiber (r = 0.67, P = 0.0001). Molar ratios of phytate to zinc were correlated with dietary fiber (r = 0.29, P = 0.004) and negatively with protein (r = –0.21, P = 0.012). When the diets of the women were classified into quartiles based on dietary zinc intake (ie, < 6.7 mg/d, 6.7–9.0 mg/d, > 9.0–10.9 mg/d, and > 10.9 mg/d), those in the lowest quartile for dietary zinc had the poorest diets in terms of absolute as well as energy-adjusted protein, fat, calcium, iron, and copper intakes. Their intakes of dietary fiber and phytate were also lower and there were no significant differences in phytate-zinc molar ratios (**Table 3**).

Classification of women with dietary phytate-zinc molar ratios above and below the median, ie, 17, showed that those with phytate-zinc molar ratios above the median had significantly higher median intakes (g/d) of grain products and vegetables but significantly lower intakes of flesh foods, fats, and sweets than those with phytate-zinc molar ratios below the median. As a result, their intakes of calcium, phosphorus, and protein from flesh foods were significantly lower whereas those for copper, phytate, and dietary fiber were higher as shown in **Table 4**. Note that intakes of energy, total fat, and protein were comparable.

The percentage of women consuming less than two-thirds of the FAO/WHO/UNU's recommended intakes (16) for energy were 92% (unadjusted for digestibility) and 95% (adjusted for digestibility). Probability analyses revealed that 27% and 36% of

TABLE 1
Median and first and third quartiles of energy and selected nutrient and antinutrient intakes of 141 rural Malawian women at 24 wk gestation

	Per day			Per MJ		
	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile
Energy (kJ)	6089	5149	7153	—	—	—
Protein (g)	46.9	38.8	56.6	7.7	7.1	8.5
Animal protein (g/d)	5.6	0	13.2	—	—	—
Fat (g)	15.2	10.1	19.3	2.5	1.8	3.1
Carbohydrate (g)	287	239	339	47	45	50
Vitamin C (mg)	94	58	140	15.9	10.0	23.2
Calcium (mg)	415	265	736	74	47	117
Iron (mg)	14.8	12.1	18.1	2.5	2.3	2.6
Zinc (mg)	9.0	6.7	10.9	1.5	1.2	1.7
Available zinc (%)	20.6 ± 7.3[1]	—	—	—	—	—
Copper (mg)	1.0	0.8	1.3	0.17	0.14	0.19
Dietary fiber (g)[2]	28.7	19.4	36.4	4.7	3.6	5.5
Phytic acid (mg)	1393	942	2115	240	168	325
Phytate: zinc[3]	17.3	13.0	20.7	—	—	—

[1] $\bar{x}$ ± SD.
[2] As nonstarch polysaccharide.
[3] Molar ratio.

TABLE 2
Mean percentages of energy, protein, and zinc intakes provided by six food groups for 141 rural Malawian women at 24 wk gestation[1]

	Grains	Legumes	MPF[2]	Sweets	Vegetables	Fruit
Energy	78.5	6.0	4.0	4.6	3.6	2.4
Protein	60.6	12.4	16.1	< 1	8.2	< 1
Iron	50.0	13.3	9.3	5.2	20.2	1.7
Zinc	61.1	8.9	20.0	< 1	8.9	< 1

[1] Fats, milk, eggs, and miscellaneous each contributed < 1% of total energy intake.
[2] Meat, poultry, and fish (meat means goat and poultry means chicken).

the women were predicted to be at risk of inadequate intakes of dietary protein (adjusted) and zinc, respectively, when consuming the FAO/WHO/UNU's requirements (16) for protein and the WHO's basal requirement (18) estimates for zinc. The corresponding risk estimate for zinc based on the WHO's normative requirement was 56%. When a fixed cutoff approach was adopted and the risk of inadequate intakes was based on the proportion of women with intakes less than two-thirds of the recommended intake, the prevalence estimate for inadequate intakes for protein (adjusted) was 27%. Estimates for zinc ranged from 30% for the basal requirement estimate to 58% for the normative requirement.

Anthropometric assessment

Mean (±SD) weight, elbow breadth, and height for the women were 53.3 ± 6.8 kg, 6.0 ± 0.3 cm, and 154.5 ± 5.5 cm, respectively. The mean *z* score for height was –1.30 ± 0.9; 12.5% of the women had a height-for-age *z* score < –2, the value considered indicative of stunting (20).

Biochemical assessment

Mean (±SD) values for biochemical indexes of zinc, albumin, and infection status are presented in **Table 5** (24). Of the women, 37% had plasma zinc values below the cutoff value specific for 24 wk gestational age compiled by Hambidge et al (7); 46% had hair zinc concentrations < 1.68 μmol/g, a season-specific cutoff point used for hair zinc concentrations in children; no cutoff points have been defined for women during pregnancy. No significant correlations existed between plasma and hair zinc, although serum albumin was correlated with plasma zinc (r = 0.35, P = 0.0001).

The prevalence of malaria was high in these rural Malawian women: 31.3% tested positive for malaria, 6% had a positive test for C-reactive protein (ie, > 15 mg/L), and 2% had elevated WBCCs (ie, > 11×10^9/L). When women were classified separately according to a positive or negative result for infection or inflammation based on each test, there were no significant differences between plasma zinc, hair zinc, or serum albumin concentrations between the two groups.

Interrelations among demographic, dietary, anthropometric, biochemical, and infection indexes

Of the dietary variables listed in Table 1, only phytate-zinc molar ratios had a significant effect on the biochemical zinc status of the women. Consequently, we present in **Table 6** data on selected demographic, anthropometric, biochemical, and infection indexes for women classified by dietary phytate-zinc molar ratios above and below the median. Women consuming diets with higher phytate-zinc molar ratios were significantly older and had a higher number of pregnancies and significantly lower hair zinc concentrations than their counterparts with low phytate-zinc molar ratios. No comparable relation was noted for plasma zinc, body weight, height, and serum albumin concentrations, or any of the measures of infection, including the prevalence of malaria.

In contrast, the number of pregnancies had a significant effect on the prevalence of malaria, as shown in **Table 7**. Primagravida had the highest prevalence of malaria, although their average WBCCs were similar to those of the other women. Also, primagravida were significantly younger and had higher serum albumin and hair zinc concentrations than the other women but significantly lower body weights, probably in part because they tended to be shorter; their plasma zinc concentrations, however, were not significantly different.

Some differences in intakes of major food groups, nutrients, and antinutrients by the number of preganncies were also observed. Of

TABLE 3
Mean intakes of energy and nutrients and mean hair and plasma zinc concentrations for rural Malawian women at 24 wk gestation classified by dietary zinc quartiles

	< 6.7 mg Zn/d (*n* = 36)		6.7–9.0 mg Zn/d (*n* = 35)		> 9.0–10.9 mg Zn/d (*n* = 35)		> 0.9 mg Zn/d (*n* = 35)	
Nutrient	Per day	Per MJ	Per day	Per MJ	Per day	Per MJ	Per day	Per MJ
Energy (kJ)	4867	—	5725	—	6587	—	7223	—
Protein (g)	33.7	6.9	43.3	7.6	51.7	7.8	62.3	8.6
Fat (g)	8.9	1.8	13.1	2.3	16.7	2.5	22.6	3.1
Carbohydrate (g)	241	50	272	48	315	48	342	47
Dietary fiber (g)[1]	19.1	3.9	26.7	4.7	33.2	5.0	36.5	5.1
Calcium (mg)	321	66	400	70	557	85	862	119
Iron (mg)	11.5	2.4	14.3	2.5	16.2	2.5	18.1	2.5
Copper (mg)	0.72	0.15	0.96	0.17	1.16	0.18	1.30	0.18
Phytate (mg)	848	174	1332	233	1700	258	2192	303
Phytate: zinc[2]	16.1	—	17.3	—	16.9	—	16.5	—
Hair zinc (μmol/g)	2.06 ± 0.90		1.62 ± 0.57		1.80 ± 0.66		1.89 ± 0.55	
Plasma zinc (μmol/L)	8.56 ± 2.53		8.43 ± 2.41		8.00 ± 2.10		7.82 ± 1.87	

[1] As nonstarch polysaccharide.
[2] Molar ratio.

TABLE 4
Intakes (median and first and third quartiles) of energy, selected nutrients, antinutrients, and major food groups of rural Malawian women consuming diets with molar ratios of phytate to zinc below and above the median

	Below the median (n = 71)			Above the median (n =70)			
	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	P[1]
Energy (kJ)	5962	5045	7069	6154	5199	7272	NS
Fat (g)	14.1	9.5	19.5	16	10.7	19.3	NS
Protein (g)	49.0	39.7	59.1	44.7	37.2	55.5	NS
Carbohydrate (g)	292.1	233.8	342.3	284.6	241.3	323.2	NS
Calcium (mg)	584.1	369.6	881.1	310.1	229.7	562.6	< 0.0001
Phosphorus (mg)	1153	903	1545	1540	1133	1840	< 0.0001
Iron (mg)	14.6	11.6	18.4	15.2	12.2	17.9	NS
Zinc (mg)	8.3	6.4	10.7	9.5	7.1	11.3	NS
Copper (mg)	0.9	0.7	1.3	1.1	0.9	1.3	0.018
Vitamin C (mg)	85.9	54.8	130.9	105.4	60.4	147.1	NS
Dietary fiber (g)	21.6	15.9	31	32.3	26	40	< 0.0001
Phytate (mg)	992	738	1262	1978	1496	2339	< 0.0001
Protein from flesh foods (g)	10.1	2.6	16.7	0	0	8.3	< 0.0001
Grains (g)	314	269	368	358	296	400	0.030
Legumes (g)	16	0	43	5	0	44	NS
Meat, poultry, and fish (g)	24	5.5	41.5	0	0	18	0.000
Sweets (g)	50	6	126	13	0	117	0.049
Vegetables (g)	106	69	153	132	83	191	NS

[1] Significant difference between groups (above compared with below the median), Kruskal-Wallis test.

the women, the primagravida and women with gravida II–IV consumed more flesh foods (26.4 compared with 26.8 compared with 10.1 g/d; $P = 0.02$) and fats and oils (1.4 compared with 0.3 compared with 0.2 g/d; $P = 0.04$) than their counterparts with gravida > IV. These trends resulted in correspondingly higher median intakes of animal protein (7.8 compared with 7.0 compared with 0 g/d; $P < 0.03$), calcium (584 compared with 501 compared with 282 mg/d; $P < 0.01$), and vitamin C (103.6 compared with 97.9 compared with 71.6 mg/d; $P = 0.05$), and diets with lower median phytate-zinc molar ratios (ie, 15.0 compared with 17.3 compared with 18.7; $P < 0.05$) for the primagravida and women with gravida II–IV compared with those with gravida > IV.

Two-way ANOVA also confirmed that zinc nutriture, as indicated by hair zinc concentrations, was significantly controlled by both the number of pregnancies and malaria infection (as indicated by the prevalence of malaria) (**Table 8**). The known tendency for multigravida to be less susceptible to malaria (25) probably explains the large interaction term in the ANOVA. The comparable analysis using plasma zinc concentration as the dependent variable was not significant.

DISCUSSION

Diagnosis of suboptimal zinc nutriture is hampered by the lack of a single, sensitive biochemical index of zinc status. Therefore, a combination of hair and plasma zinc concentrations was used. Hair zinc reflects chronic zinc status over the period of hair growth and is much more stable than plasma zinc. Plasma zinc is affected by many other factors besides zinc status, including disease states, diurnal variation, fasting, length of time before the separation of the plasma, etc. In addition, plasma zinc is an acute index of zinc status, which is particularly difficult to interpret during pregnancy. There is a decline in plasma zinc during the first trimester, which continues throughout pregnancy, even in the presence of optimal zinc nutriture (6, 10, 12).

Of the women in this study, more than one-third had biochemical evidence of zinc deficiency based on low hair and plasma zinc concentrations. The existence of suboptimal biochemical zinc status was attributed to three major etiologic factors: low intakes of poorly bioavailable zinc, frequent reproductive cycling, and malarial infection, each of which is discussed below.

Low intakes of poorly available iron and zinc

Our results indicate that a high proportion of the pregnant women were at risk of inadequate intakes of zinc, arising from a combination of low intakes and poor bioavailability of zinc. Probability estimates for inadequacy were 36% and 56% for zinc when the basal and normative requirement estimates (18), respectively, were used. These findings are not surprising. These rural Malawian diets were predominantly plant based; cereals provided a large proportion of the energy (78%), protein (61%), and zinc (61%). Intakes of flesh foods (mainly fish) were low, averaging 5.6 g/d and contributing only 4% of the energy and 20% of the zinc (Table 2). Flesh foods are a rich source of readily available zinc, whereas cereals, especially unrefined corn—

TABLE 5
Hair and plasma zinc and serum albumin concentrations and white blood cell counts (WBCC) for 151 rural Malawian women at 24 wk gestation

Biochemical index	Cutoff value[1]	$\bar{x} \pm$ SD
Hair zinc (μmol/g)	ND[2]	1.8 ± 0.7
Plasma zinc (μmol/L)	< 7.1(7)	8.1 ± 2.3
Serum albumin (g/L)	33[3] (24)	31 ± 5
WBCC (× 10^9/L)	> 11 (6)	6.5 ± 1.8

[1] Reference numbers in parentheses.
[2] The cutoff value for hair zinc used as indicative of suboptimal zinc status is 1.68 μmol/g (*see* text).
[3] Mean reference value.

TABLE 6
Selected demographic, anthropometric, infection, and biochemical zinc indexes for rural Malawian women at 24 wk gestation classified by molar ratios of phytate to zinc above and below the median[1]

	Below the median (*n* = 71)			Above the median (*n* =70)			
	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	P^2
Age (y)	20	18.5	25.5	24	20	28	0.018
Gravida	2	1	4	3	2	6	0.024
Weight (kg)	52.2	49	58.2	52.2	48.8	56.9	NS
Height (cm)	153.9	149.8	157.4	155.2	151.9	158.1	NS
Elbow breadth (cm)	6	5.8	6.2	6	5.8	6.2	NS
Hair zinc (μmol/g)	1.8	1.6	2.2	1.6	1.4	1.9	0.027
Plasma zinc (μmol/L)	7.9	6.8	9.7	7.9	6.6	9.4	NS
WBCC[3] ($\times 10^9$/L)	6.6	5.4	7.6	5.9	5.2	7.7	NS

[1] Malaria prevalence was 31% and 30% in the below- and above-the-median groups, respectively.
[2] Significant difference between groups (above compared with below the median), Kruskal-Wallis test.
[3] White blood cell count.

the primary staple in Malawi—contain high concentrations of phytic acid (myoinositol hexaphosphate), the most potent inhibitor of zinc absorption (26).

Low energy intakes were partly responsible for the low zinc intakes observed. Energy and zinc intakes were positively correlated ($r = 0.69$, $P = 0.0001$) and there was a consistent decline in energy intakes with decreasing quartiles of dietary zinc (Table 3). We suggest that these low intakes of energy and zinc were not the result of underreporting, but arose because the dietary recall data were collected during the food-shortage season, a period when some of these women actually lost weight during pregnancy (6). Such low zinc intakes are cause for concern because women with diets with a zinc content within the lowest quartile also had lower absolute and energy-adjusted intakes of several other essential nutrients, confirming the poor overall quality of their diets. Furthermore, the amount of zinc in the lowest quartile (ie, < 6.7 mg) was comparable with that associated with a higher risk of low birth weight and preterm delivery in a study of US women (27). Nevertheless, despite such low intakes of dietary zinc, these women had hair and plasma zinc concentrations comparable with those of the other three dietary zinc groups (Table 3).

The adequacy of dietary zinc depends on its bioavailability as well as on the daily supply (18). Our results confirm the critical effect of zinc bioavailability as indicated by the influence of phytate-zinc molar ratios on biochemical zinc status. Those women with dietary phytate-zinc molar ratios above the median had significantly lower median hair zinc concentrations than their counterparts with diets with phytate-zinc molar ratios below the median (ie, < 17) (Table 6). This is important because ratios > 15 have been associated with zinc deficiency in humans (28–30), and 62% of the women had diets with phytate-zinc molar ratios ≥ 15. Other investigators have also reported comparable relations between hair zinc concentrations and phytate-zinc molar ratios in studies of children (31–33), providing further support for the dietary and biochemical relations observed here.

Not surprisingly, the overall quality of the diets with phytate-zinc molar ratios above the median was poorer: women consumed significantly more grains and vegetables but less flesh foods (mainly fish) and fats than those with diets with lower dietary phytate-zinc molar ratios. As a consequence of such trends in food-consumption patterns, intakes of calcium, phosphorus, and protein from flesh foods were lower whereas those of phytate and dietary fiber were higher (Table 4). Flesh foods are a rich source of readily available zinc and inclusion of even small amounts can increase the apparent absorption of zinc and counteract the negative effect of phytic acid on zinc absorption (34). In addition, low-fat diets have been reported to inhibit zinc absorption in some studies (35). Fat intakes were low in these pregnant women, providing only 9% of energy on average;

TABLE 7
Selected demographic, anthropometric, biochemical, and infection indexes of pregnant Malawian women by gravida class[1]

	Gravida = I (*n* = 46)			Gravida = II-IV (*n* = 66)			Gravida > IV (*n* = 40)			
	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	Median	1st Quartile	3rd Quartile	P^2
Age (y)	18	16	19	21.5	20	26	30	28	32	0.000
Weight (kg)	51.2	46.4	53.3	53.8	49.7	58.7	54.1	49.8	60	0.007
Height (cm)	153.6	149.9	156.6	154.4	149.6	159.4	155.5	152.6	159	NS
Hair zinc (μmol/g)	2.0	1.7	2.3	1.6	1.4	2.0	1.6	1.4	2.0	0.007
Plasma zinc (μmol/L)	8.1	6.8	10.2	7.6	6.7	9.3	7.8	6.5	9.2	NS
Serum albumin (g/L)	3.1	2.8	3.3	3.1	2.9	3.3	3.0	2.8	3.1	0.035
WBCC[3] ($\times 10^9$/L)	6.2	5.2	7.7	6.2	5.4	7.3	6.3	5.1	8.2	NS

[1] Malaria prevalence was 46%, 21%, and 28% for gravida I, II-IV, and > IV, respectively. *See* table 8 for further details.
[2] Significant difference among groups, Kruskal-Wallis test.
[3] White blood cell count.

TABLE 8
ANOVA of the influence of gravida and malaria infection on zinc nutriture, as measured by hair zinc[1]

Source	Sum of squares	Degrees of freedom	Mean square	*F* ratio	*P*
Malaria	3.027	1	3.027	6.88	0.010
Gravida	3.738	2	1.869	4.25	0.016
Malaria × gravida	2.175	2	1.088	2.47	0.088
Error	64.234	146	0.440		

[1] $n = 152$. Dependent variable is hair zinc. Multiple $r = 0.344$, multiple $r^2 = 0.118$.

intakes were especially low for women with zinc intakes in the lowest quartile (Table 3).

Frequent reproductive cycling

Frequent reproductive cycling was also a significant etiologic factor influencing the biochemical zinc status of these rural pregnant Malawian women. Women with a gravida ≥IV had significantly lower hair zinc concentrations than those with a gravida II–IV; primagravidas had the highest hair zinc concentrations (Table 7). Such a trend was not apparent for plasma zinc concentrations although primagravida tended to have a lower median plasma zinc concentration than their multigravida counterparts. The extent to which this trend in hair zinc concentrations was induced by depletion of tissue zinc associated with frequent reproductive cycling compared with diets of poorly available zinc cannot be quantified. The quality of the diets for the multigravida was consistently poorer than that of the primagravida. Women with a gravida ≥IV consumed significantly less flesh foods (mainly fish), but more cereals, and as a consequence their diets had higher median phytate-zinc molar ratios (ie, 18.7 compared with 15.0; $P < 0.05$) but lower intakes of calcium (282 compared with 584 mg/d; $P < 0.01$) and protein from flesh foods (0 compared with 47.7 g/d; $P < 0.03$). Low SES exacerbated by households in which many people lived in relation to the available food supply may have been the cause of this dietary pattern, although no such relation was shown statistically, probably because most of the women (75%) fell into the lower SES categories, with only 4.2% in the highest SES category.

Malarial infection

The prevalence of malarial infection in these rural, pregnant Malawian women was high (31.3%), a finding that was not unexpected. The study was undertaken during the rainy season. In addition, during pregnancy women are known to have an increased susceptibility to *Plasmodium falciparum* infection, and hence, experience a higher frequency and density of parasitemia, although the mechanisms are not yet clear. Two-way ANOVA results confirmed that the prevalence of malaria had a significant effect on zinc concentrations in hair (but not in plasma) when the number of pregancies was taken into account (Table 8). Those women with malaria had a lower zinc status, based on hair zinc concentrations, probably induced by chronic hemolysis, resulting in increases in urinary excretion of zinc (36). A relation between zinc nutriture (based on hair zinc) and malaria was reported earlier in children from Papua New Guinea (37).

The significant interaction between the number of pregnancies and malaria (Table 8) has been well documented (25). Primagravida are at particular risk for malarial infection (38, 39). Hence, it is not surprising that primagravida had a higher prevalence of malaria than multigravida (46% compared with 28%) (Table 7).

Conclusions

In summary, our results confirm that zinc deficiency in rural, pregnant Malawian women is associated with low intakes of poorly available zinc, frequent reproductive cycling, and an increased prevalence of malaria. In this rural African population, the most practical methods to alleviate zinc deficiency include modifying the diets to improve the bioavailability rather than the overall content of dietary zinc, encouraging effective methods of contraception to reduce frequent reproductive cycling, and implementing low-cost methods (eg, bed nets) to reduce malaria parasitemia.

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