Original Communication

A High Prevalence of Zinc- but not Iron-Deficiency among Women in Rural Malawi: a Cross-Sectional Study

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Abstract: *Background:* Zinc deficiency is often associated with nutritional iron deficiency (ID), and may be exacerbated by low selenium status. *Aim:* To investigate risk of iron and zinc deficiency in women with contrasting selenium status. *Methods:* In a cross-sectional study, 1-day diet composites and blood samples were collected from self-selected Malawian women aged 18–50 years from low- (Zombwe) (n=60) and high-plant-available soil selenium (Mikalango) (n=60) districts. Diets were analyzed for trace elements and blood for biomarkers. *Results:* Zinc deficiency (>90 %) was greater than ID anemia (6 %), or ID (5 %), attributed to diets low in zinc (median 5.7 mg/day) with high phytate:zinc molar ratios (20.0), but high in iron (21.0 mg/day) from soil contaminant iron. Zombwe compared to Mikalango women had lower ($p<0.05$) intakes of selenium (6.5 vs. 55.3 μg/day), zinc (4.8 vs. 6.4 mg/day), iron (16.6 vs. 29.6 mg/day), lower plasma selenium (0.72 vs. 1.60 μmol/L), and higher body iron (5.3 vs. 3.8 mg/kg), although plasma zinc was similar (8.60 vs. 8.87 μmol/L). Body iron and plasma zinc were positive determinants of hemoglobin. *Conclusion:* Risk of zinc deficiency was higher than ID and was shown not to be associated with selenium status. Plasma zinc was almost as important as body iron as a hemoglobin determinant.

Key words: Malawi, women, diet composites, plasma Zn, Se status, body iron, anemia

Introduction

Iron deficiency is recognized as the most common micronutrient deficiency in low-income countries. Women of child-bearing age are especially at risk because pregnancy entails a high demand for iron. A major factor contributing to iron deficiency in these settings is inadequate intakes of bioavailable iron. Such deficits arise because diets are predominantly plant-based, and intakes of readily available heme iron from flesh foods are generally low. These dietary patterns are also likely to be inadequate in absorbable zinc because zinc and iron have a similar distribution in the food supply and are affected by many of the same dietary absorption modifiers [1]. Indeed, low iron stores have been identified as a risk factor for suboptimal zinc status [2]. Furthermore, a compromised zinc status may have a negative impact on hemoglobin concentrations, independent of iron status, as has been reported previously among pregnant women in Ethiopia [3]. Deficiencies of iron and zinc can have numerous adverse effects on women's health, including impairments in physical work capacity [4], cognitive function [5], poor pregnancy outcomes [6, 7], and increased risk of morbidity and mortality [8].

An additional factor with the potential to compromise the zinc status of Malawian women is a low selenium status. Our earlier cross-sectional study reported low levels of selenium in the diets and plasma of rural Malawian women living in a region where plant-available selenium content of soils and concentrations in staple foods are low [9]. There is accumulating evidence that selenium interacts with zinc via several mechanisms [10–12]. Therefore in this study, we have determined the risk of zinc deficiency in two groups of rural Malawian women of child-bearing age, living in districts with contrasting plant-available selenium concentrations in the soil. We used two recommended indicators for assessing population zinc status: the prevalence of inadequate zinc intakes and low plasma zinc concentrations [13]. In addition, we have examined the risk of iron deficiency in these women, using hemoglobin, mean cell volume, and total body iron calculated from plasma ferritin and soluble transferrin receptor, in an effort to provide a more accurate reflection of the prevalence and severity of nutritional iron deficiency among these rural Malawian women. We also explored possible determinants of plasma ferritin, total body iron, hemoglobin, and plasma zinc.

Subjects and Methods

Study site and subjects

The women were a convenience sample recruited in March 2011 from six rural villages situated in a district with acidic soils with low available selenium in the Extension Planning Area (EPA) of Zombwe (n = 60) in Mzuzu Agricultural Development District in the north, and six villages in Mikalango EPA (n = 60), a district with calcareous soils with highly available selenium in the Shire Valley in the south of Malawi. December to the end of March is the pre-harvest period and rainy season in Malawi, when most of the farming activities take place and food intakes are lowest. Women (non-pregnant) aged 18 to 50 years, apparently healthy with no evidence of acute or chronic illnesses, and with an initial hemoglobin > 80 g/L, were eligible to participate. Only one woman per household was recruited. The sample size was sufficient to detect a difference in the prevalence of zinc deficiency of 15 % between the women in the two EPAs with a confidence level of 95 % [14].

The study protocol was approved by the National Health Sciences Research and Ethics Committee, Malawi. Verbal informed consent was obtained from the traditional authorities in the villages and from the participants. After recruitment, the women were interviewed in their homes by trained research assistants using a pretested questionnaire to obtain demographic and socio-economic data, and information on the health and family characteristics of the women. From these data, a socio-economic status (SES) index was developed based on a total possible maximum score of 14 [15].

Anthropometry

Weight and height were measured using standardized techniques and calibrated equipment with participants wearing light clothing and no shoes, from which body mass index (BMI) [weight (kg)/height $(m)^2$], and the proportions with height-for-age Z scores < -2 SD and height < 145 cm were calculated.

Collection of duplicate diet composites

Trained research assistants collected one-day-weighed duplicate diet composites (including drinking water) from the women in their homes using digital scales accurate to ± 1 g (Salter, UK); details are described

in Hurst *et al.* [9]. Research assistants also recorded the types of foods consumed. Weekend and weekdays were proportionately represented to account for any day-of-the-week effects on food and/or nutrient intakes in the group. Diet composites were transported chilled to a central laboratory in Lilongwe for processing. Women were instructed not to change their normal dietary pattern during the diet-composite day, and were reimbursed for the cost of the food.

Analyses of diet composites

The weighed diet composites were blended to a homogeneous slurry, after which aliquots were withdrawn into trace-element-free polyethylene containers, frozen at –20 °C, and then shipped on dry ice to the University of Nottingham, where they were freeze-dried to constant weight. The diet composites were microwave-digested, and the digests analyzed for Fe, Zn, Se, Ca, Al, and Ti by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher X Series $^{(II)}$ model) in the laboratory of SY, as described elsewhere [9]. Analysis of phytate [as inositol penta-(IP5) and hexa-(IP6) phosphates] was performed by high-performance liquid chromatography (HPLC) in the laboratory of RSG using the method of Lehrfeld [16], as modified by Hotz and Gibson [17]. Molar ratios of phytate:zinc were calculated for each diet composite from the analyzed values. Accuracy and precision of the diet composite assays were established using certified reference materials for the minerals [9] and an inter-laboratory comparison for phytate [17].

Calculation of the prevalence of inadequate intakes of zinc

The distribution of observed intakes of zinc based on the analyzed one-day diet composites were adjusted to usual intakes by using PC-SIDE (PC software for intake distribution estimation) in conjunction with the intake, monitoring, assessment and planning program (IMAPP) [18]. An external variance component ratio for zinc (0.6993) calculated from dietary data for pregnant Malawian women [19] was used for this step because only one-day diet composites were collected. The prevalence of inadequate intakes of zinc, a recommended indicator for population risk of zinc deficiency [13], was calculated using the Estimated Average Requirement (EAR) cut-point method and the EAR for an unrefined cereal-based diet set by the International Zinc Consultative Group [20]. The prevalence of inadequate intakes of iron was not estimated in view of the uncertainty about the bioavailability of iron in these diets [21].

Laboratory assessment

Fasting morning venipuncture blood samples were drawn into trace-element-free heparinized evacuated tubes (Becton Dickinson, UK Ltd, Plymouth, UK) from all women, in the sitting position. Blood samples were stored in cooler boxes with ice packs as soon as possible after collection, and transported to the laboratories of the nearby district hospitals, where one aliquot of whole blood was analyzed for a complete blood cell count performed by an electronic counter. A second aliquot was separated by centrifugation using trace-element-free techniques based on the recommended procedures of the International Zinc Nutrition Consultative Group (IZiNCG) [20], and the plasma stored frozen at –80 °C in trace-element-free polypropylene vials. The frozen plasma samples were shipped to the UK on dry ice for analysis.

Plasma ferritin, soluble transferrin receptor (sTfR), α-1-acid glycoprotein (AGP), and C-reactive protein (CRP) were analyzed in duplicate in the laboratory of SFT by enzyme immunoassays using commercial kits manufactured by Ramco Laboratories (Houston, TX) for ferritin and sTfR, and R&D Systems (Minneapolis, MN, USA) for the inflammatory biomarkers. Plasma zinc and selenium were analyzed using ICP-MS (Thermo Fisher X Series$^{(II)}$ model) in the laboratory of SDY, as described previously [9]. A pooled plasma sample together with manufacturer's controls (for Hb, ferritin, AGP, and CRP) or certified reference materials (for plasma zinc, and selenium) [8] were used to check the precision and accuracy of the analytical methods. The WHO international reference material (Recombinant sTfR, NIBSC code: 07/202) was used for the sTfR assay [22]. The between-assay coefficients of variation (CVs) for serum ferritin, sTfR, CRP, AGP, zinc, and selenium were 10 %, 4 %, 7 %, 6 %, 1.3 %, and 1.9 %, respectively. Values for the controls fell within the certified ranges for plasma ferritin, sTfR, AGP, CRP, zinc, and selenium.

Plasma concentrations of AGP > 1.0 g/L and CRP > 5 mg/L were used to indicate the presence of chronic and acute inflammation, respectively [23] and to adjust the ferritin values for subclinical inflammation [24]. Anemia was assessed by Hb < 120 g/L [23], microcytic anemia by mean cell volume (< 82 fL), depleted iron stores as plasma ferritin < 12 μg/L (adjusted for

inflammation) in the absence of anemia [23], and iron deficiency (ID) as total body iron < 0 mg/kg, adjusted for inflammation [25]. The algorithm of Cook *et al.* [25] was used to calculate total body iron because the ratio sTfR to ferritin was measured using the Ramco assays [26]. Iron deficiency anemia (IDA) was defined as iron deficiency concurrent with anemia. Zinc deficiency was defined as plasma zinc < 10.7 μmol/L [20,27]. No universal interpretive criteria are available for plasma selenium levels because they vary markedly with geographic location [28]. In this study, plasma selenium concentrations were considered low when below the level of optimal activity of at least one of the selenoproteins (i.e. < 0.82 μmol/L) [29].

Statistical Analysis

All p values are 2-sided and not adjusted for multiple testing. Distribution of data was checked for normality using the Shapiro-Wilkes test. For consistency and comparability with earlier data, intakes of iron and zinc are reported as medians (1st, 3rd quartiles). Values for ferritin (adjusted for inflammation), sTfR, CRP, and AGP were log-transformed to normalize their distributions and expressed as geometric means (95th Confidence Interval). Differences in median intakes, arithmetic, or geometric mean (ferritin, CRP, and AGP) biomarker concentrations in the two EPAs were assessed using the Mann-Whitney *U* test and Student's two sample *t*-tests, respectively, whereas differences between the two EPAs for the proportion with storage iron depletion, ID, IDA, iron sufficiency, non-iron deficiency anemia, and zinc deficiency were examined using Fisher's exact test. Spearman's rank correlation coefficients were used to explore associations between socio-demographic variables, hemoglobin, and the plasma biomarkers.

Multiple regression analysis was used to examine the independent predictors of adjusted log ferritin, adjusted total body iron, hemoglobin, and plasma zinc. The explanatory variables investigated in the regression models were those that were known or suspected [3] to be biologically important. Dietary intake data could not be included in the models because they were based on one-day intakes per person [30]. There was no evidence in the multiple regression models of multiple co-linearity for the independent variables. Statistically significant differences are indicated by $p < 0.05$. All statistical analyses were carried out using STATA-11.0 (Stata Corp, College Station, TX).

Results

Socio-demographic characteristics of study population

All women approached agreed to participate in the study and were predominantly subsistence farmers. Significantly more women from the high selenium EPA (i.e. Mikalango) had little or no primary education, were classified into the two lowest SES categories

Table I: Selected socio-demographic and anthropometric characteristics of Malawian women with a low plasma selenium (Zombwe) and high plasma selenium (Mikalango) status.

	Zombwe		Mikalango		p
Households are subsistence farmers [2] (%)	53/60	88	46/60	77	0.249
Respondents with no or only primary education [2](%)	6/60	10	19/60	32	0.049
Household SES score 7 or less on scale of 1-14[2] (%)	16/60	27	35/60	58	0.002
Number of persons in household[1,3]	4.8	1.6	4.7	1.6	0.610
Age (y) [1,3]	39.0	11.0	34.8	9.6	0.026
Height, cm[1,3]	156.5	5.5	152.8	5.9	0.001
Weight, kg[1,3]	55.4	11.6	52.8	8.0	0.173
BMI, kg/m^2 [1,3]	22.5	4.1	22.5	3.3	0.977
BMI less than 18.5[2] (%)	6/53	11	4/55	7	0.523
Stunted (HAZ < −2.0)[2] (%)	4/53	8	5/55	9	0.524
Height less than 145 cm[2] (%)	2/53	4	3/55	5	0.518

[1] Values are mean and standard deviation
[2] Fisher's exact test
[3] Students *t*-test

(i.e., SES score 1–3 and 4–7), and had a lower mean age and height than the women from the low selenium EPA (i.e. Zombwe) ($p< 0.05$). In contrast, there were no significant differences in the mean number of persons per household, body weight, and BMI of the women in the two districts, with < 15 % in each district with a BMI below 18.5 (termed "mild underweight") (Table I). Of the women, less than 10 % were stunted, with very few (≤ 5 %) with a height below 145 cm, the critical height to limit the risk of delivery complications as a result of cephalo-pelvic disproportions [31].

Intakes from analyzed diet composites

Maize was the dominant cereal in Zombwe whereas in Mikalango, sorghum and millet replaced maize. Consumption of both cellular animal protein and dairy products were low in both groups, with the exception of a small amount of milk consumed with tea. Indeed, during the diet composite collection day, milk was consumed in tea by the women in Mikalango on only one of the nine tea-drinking- occasions, compared to eight of the 51 tea-drinking-occasions in Zombwe. Median (1^{st}, 3^{rd} quartile) analyzed intakes (Table II) were significantly higher ($p < 0.05$) for iron, zinc, selenium, titanium, phytate, and molar ratios of phytate:zinc in Mikalango compared to Zombwe, although median intakes of aluminum were not significantly different in the two EPAs.

The prevalence of inadequate intakes of zinc was 54 % in Mikalango EPA and 89 % in Zombwe EPA, based on an EAR set for unrefined cereal-based diets [20].

Biochemical status

In those women with elevated CRP levels indicative of acute inflammation (CRP >5 mg/L, 17.5 %; 21/120), higher concentrations of log plasma ferritin ($p = 0.048$) and a tendency for lower values for plasma zinc ($p = 0.061$) were observed; hemoglobin, plasma sTfR, and plasma selenium did not differ. Elevated AGP concentrations had no significant association with plasma ferritin or zinc.

In Zombwe, the mean plasma selenium was lower ($p < 0.001$) as noted earlier [9], whereas the mean cell volume, geometric mean ferritin, mean total body iron (mg/kg) (both adjusted for inflammation), and the geometric mean for CRP (but not AGP) were higher ($p < 0.05$) in Zombwe, although the proportions of women with elevated CRP and AGP levels in the two districts were not significantly different (Table III).

The overall prevalence of anemia and iron deficiency anemia was 21 % (25/119) and 6 % (7/115), respectively. There were no significant differences between the two districts (Tables III and IV). Of the anemic women, 14 % (17/119) had a low mean cell volume (i.e. < 82 fL). Both storage iron depletion and iron deficiency (in the absence of anemia) were low in both districts (Table IV). Low plasma zinc concentrations were observed in 92 % and 95 % of the women from Zombwe and Mikalango, respectively, with no significant differences between the two districts. As expected, 82 % of women in Zombwe compared to none in Mikalango had plasma selenium concentrations <0.82 μmol/L.

Table II: Intakes of iron, zinc, selenium, phytate, molar ratios of phytate:zinc, aluminum, and titanium, based on analyzed diet composites for Malawian women with a low plasma selenium (Zombwe) and a high plasma selenium (Mikalango) status.

	Zombwe (n = 55)	Mikalango (n = 58)	
	Median ($1,3^{rd}$ Q)	Median ($1,3^{rd}$ Q)	P[1]
Iron (mg/day)	16.6 (10.9, 26.0)	29.6 (15.9, 49.3)	< 0.001
Zinc (mg/day)	4.8 (3.5, 6.4)	6.4 (4.4, 8.5)	0.003
Selenium (μg/day)	6.6 (4.5, 10.1)	55.3 (31.4, 89.8)	< 0.001
Phytate (mg/day)	846 (525, 1197)	1564 (1094, 2236)	< 0.001
Phytate:zinc[2]	17 (14,20)	24 (20,31)	< 0.001
Aluminum (mg/day)	15.6 (11.3, 27.6)	16.7 (10.0, 29.3)	0.827
Titanium (mg/day)	0.18 (0.12, 0.31)	0.32 (0.22,0.44)	< 0.001

(1,3rd Q): 1st, 3rd Quartile
[1]Mann Whitney-*U* test
[2]Molar ratio

Table III: Plasma biomarkers, hemoglobin, and mean cell volume for Malawian women with a low plasma selenium (Zombwe) and a high plasma selenium (Mikalango) status.

	Zombwe	Mikalango	
	Mean (95% CI)	Mean (95% CI)	p
Zinc, μmol/L[1,4]	8.60 (8.22, 8.99)	8.87 (8.55, 9.19)	0.289[1]
Zinc < 10.7 μmol/L, *n (%)*[5]	55/60 (92%)	57/60 (95%)	0.464
Selenium, μmol/L[1,4]	0.72 (0.68, 0.75)	1.60 (1.52, 1.68)	<0.001
Selenium < 0.82 μmol/L[5], *n (%)*	49/60 (82%)	0/60 (0%)	<0.001
Hemoglobin, g/L[1,4]	138.9 (132.4,145.4)	132.8 (128.6, 137.0)	0.119
Hemoglobin < 120 g/L[5], *n (%)*	14/60 (23%)	11/59 (19%)	0.530
MCV, fL[1,4]	90.2 (87.6, 92.9	85.3 (82.3, 87.9)	0.009
MCV <82 fL[5], *n (%)*	14/60 (23%)	24/59 (41%)	0.042
Ferritin, μg/L[2,3]	38.6 (30.6, 48.7)	26.3 (20.5, 33.8)	0.027
Ferritin <12.0 μg/L[5], *n (%)*	5/60 (8%)	11/56 (20%)	0.078
Body iron (mg/kg)[1,4]	5.3 (4.3, 6.4)	3.8 (2.7, 4.9)	0.046
Body iron<0 mg/kg[5], *n* (%)	6/60 (10%)	8/56 (14%)	0.479
CRP, mg/L[2,4]	1.6 (1.1, 2.4)	0.7 (0.5, 1.1)	0.004
CRP > 5 mg/L[5], *n (%)*	13/60 (22%)	8/60 (13%)	0.230
AGP, g/L[2,4]	0.5 (0.5, 0.6)	0.6 (0.5, 0.6)	0.326
AGP >1 g/L[5], *n (%)*	6/60 (10%)	4/59 (7%)	0.527

MCV: Mean cell volume; CRP: C-reactive protein; AGP: α-1-acid glycoprotein
[1]Values are arithmetic means
[2]Values are geometric means
[3]Ferritin values are adjusted for inflammation [24]
[4]Students *t*-test
[5]Fisher's exact test

Table IV: Prevalence of depleted iron stores, iron deficiency, iron deficiency anemia, iron sufficiency, and non-iron deficiency anemia for Malawian women with a low plasma selenium (Zombwe) and a high plasma selenium (Mikalango) status.

Stages of iron deficiency	Zombwe	Mikalango	
	n (%)	n (%)	p[2]
Storage iron depletion[1,2]			
Ferritin <12 μg/L+ Hb ≥120 g/L	2/60 (3%)	5/55 (9%)	0.269
Iron deficiency [1,2]			
Body iron <0 mg/kg + Hb ≥120 g/L	4/60 (7%)	2/55 (4%)	0.683
Iron deficiency anemia[1,2]			
Body iron <0 mg/kg + Hb <120 g/L	2/60 (3%)	5/55 (9%)	0.269
Iron sufficient (non-anemic)[1,2]			
Body iron > 0 mg/kg + Hb ≥120g/L	42/60 (70%)	43/55 (78%)	0.699
Non-iron deficiency anemia[1,2]			
Body iron > 0 mg/kg + Hb <120g/L	12/60 (20%)	5/55 (9%)	0.196

Hb: Hemoglobin
[1]Ferritin values are adjusted for inflammation [24]
[2]Fisher's exact test

Interrelations among biomarkers and socio-demographic variables

Significant Spearman's rank correlations were observed between values for all the women for adjusted log ferritin and hemoglobin ($r = 0.245$, $p = 0.008$), log sTfR, and hemoglobin ($r = -0.320$; $p = 0.005$), adjusted total body iron and hemoglobin ($r = 0.388$, $p < 0.001$), adjusted log ferritin and log sTfR ($r = -0.554$, $p < 0.001$), and plasma zinc and hemoglobin ($r = 0.291$, $p = 001$). No significant relationships were observed between plasma biomarkers and the socio-demographic or anthropometric characteristics of the women

In view of the very large differences in the plasma selenium concentrations in the two EPAs [9], associations between plasma selenium and other biomarkers were examined separately for each EPA. In the low-selenium Zombwe EPA, the Spearman's rank correlation between plasma selenium and zinc was positive ($r = 0.253$; $p = 0.052$, $n = 60$), whereas in the high-selenium Mikalango EPA the correlation was negative ($r = -0.273$; $p = 0.035$, $n = 60$). Plasma selenium was not associated with any of the other biomarkers or the socio-demographic variables of the women in each EPA.

Determinants of plasma ferritin, total body iron, plasma zinc and hemoglobin

Plasma CRP (but not AGP) was a significant and positive determinant of unadjusted log ferritin ($p = 0.004$; $R^2 = 0.10$) based on linear regression analysis. Neither age, household socio-economic status, education level of the respondent, BMI, or district were significant determinants of adjusted log ferritin or adjusted total body iron, and for plasma zinc when log CRP or log AGP was also included as an additional independent variable in the regression analysis. In the multiple regression model for hemoglobin, total body iron adjusted for inflammation had the largest standardized beta coefficient, followed closely by plasma zinc; neither log AGP, household SES score, age, or district (high or low selenium status area) was significant (Table V).

Discussion

A striking feature of the results is the very high prevalence of zinc deficiency, which was independent of the selenium status, and the contrasting much lower prevalence of iron deficiency among these Malawian women. This result was unexpected in view of the low intakes of readily available heme iron from cellular animal protein and the high phytate:iron molar ratios in the diets. Furthermore, the multiple regression results with hemoglobin as the dependent variable emphasized that the zinc status of these women was almost as important as total body iron (mg/kg body weight) as a determinant of hemoglobin.

Zinc status

In this study the prevalence of inadequate intakes of zinc was high, especially among the women from Zombwe, suggesting that dietary-induced zinc deficiency most likely contributed at least in part to the high prevalence of zinc deficiency noted here, a finding consistent with earlier reports of rural Malawian pregnant women [15]. This finding is not unexpected as the diets of the women in both EPAs were low in zinc with high phytate-to-zinc molar ratios, and the inhibitory effect of phytate on zinc absorption in adults is now thought to be much larger than previously estimated

Table V: Multiple linear regression analysis with hemoglobin as the dependent variable.

Independent variable	β coefficient (95% CI)	Standardized β	p > t
Total body iron, mg/kg[1]	0.183 (0.095, 0.272)	0.354	0.000
Plasma zinc, μmol/L	0.007 (0.003, 0.011)	0.293	0.001
Log AGP, g/L	−0.642 (−1.478, 0.195)	−0.126	0.131
Household SES score	−0.090 (−0.325, 0.145)	−0.069	0.448
Age, y	−0.026 (−0.061, 0.009)	−0.126	0.148
District code	−0.682 (−1.482, 0.118)	−0.159	0.094

n=115, $R^2 = 0.252$
[1]Plasma ferritin adjusted for inflammation [24]

[32]. Moreover, there is no evidence of an adaptive response to habitual high phytate intakes on zinc absorption [33]. Nevertheless, the predicted risk of zinc deficiency based on low plasma zinc concentrations was higher than predicted from zinc intakes. Several factors may account for this lack of concordance, including the existence of tropical enteropathy, which has been reported earlier to exacerbate risk of zinc deficiency among Malawian children [34].

The prevalence of low plasma zinc concentrations among the women was unexpectedly high in the two districts, with a difference of only 3 %, despite their contrasting plant-available soil selenium concentrations. Our sample size was too small to detect whether this was a significant difference in the prevalence of zinc deficiency.

Iron status

Less than 15 % of the women had iron deficiency (defined by total body iron <0 mg/kg), despite the high prevalence of zinc deficiency (i.e. > 90 %) in both EPAs. Indeed, total body iron for the Zombwe women, unlike the women in Mikalango EPA, was very comparable to that for US women of child bearing age (20 to 49 years) in the US National Health and Nutrition Examination Survey 2003–2006 [35]. A lack of concordance between the prevalence of iron and zinc deficiency has also been reported earlier among pregnant women in Ethiopia whose diets, like those reported here, contained negligible amounts of readily absorbable heme iron but high levels of non-heme iron [3]. Contaminant iron from soil was probably responsible, at least in part, for the high iron content of the Malawian diets, as reported earlier in Ethiopia [36, 37]. This suggestion is supported by the high concentrations of aluminum and titanium in the analyzed diet composites, well recognized markers of soil contamination [38], and their positive correlation ($p < 0.05$) with the iron concentration of the diet composites. These findings highlight the importance of assessing iron intakes from analyzed duplicate diet composites in this setting. In Malawi, iron contamination from soil may arise from using threshing sticks to thresh cereal grains. During this practice, soil adheres to the outer surface of the cereal grains, especially those with small grains such as sorghum and millet, the major cereals consumed in the Mikalango EPA.

The low prevalence of depleted iron stores, especially among the Zombwe women (i.e. 3 %), even after adjusting ferritin for inflammation, suggests that at least some of the contaminant iron may have joined the common non-heme iron pool (i.e. is exchangeable) and thus been available for absorption as postulated in Ethiopia [36], although we recognize that predicting the exchangeability of contaminant iron is difficult [39]. Moreover, the low prevalence of iron deficiency anemia overall (i.e. 6 %) suggests that the few women identified with low iron stores may have upregulated iron absorption from their predominantly cereal-based diets to preserve hemoglobin mass.

The women in the high-selenium EPA, Mikalango, had significantly higher intakes of total iron compared to those in the low-selenium EPA, yet significantly lower ($p < 0.05$) adjusted values for plasma ferritin and total body iron (Tables II and III). Several factors may account for this discrepancy, including the significantly higher phytate (Table II), and probably higher polyphenol content of the brown finger and bullrush millets and brown sorghum consumed in Mikalango, compared to the maize in Zombwe, which has a low polyphenol content [40]. Both phytate and polyphenols inhibit absorption of the non-heme iron intrinsic to food as well as any exchangeable contaminant iron [1,39]. Consumption of polyphenol-containing tea was not an additional inhibitory factor because the women in Mikalango consumed less tea than those in Zombwe.

It is also possible that the proportion of exchangeable contaminant iron was lower in the diets in Mikalango than Zombwe due to differences in soil mineralogy. Mikalango EPA is characterized by areas of calcareous Eutric Vertisols (median pH = 7.8), whereas in Zombwe EPA soils have a low pH (median 5.2) [9]. Certainly, the proportion of soil contaminant iron that is available for absorption is said to vary widely [39]. Reduction in iron absorption as a consequence of inflammation [41] is unlikely to be responsible for the discrepancy because there were no differences in the prevalence of elevated concentrations of CRP and AGP in the two EPAs.

Determinants of plasma ferritin, total body iron, hemoglobin, and plasma zinc

We were unable to explore the role of the dietary intakes as determinants of iron, hemoglobin, or zinc status of the women in our multiple regression models because only one-day diet composites were collected. Of the dependent variables investigated, only significant determinants of hemoglobin were identified. These were adjusted total body iron and plasma zinc, with plasma zinc being almost as important as total body iron (based on the absolute values of the

standardized ß-coefficients). Furthermore, there was no significant correlation linking these two independent variables (i.e. total body iron and plasma zinc) (data not shown), confirming that a comparable distribution of iron and zinc in the food supply was not responsible for the positive association between zinc and hemoglobin noted here.

Both dietary and non-dietary factors probably contributed to the positive relationship observed here between adjusted total body iron and hemoglobin. As emphasized earlier, total iron intakes were unexpectedly high, in part due to contaminant iron from soil, although the extent to which the contaminant iron is exchangeable is uncertain. Consequently, we did not calculate the prevalence of inadequate intakes of iron.

It is of interest that 15 % of the cases of anemia overall among these Malawian women were not attributable to iron deficiency (Table IV). Other factors that have been reported in earlier studies to play a role in anemia in Malawi include deficiencies of vitamin B-12 and vitamin A [42], as well as malaria, glucose-6-phosphate dehydrogenase deficiency (G6PD), α-thalassemia, and sickle cell disease [43]. Most of these factors were not investigated in this study with the exception of macrocytosis (i.e. mean cell volume > 99 fL) [44], a characteristic of vitamin B-12 deficiency, which was seen in 8 % of the women.

Plasma zinc was found to be an important determinant of hemoglobin in an earlier study of pregnant Ethiopian women [3]. Several mechanisms exist whereby zinc has a role in hemoglobin concentrations. For example, hemoglobin synthesis depends on several zinc-dependent enzyme systems, specifically aminolevulinic acid dehydrase [45] and thymidine kinase and DNA polymerase [46], whereas a zinc finger transcription factor – GATA-1 – is essential for normal hematopoiesis [47]. Zinc may also play a role in stabilizing red cell membranes and in increasing plasma IGF-1 levels, which may in turn stimulate erythropoiesis [48].

Despite the tendency for plasma zinc and selenium to be positively correlated among women in the low-selenium district of Zombwe and negatively correlated in the high-selenium district of Mikalango, district was not a significant determinant of plasma zinc in our multiple regression model. The reason for these findings is uncertain.

Strengths and weaknesses of study

We believe this study has several strengths. The analysis of the duplicate diet composites improved the estimates of trace element and phytate intakes, and confirmed the presence of contaminant iron from soil, although because the diet composites were based on only one day, the data could not be included in the multiple regression models. Furthermore, our estimates for the prevalence of inadequate zinc intakes were calculated from the distribution of usual intakes, whereas that of iron deficiency was based on total body iron adjusted for inflammation to provide a more accurate reflection of the true prevalence and severity of nutritional iron deficiency [49]. Finally, analysis of plasma zinc and selenium as well as multiple iron biomarkers allowed the assessment of inter-relationships among these micronutrients in Malawian women, which has not been possible in earlier reports.

Nevertheless, we were not able to assess the presence of malaria parasitemia or genetic hemoglobin disorders, all of which also have been implicated in anemia in Malawi [43] and also have the potential to confound total body iron assessment by elevating sTfR in response to an increased rate of erythropoiesis [49, 50]. Further, we used the correction factors of Thurnham *et al.* [24] to adjust for the effect of subclinical inflammation on ferritin and body iron because our sample size was too small to yield reliable estimates based on internally generated correction factors.

We also recognize that our results are based on a convenience sample of women and therefore not representative of women of child-bearing age living in Zombwe and Mikalango EPAs of Malawi. However, in an effort to minimize bias, women from six villages in Zombwe EPA and six villages in Mikalango were approached, all of whom were eligible and agreed to participate in the study. A further limitation is that our small sample size may have accounted for our inability to both detect any difference in the prevalence of low plasma zinc among the women in the two districts and explain none of the variance in ferritin, total body iron, and plasma zinc concentrations. Nonetheless, some of the nutritional status variables reported here were comparable to those reported in the same geographical areas in the 2010 Malawian Demographic and Health Survey [51]. For example, in Zombwe and Mikalango EPAs, both the mean BMI (Zombwe, 22.4 vs. 22.5; Mikalango, 21.6 vs. 22.5) and the proportion with BMI < 18.5 (Zombwe, 5.6 vs. 10 %; Mikalango, 7.8 vs. 15 %) were reasonably comparable to those reported here. Likewise, for Zombwe, the prevalence of anemia in the DHS survey was similar (26 vs. 23 %), although higher for the DHS survey in Mikalango (37 %) than that reported in this study (19 %). Finally, because our study was observational, we cannot exclude the possibility of unmeasured confounding.

Conclusion

Risk of zinc deficiency among these Malawian women was much higher than that of iron deficiency in both districts. This finding was attributed to diets low in absorbable zinc but with a high total iron content due in part to contaminant iron from soil. We were unable to show any differences in the prevalence of zinc deficiency among the women living in districts with low and high plant-available soil selenium concentrations. Plasma zinc was almost as important as total body iron as a determinant of hemoglobin. Our findings suggest that at least some of contaminant iron from soil was exchangeable and thus available for absorption, and highlight the utility of analyzing diet composites to assess total iron intakes in this setting.

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