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Concurrent Micronutrient Deficiencies Are Low and Micronutrient Status Is Not Related to Common Health Indicators in Ghanaian Women Expecting to Become Pregnant

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ABSTRACT

Background: Micronutrients are important for reproductive health and pregnancy, but the status of multiple vitamins and minerals is rarely measured in women before pregnancy.

Objectives: We aimed to assess the status and concurrent deficiencies of micronutrients among women before pregnancy and their relation with common health indicators.

Methods: This was a cross-sectional study that recruited women who expected to become pregnant within the next 6 mo in Asesewa, Ghana, a semi-urban community. Women self-reported demographics and health history. We measured blood pressure, height, and weight and conducted a blood draw and hemoglobin assessment ($n = 98$). We measured serum/plasma concentrations of ferritin, iron, total iron binding capacity, zinc, copper, retinol, and 25-hydroxyvitamin D, in addition to markers of inflammation. We used established cutoffs for deficiency and insufficiency/low status for each micronutrient after adjusting ferritin, zinc, and retinol for inflammation. We compared biomarker distributions by common health indicators.

Results: Forty percent of women had overweight/obesity, 33% were anemic, and 23% had elevated blood pressure. Overall, 27% had ≥1 deficiencies, whereas only 4% had 2 deficiencies. Fifty-eight percent of women had ≥1 insufficiencies and 18% had ≥2 insufficiencies. Prevalence of individual deficiencies was 12%, 7%, 7%, 4%, and 0% and prevalence of individual insufficiencies was 18%, 12%, 29%, 13%, and 13% for iron, copper, vitamin A, zinc, and vitamin D, respectively. Iron biomarkers and retinol concentrations differed by anemia status, and copper was higher in those with elevated blood pressure. Micronutrient concentrations were not associated with self-reported medical history (parity or history of anemia, malaria, or night blindness).

Conclusions: In Asesewa, Ghana, there was a relatively low prevalence of individual micronutrient deficiencies, but the majority of women were insufficient in ≥1 micronutrients. Iron and vitamin A status was lower in those with anemia, but otherwise, micronutrient status did not relate to common health markers. *Curr Dev Nutr* 2019;3:nzz053.

Keywords: prepregnancy, co-occurring, concurrent, deficiency, micronutrients, status, biomarker

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Supplemental Figures 1 and 2 and Supplemental Table 1 are 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/cdn/.

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Abbreviations used: AGP, α_1-acid glycoprotein; CRP, C-reactive protein; TIBC, total iron binding capacity; TSAT, transferrin saturation; WRA, women of reproductive age; 25(OH)D, 25-hydroxyvitamin D.

Introduction

Micronutrients, particularly iron, zinc, iodine, and vitamin A, are essential for the overall health and development of women of reproductive age (WRA) (1). These micronutrients play important biological roles during the reproductive years and are vital in preparing a woman for pregnancy. Deficiencies in these micronutrients have been associated with preterm deliveries, low birth weight, fetal growth restriction, congenital abnormalities, and cognitive impairments, among other health issues (2–9). It is important for women to obtain adequate stores of these nutrients prior to pregnancy both to improve reproductive health and to meet the increased demand

during the pregnancy period. Unfortunately, numerous reports indicate a high prevalence of micronutrient deficiencies in WRA worldwide, especially in low- and middle-income countries (1, 10, 11).

Several studies, including meta-analyses, have examined a range of individual micronutrient deficiencies, including zinc, retinol, iron, iodine, folate, vitamin B-12, and vitamin A, in WRA (12–16). Meta-analyses, however, have not reported which of these micronutrient deficiencies co-occur because it is rare that co-occurrence is reported in individual studies. In reality, it is likely that individuals in developing countries often experience multiple micronutrient deficiencies simultaneously. Few studies have assessed concurrent micronutrient deficiencies; only 1 focused on nonpregnant women (17), whereas others assessed pregnant women (18, 19) and lactating mothers (20). Some grouped anemia with specific micronutrients to report concurrence, and insufficiencies were not quantified. As such, the extent of the burden of co-occurring micronutrient deficiencies is unclear and is mostly speculated (14, 15, 17). In addition, data on micronutrient status specific to prepregnancy (women expecting to become pregnant) are lacking.

Public health nutritionists have a limited set of tools to screen for potential micronutrient deficiencies in an inexpensive, quick manner. Examples include the assessment of goiter for iodine deficiency, night blindness for vitamin A, and anemia (which requires a small blood sample) as a proxy for iron deficiency. Dietary intake assessments have been used as a proxy for micronutrient status (21), but in addition to requiring extensive training to properly collect such data, there is often low correlation between the measured dietary intake of a nutrient and biochemically measured micronutrient status in an individual. Currently, simple screeners for micronutrient deficiencies are lacking.

Given the limited information on simultaneously occurring micronutrient deficiencies (and complete lack of data on insufficiencies), coupled with the lack of noninvasive tools to screen for micronutrient deficiencies, we aimed to assess the micronutrient status of Ghanaian women planning to become pregnant and to determine which of these micronutrient deficiencies co-occurred. Furthermore, we explored the relations between several commonly collected noninvasive health measures and micronutrient status.

Methods

Study setting and population

We conducted a cross-sectional study in Asesewa, a semi-urban setting in the eastern region of Ghana. The initial purpose of the study was to assess micronutrient status in WRA in the area and to pilot field methods to identify women before a pregnancy. Asesewa is the administrative capital of Upper Manya Krobo District, a predominantly farming district. More than 73% of the district population is employed in the agriculture sector (22). We recruited a convenience sample of women from churches, mosques, and women's networking groups. We worked together with religious leaders to plan the timing of visits to the churches and mosques in the study area. Trained research assistants and field supervisors addressed congregants during church services and after Muslim prayers, after which interested women were screened. Similar arrangements were made with leaders of women's groups. Later in the study, we added a door-to-door recruitment approach to enable us to reach women who did not belong to religious or women's groups. Inclusion criteria were nonpregnant females, aged 18–35 y, planning/expecting to get pregnant within 6 mo, and residing in the Asesewa area. Specifically, we asked women, "Are you planning to become pregnant in the next 6 months? Or do you expect that you could become pregnant in the next 6 months?" Women were excluded if they had been pregnant in the previous 12 mo. Eligible women were invited to the Asesewa Government Hospital for study consent and 1 visit that included data collection and a blood draw. Visits took place between October 2015 and April 2016. The study was reviewed and approved by the institutional review boards of The Pennsylvania State University and the Noguchi Memorial Institute for Medical Research, University of Ghana. Written informed consent was obtained from each woman prior to data collection. Women were reimbursed for travel costs and received a bar of soap as a token of appreciation for their voluntary participation.

Data collection

Eligible women were consented and enrolled at the beginning of the study visit, which occurred in the morning or early afternoon depending on the woman's availability. Data collection for enrolled women consisted of questions that were administered orally by trained study staff, and responses were recorded on a Samsung Galaxy Tab 4 (Model SM-T230NU; Samsung Electronics) using Open Data Kit 2.0 software (23). We adopted a questionnaire that has been used to assess household socioeconomic status and food insecurity in the study area (24). We added questions on pregnancy and health history and slightly adapted some questions related to socioeconomic status based on the interests of the current study.

Height was measured to the nearest 0.1 cm using the Seca 217 stadiometer (Seca GmbH), and weight was measured to the nearest 0.1 kg using the Seca 874 flat scale (Seca GmbH). Participants wore minimal or light clothing; they were also asked to remove shoes and any heavy clothing or objects, such as jewelry, before weighing. Each measurement was repeated twice, and the mean was calculated. Women provided a small urine sample, and a URIT 2 V reagent strip (URIT Medical Electronic Group) was read 30 s after dipping in the urine. By color, it indicated a range of 4 categories of protein: negative/trace, 30 mg/dL, 100 mg/dL, and ≥300 mg/dL.

Blood collection and processing

Women were not asked to fast before the blood draw; therefore, postprandial time was variable. A trained phlebotomist at the hospital completed the blood draws using a butterfly blood collection system. Blood was collected from an antecubital vein into 2 different 4-mL BD vacutainer venous blood collection tubes—SST serum separation tubes and lithium heparin-coated tubes (VWR). A drop of blood was transferred to a HemoCue cuvette from the butterfly tubing and placed into a HemoCue 201 analyzer to measure hemoglobin concentration. The HemoCue machine was calibrated each morning before fieldwork using control cuvettes. Lithium heparin-coated tubes were centrifuged within 30 min of blood collection at 1300 g for 15 min to obtain plasma. SST serum separation tubes were allowed to stand for 30–60 min to allow for clotting and were then centrifuged in the same way as the plasma samples to obtain serum. Plasma and serum were aliquoted into sterile cryovials and were initially frozen in a liquid nitrogen tank in the hospital for 1–2 wk before transporting to the

Noguchi Memorial Institute for Medical Research in Accra, where they were stored at −80°C until the study was completed. Samples were then shipped on dry ice to The Pennsylvania State University and stored in a −80°C freezer until analysis. To prevent vitamin A degradation from light, which could alter the measured retinol concentration, 1 of the plasma cryovials was wrapped with aluminum foil.

Biochemical analyses

We assayed plasma samples for zinc, copper, and retinol, and we assayed serum samples for 25-hydroxyvitamin D [25(OH)D], iron, ferritin, total iron binding capacity (TIBC), and the inflammation markers α_1-acid glycoprotein (AGP) and C-reactive protein (CRP). We measured serum ferritin by ELISA (Ramco Laboratories), calibrated against WHO standards, and serum iron and TIBC by colorimetric methods (25). Transferrin saturation (TSAT) was calculated as serum (iron/TIBC) × 100. For zinc and copper assays, plasma samples were diluted 5-fold as previously described (26, 27) with 0.1 N nitric acid (trace element grade; EMD Millipore), and concentrations of each were measured using flame atomic absorption spectroscopy on an AAnalyst 400 spectrometer (Perkin Elmer). Plasma retinol concentration was measured using ultra-performance LC (ACQUITY UPLC System; Waters Corporation) in Catharine Ross' laboratory. For zinc, copper, and retinol measurements, we used the National Institute of Standards and Technology reference material (SRM 1950) as control samples for our analyses. AGP and CRP were measured using radial immunodiffusion tests (Kent Laboratories) and used to adjust for micronutrient concentrations when inflammation was present. The intra-assay coefficient of variation for the biomarkers was 2.1% for TIBC, 3.1% for copper, 3.3% for zinc, 4.0% for ferritin, and 4.0% for iron.

Serum aliquots were shipped to the Analytical Facility for Bioactive Molecules, The Hospital for Sick Children, Toronto, Canada; this laboratory is certified by Vitamin D External Quality Assessment Scheme for the measurement of 25(OH)D concentrations. Samples were analyzed by LC–tandem MS using an Agilent 1290 HPLC interfaced with an AB Sciex 5500 QTRAP mass spectrometer (28, 29). 25(OH)D_3 and the C3 epimer of 25(OH)D_3 were quantified separately; these are the main circulating forms of the intermediate metabolite.

Anemia was classified as a hemoglobin concentration <12.0 g/dL; moderate anemia was <11.0 and severe was <8.0 g/dL (30, 31). The following cutoffs were used to define micronutrient deficiency and insufficiency/low status, respectively: adjusted serum ferritin <15.0 μg/L and <20.0 μg/L for iron (30), adjusted plasma retinol <20 μg/dL and <30 μg/dL for vitamin A (32), adjusted plasma zinc <66 μg/dL and <70 μg/dL for zinc (33, 34), plasma copper <70 μg/dL and <90 μg/dL for copper (34), and serum 25(OH)D (without the C3 epimer) <30 nmol/L and <50 nmol/L for vitamin D (35).

We defined signs of inflammation as high CRP (>5.0 mg/L) and/or AGP (>100 mg/L) (36). We classified women into 4 groups of inflammation/infection status: 1 (reference/healthy), normal CRP and AGP; 2 (incubation), high CRP and normal AGP; 3 (early convalescence), high CRP and high AGP; and 4 (late convalescence), normal CRP and high AGP (37). We then used correction factors for ferritin (37), zinc (38), and retinol (39) to adjust for the measured concentrations (reported as "adjusted" throughout). Correction factors were as follows (always 1 for the reference group): ferritin: 0.77, 0.53, and 0.75; retinol: 1.14, 1.31, and 1.12; and zinc: 1.08, 1.14, and 1.12 (each for groups 2, 3, and 4, respectively). Due to the small sample size, we did not internally calculate correction factors or use proposed regression methods (40).

After biomarker data were collected, we compiled results for each woman into a letter and delivered the letters to as many women as we could re-contact. Letters were written in English because it is the national language and is taught in all schools. A dietitian was available at the Nutrition Research Center in Asesewa for women who wanted to discuss their micronutrient status (∼10 women used this resource).

Statistical analyses

We estimated the prevalence of single and co-occurring micronutrient deficiencies/insufficiencies as the percentage of women below established cutoffs for each biomarker (using inflammation-adjusted concentrations for ferritin, retinol, and zinc). For continuous biomarker concentrations, we visually examined the normality of each distribution using kernel density and quantile–quantile plots. We used bivariate analysis to examine differences in nutrient status (biomarker concentration) for several measured and self-reported health indicators divided into dichotomous categories. If a biomarker distribution was normal, we tested for equal variance (i.e., SDs) between groups. We tested for a difference between means using a 2-sample independent *t* test, with or without an "unequal" option in Stata to relax the equal variance assumption, depending on the variance test. If the biomarker distribution was not normal, we tested the difference using the Wilcoxon–Mann–Whitney test. Stata 15 (Stata) was used for analyses, and a P value <0.05 was considered statistically significant, without adjusting for multiple testing.

Results

We enrolled 100 eligible women, and analysis was conducted for 98 women with complete data. Just over half of households of women owned their home, and a third owned agricultural land (**Table 1**). Almost 20% of households had a female head. Most women drank commercially packaged water (either bottled or "sachet"). Almost every household had electricity and a mobile phone, yet few had a flush toilet or a specific place for handwashing. Although <40% of women reported being married (government process), almost all other women were in a traditional/customary marriage. Just over half of women reported ≤1 previous births. Few women reported being diagnosed with high blood pressure or other heart problems, but a similar number of women had been diagnosed with anemia or night blindness (14% and 15%, respectively). More than half of women had malaria diagnosed in the past. Only 2 women reported taking a vitamin or mineral supplement.

Forty percent of women were overweight or obese (Table 1), and only 1 woman was classified as underweight. Four women had blood pressure in the hypertensive range; however, none of these women reported being diagnosed with high blood pressure previously. All women had negative/trace results for urine protein except 1 woman

TABLE 1 Sociodemographic, pregnancy history, and health characteristics of women of reproductive age and their households in Asesewa, Ghana [1]

Characteristics	
Household sociodemographics	
Own home, %	52.0
Own agricultural land, %	32.7
Female head of household, %	19.4
Participant is head, %	13.3
Number of adults in household[2]	2 [2, 4]
1, %	7.1
2, %	48.0
≥3, %	44.9
Number of children in household[2]	1 [0, 3]
0, %	29.6
1, %	24.5
≥2, %	45.9
Drinking water source,[3] %	
Piped/public tap	42.9
Sachet	55.1
Toilet facility, %	
Flush	17.4
Ventilated improved pit latrine	36.7
Pit latrine or other	45.9
Specific place to wash hands, %	17.4
Has refrigerator/freezer, %	43.9
Has mosquito net(s), %	82.7
Participant sociodemographics	
Age, y	26.5 ± 5.1
Education,[4] y	9 [8, 12]
Married,[5] %	38.8
Generates income, %	70.4
Pregnancy history, %	
Parity	
0	46.9
1	26.5
≥2	26.5
Spontaneous miscarriage (≥1)	19.3
Induced abortion (≥1)	24.6
Self-reported health history, %	
Anemia	14.3
Malaria	55.1
High blood pressure	3.1
Other heart problems	9.2
Night blindness	15.3
Measured health markers	
Height,[6] cm	160.0 ± 6.3
BMI, kg/m^2	23.8 [21.5, 29.0]
Normal (18.5 to <25.0), %	60.2
Overweight (25.0 to <30.0), %	21.4
Obese (≥30.0), %	18.4
Systolic blood pressure, mm Hg	109.5 ± 11.6
Diastolic blood pressure, mm Hg	70.2 ± 10.4

[1]Values are percentages for categorical variables, means ± SDs for continuous variables with a normal distribution, or medians [IQRs] for continuous variables with a nonnormal distribution; $n = 98$.
[2]Highest number of adults and children reported in household was 7 and 8, respectively.
[3]Two women reported "tube well/borehole."
[4]Six women reported 0 y of schooling; 11 women reported completing senior secondary school; no one reported completing higher education (university or polytechnics).
[5]Although few women reported being "married," 96 women reported being in a committed relationship; "customary marriage" is the most common/traditional union in Ghana but is often not called "marriage."
[6]Minimum and maximum were 141 cm and 176 cm, respectively.

with a 30 mg/dL result. One-third of women were anemic; of these, 36% ($n = 12$) were moderately anemic, and 1 woman was severely anemic. For inflammation status, 4% of women were in an "incubation" stage, and 11% of women were in the "late convalescence" stage (no one was in "early convalescence").

Using deficiency cut points, there were no women with vitamin D deficiency, whereas 4% of women had zinc deficiency, 7% of women had copper or vitamin A deficiency, and 12% had iron deficiency (**Figure 1**). Using other classifications for iron deficiency, 29% of women had TSAT <16%, and 7% of women had both ferritin <15 μg/L and TSAT <16%. Eight percent of women had a ferritin concentration of <12 μg/L, another common cut point for iron deficiency. Although 27% of women had ≥1 deficiencies, there was not much overlap, and only 4% had 2 deficiencies (none had >2).

There was some overlap between women who were classified as anemic and those with a micronutrient deficiency: 45% of women with anemia also had ≥1 of the 5 deficiencies we examined (**Supplemental Figure 1**). On the other hand, 17% of women who were not anemic had ≥1 deficiencies. Thus, hemoglobin was not a good predictor for the presence or absence of these 5 micronutrient deficiencies. The prevalence of iron deficiency anemia was low. Only 24% of all anemia (8 of 33 cases) appeared to be due to iron deficiency (using ferritin <15 μg/L; 48% if using TSAT <16%).

Using cut points for insufficient/low status, the prevalence of poor micronutrient status was higher, with low vitamin A the most common at 29% (Figure 1). More than half of women had low status in ≥1 micronutrients, 18% had at least 2 insufficiencies, and 9 women had 3 insufficiencies. The prevalence of each deficiency and insufficiency was similar across overweight and obesity status, with no meaningful differences ($P > 0.05$) except that vitamin A insufficiency was higher in normal-weight than in overweight/obese women (36% compared with 18%, $P = 0.04$).

We also examined the prevalence of the concurrence of 2 insufficiencies to identify which micronutrients were overlapping in insufficient status (**Supplemental Table 1**). There was low overlap overall; however, vitamin A had the highest prevalence of co-occurrence with iron (7% of women had both) and, surprisingly, with zinc (5%), copper (6%), and vitamin D (6%) as well. Anemia was slightly more predictive of insufficiencies compared with deficiencies: 64% of those with anemia had ≥1 insufficiencies (Supplemental Figure 1).

We presented conventional summary statistics for each biomarker concentration (**Table 2**) along with distributions by kernel density (**Supplemental Figure 2**). Differences in nutrient biomarkers by measured (**Table 3**) and self-reported health (**Table 4**) indicators are shown by means or medians. Few biomarkers were associated with health indicators. As expected, there was lower TSAT and higher TIBC in those with anemia, but in this study, ferritin and iron concentrations did not differ by anemia classification (Table 3). However, anemia was associated with lower retinol concentrations. AGP concentrations were higher in those with anemia and those classified as overweight. Higher copper concentrations were observed in women with elevated blood pressure. Self-reported health characteristics—parity and history of anemia, malaria, or night blindness—were not related to biomarker concentrations except that AGP was higher among women who reported being diagnosed with anemia (Table 4).

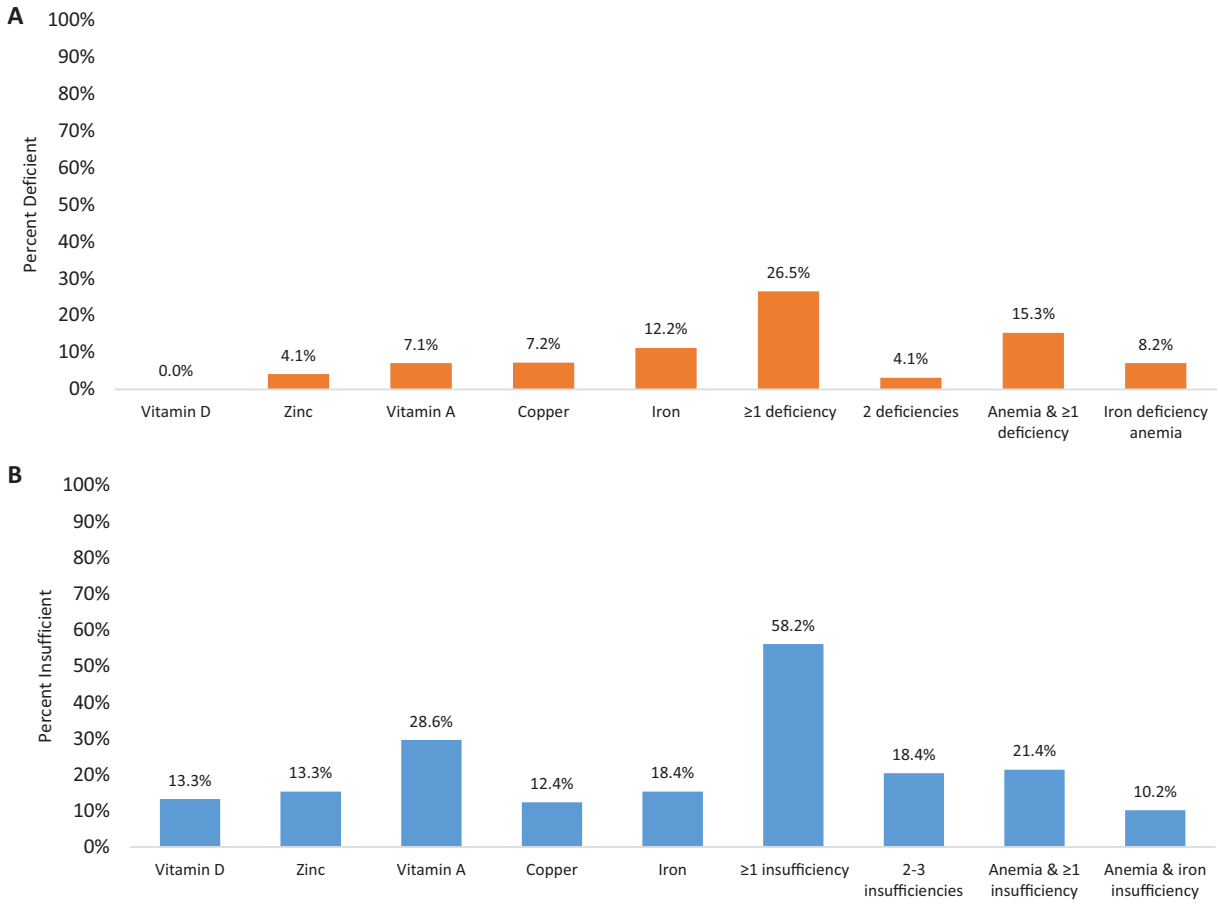

FIGURE 1 Prevalence of micronutrient deficiencies (A) and insufficiencies (B) of women expecting to become pregnant in Ghana ($n = 98$). Cutoffs used to define deficiency and insufficiency, respectively: 25-hydroxyvitamin D <30 nmol/L and <50 nmol/L (vitamin D); adjusted zinc <66 μg/dL and <70 μg/dL; adjusted retinol <20 μg/dL and <30 μg/dL (vitamin A); copper <70 μg/dL and <90 μg/dL ($n = 97$); and adjusted ferritin <15 μg/L and <20 μg/L. Adjustments were done using correction factors from references 37–39.

Discussion

In this cross-sectional study in a semi-urban area of Ghana, we examined the status of 5 micronutrients in WRA who were expecting to become pregnant in the next 6 mo. The prevalence of each individual deficiency was low, but more than one-fourth of women had an overt deficiency. We also found that more than half of women were insufficient in ≥1 micronutrients, and one-third had anemia. In examining health factors that related to micronutrient status, there were few associations beyond anemia's relation to iron and vitamin A status biomarkers, yet anemia had a low positive predictive value (45%) for micronutrient deficiencies. This is a setting in which most women are raising food at their homesteads, malaria is endemic, and the overall number of births per woman is moderate. Recent projects designed to reduce food insecurity, including Nutrition Links (24), have raised awareness and availability of animal source foods, and have increased nutritional knowledge in this area. The effect of these projects may account for the relatively low prevalence of micronutrient deficiencies we observed in this area compared with other low-resource settings.

Although there are numerous studies examining micronutrient status in women of reproductive age, few have used blood-based biomarkers, and of those, it is rare that studies report multiple micronutrients and concurrent deficiencies. There are 3 studies, similar to ours, that measured a relatively small number of micronutrients (3–6 each) on a small number of women (109–283 women) (17, 19, 20) in India and Indonesia. Another more comprehensive study was conducted in Nepal on >1100 pregnant women and measured the status of 11 vitamins and minerals (18). Overall, similar cut points were used to define deficiency, but insufficiency/low status was not reported in addition to deficiency prevalence (with the exception of low vitamin A status being reported in the Nepal study). In general, the prevalence of deficiencies, and concurrent deficiencies, in the women in Ghana was lower than that reported in the other 4 studies; we could not compare the prevalence of insufficiencies to the larger context because they are so rarely reported. It was quite surprising to us that so few studies have reported the overlap of biomarker-based micronutrient status, and we find it urgent and important that more studies measure and report the concurrence of multiple micronutrient deficiencies in WRA. Future reviews and meta-analyses of micronutrient deficiencies should

TABLE 2 Micronutrient and inflammation blood biomarker concentrations of women expecting to become pregnant in Asesewa, Ghana[1]

Biomarker	Mean (SD)	Geometric mean (95% CI)	Median [IQR]	Range
Serum				
α_1-acid glycoprotein,[2] mg/dL	68.9 (41.3)	60.5 (54.7, 66.8)	58.0 [42.1, 81.0]	18.2–306.9
Iron, μg/dL	77.4 (42.2)	65.8 (57.8, 74.8)	75.0 [48.0, 96.0]	3.0–275.0
Ferritin, μg/L	74.4 (60.6)	51.4 (42.3, 62.4)	51.5 [32.7, 101.6]	1.4–284.1
Adjusted ferritin,[3] μg/L	71.3 (57.9)	49.2 (40.4, 59.9)	51.5 [31.5, 98.5]	1.4–284.1
Total iron binding capacity, μg/dL	312.8 (71.7)	306.0 (293.6, 318.9)	302.5 [271.0, 334.0]	164.0–721.0
Transferrin saturation, %	25.5 (13.6)	21.1 (18.2, 24.3)	25.5 [15.0, 33.0]	1.0–67.0
25(OH)D,[4] nmol/L	65.1 (14.2)	63.5 (60.7, 66.5)	65.0 [53.9, 73.1]	31.0–102.6
25(OH)D C3 epimer, nmol/L	2.9 (1.4)	2.6 (2.4, 2.9)	2.7 [1.9, 3.7]	0.8–7.3
Total 25(OH)D, nmol/L	68.0 (15.0)	66.3 (63.4, 69.4)	68.7 [56.1, 75.9]	32.1–107.6
Plasma				
Copper,[5] μg/dL	114.8 (25.2)	111.9 (106.7, 117.2)	114.0 [101.8, 127.4]	60.2–195.6
Zinc, μg/dL	81.6 (12.0)	80.7 (78.4, 83.1)	79.6 [71.6, 91.6]	59.2–119.6
Adjusted zinc,[6] μg/dL	83.1 (13.9)	82.0 (79.4, 84.7)	79.9 [72.1, 93.2]	59.2–134.0
Retinol, μg/dL	37.3 (13.8)	35.1 (32.7, 37.6)	35.6 [28.8, 43.4]	14.4–105.2
Adjusted retinol,[7] μg/dL	38.0 (14.2)	35.7 (33.3, 38.3)	36.2 [29.0, 44.3]	14.4–105.2
Whole blood				
Hemoglobin, g/dL	12.3 (1.3)	12.2 (11.9, 12.5)	12.4 [11.7, 13.2]	6.1–14.8

[1] $n = 98$. 25(OH)D, 25-hydroxyvitamin D.
[2] Marker of inflammation; 11 α_1-acid glycoprotein values were >100 mg/dL.
[3] Adjustment using correction factors from Thurnham et al. (37).
[4] Marker of vitamin D status; total 25(OH)D is 25(OH)D + the C3 epimer fraction.
[5] $n = 97$ due to 1 missing value.
[6] Adjustment using correction factors from Mburu et al. (38).
[7] Marker of vitamin A status; adjustment using correction factors from Thurnham et al. (39).

examine the potential impact of publication bias (i.e., Are papers only published if prevalence is high?) on reporting.

Here, as we aimed to draw attention to the issues of co-occurring deficiencies, we also highlight that although often mentioned as a major concern, the negative health consequences of overlapping micronutrient deficiencies are not well documented. It is known that when mineral deficiencies are extreme or overlapping, there is a higher risk of lead, arsenic, or other toxic heavy metal uptake, causing a double hit of the burden of deficiencies (41, 42). When both calcium and vitamin D are deficient, there is a much higher risk for poor bone health outcomes (43). Vitamin A, zinc, and other micronutrient deficiencies can exacerbate iron deficiency anemia by disrupting iron transport or hematopoiesis, and ultimately they can cause continued anemia even with iron repletion (44, 45). In this study in Ghana, vitamin A insufficiency was most commonly co-occurring with other insufficiencies (iron, zinc, and vitamin D). In the study of pregnant Nepalese women, zinc had the highest overlap with other deficiencies (vitamin A, vitamin B-6, vitamin B-12, riboflavin, and iron) (18). Yet, none of the studies we found connected co-occurring deficiencies with specific health outcomes in WRA or during pregnancy. Because poor reproductive and pregnancy outcomes (e.g., miscarriage, stillbirth, preterm birth, and fetal growth restriction) remain an enormous public health burden, understanding the relation between multiple deficiencies and higher risk of adverse outcomes is paramount. On a positive note, there is now substantial evidence to show a benefit to healthy pregnancy outcomes when multiple micronutrients are given as a supplement to women during pregnancy (compared with only iron and folic acid) (46, 47).

Because there are regional and local-level differences in nutritional status, it is important to be able to assess the micronutrient status of individuals and communities. Although this study was small, we did not find common health indicators or self-reported markers that would provide insight into micronutrient status, aside from the known relations between anemia, iron, and vitamin A. As part of the vitamin D status assessment, we examined the C3 epimer fraction of 25(OH)D and did not find that its inclusion impacted our results (we chose to report it because of the emerging interest in the potential differences in concentrations by age and race/ethnicity). When iron biomarkers are not measured, it is typically estimated that half of anemia is due to iron deficiency. Here, we found a lower ratio of iron deficiency within anemia (depending on the classification for iron deficiency, 24–48% of those anemic) and in general found anemia status as a poor positive or negative predictor of deficiencies or insufficiencies. Simple, common health screening data, similar to those reported in this article, should be tested in populations with a higher prevalence of micronutrient deficiencies. Because it is difficult and costly to measure and interpret micronutrient biomarkers, we recommend that resources be focused on identifying potential screening tools in addition to developing inexpensive and less invasive methods for more directly measuring micronutrient status.

In this study, we were most interested in examining the prepregnancy health and nutritional status of women who might become pregnant soon. Because this study was planned to pilot field methods for identifying women to follow through to pregnancy, the sample size was small and we did not measure dietary intake. We did, however, use rigorous methods for assessing micronutrient

TABLE 3 Micronutrient blood biomarker concentrations of women expecting to become pregnant in Asesewa, Ghana, by common health indicators measured in the study[1]

	Hemoglobin		BMI		Blood pressure	
Biomarker	**≥12.0 g/dL (*n* = 65)**	**<12.0 g/dL (*n* = 33)**	**<25 kg/m^2 (*n* = 59)**	**≥25 kg/m^2 (*n* = 39)**	**Normal (*n* = 75)**	**Elevated (*n* = 23)**
Serum						
α_1-acid glycoprotein,[2] mg/dL	51.9 [42.1, 78.9]*	78.9 [51.9, 86.2]*	51.9 [41.6, 68.5]*	78.9 [51.9, 86.2]*	51.9 [42.1, 81.0]	64.0 [51.9, 95.6]
Iron, μg/dL	81.2 (33.9)	70.0 (54.9)	81.2 (47.3)	71.7 (32.6)	78.1 (43.3)	75.2 (39.0)
Adjusted ferritin,[3] μg/L	55.4 [41.0, 100.9]	36.7 [16.1, 86.1]	47.4 [31.5, 87.5]	66.5 [24.5, 122.2]	49.8 [31.5, 96.8]	66.5 [19.1, 115.1]
TIBC, g/dL	301.0 [261.0, 315.0]*	329.0 [285.0, 383.0]*	306.0 [278.0, 342.0]	301.0 [261.0, 329.0]	301.0 [264.0, 334.0]	313.0 [285.0, 363.0]
Transferrin saturation, %	28.0 (12.1)*	20.4 (15.2)*	26.3 (14.9)	24.3 (11.5)	25.8 (13.9)	24.3 (12.9)
25(OH)D,[4] nmol/L	65.3 (14.9)	64.7 (12.9)	65.0 (15.3)	65.3 (12.5)	64.4 (14.5)	67.4 (13.0)
25(OH)D C3 epimer, nmol/L	2.9 (1.3)	3.0 (1.4)	2.9 (1.4)	2.9 (1.4)	2.8 (1.4)	3.1 (1.4)
Total 25(OH)D, nmol/L	68.2 (15.8)	67.7 (13.6)	67.8 (16.3)	68.3 (13.1)	67.2 (15.4)	70.5 (13.7)
Plasma						
Copper,[5] μg/dL	115.8 (25.2)	112.6 (25.4)	115.6 (24.9)	113.4 (25.9)	111.6 (23.8)*	124.9 (27.4)*
Adjusted zinc,[6] μg/dL	84.2 (13.0)	80.9 (15.6)	81.1 (12.6)	86.1 (15.3)	83.6 (14.0)	81.3 (13.8)
Adjusted retinol,[7] μg/dL	40.2 (15.3)*	33.6 (10.8)*	35.6 (11.5)	41.7 (17.0)	37.2 (12.4)	40.6 (19.0)
Whole blood						
Hemoglobin, g/dL	13.0 (0.7)*	10.9 (1.1)*	12.1 (1.4)	12.5 (1.1)	12.2 (1.4)	12.4 (0.9)

[1] Data are presented as arithmetic means (SDs) or medians [IQRs]; $n = 98$. * $P < 0.05$ for *t* test or Wilcoxon–Mann–Whitney test if distribution was normal or nonnormal, respectively. TIBC, total iron binding capacity; 25(OH)D, 25-hydroxyvitamin D.
[2] Markers of inflammation; 11 α_1-acid glycoprotein values >100 mg/L.
[3] Adjustment using correction factors from Thurnham et al. (37).
[4] Marker of vitamin D status.
[5] $n = 97$ due to 1 missing value.
[6] Adjustment using correction factors from Mburu et al. (38).
[7] Marker of vitamin A status; adjustment using correction factors from Thurnham et al. (39).

TABLE 4 Micronutrient blood biomarker concentrations of women expecting to become pregnant in Asesewa, Ghana, by self-reported health history[1]

	Parity		Anemia[2]		Malaria[2]		Night blindness[3]	
Biomarker	0 ($n = 46$)	≥1 ($n = 52$)	No ($n = 83$)	Yes ($n = 14$)	No ($n = 44$)	Yes ($n = 54$)	No ($n = 83$)	Yes ($n = 15$)
Serum								
α_1-acid glycoprotein,[4] mg/dL	55.0 [41.6, 81.0]	59.7 [43.8, 81.0]	51.9 [42.1, 81.0]*	81.0 [58.0, 133.4]*	58.0 [43.8, 81.0]	61.5 [42.1, 81.0]	61.5 [42.1, 81.0]	51.9 [42.1, 64.0]
Iron, μg/dL	82.2 (48.4)	73.2 (35.7)	76.6 (44.0)	82.4 (32.3)	77.6 (49.9)	77.2 (35.1)	76.7 (38.7)	81.3 (59.5)
Adjusted ferritin,[5] μg/L	59.7 [34.0, 91.6]	49.4 [30.2, 106.1]	50.6 [24.5, 101.0]	58.2 [47.4, 91.7]	50.8 [31.6, 117.9]	52.1 [28.8, 89.4]	51.3 [31.7, 100.9]	51.7 [24.1, 94.0]
TIBC, g/dL	301.0 [268.0, 334.0]	306.0 [280.5, 337.0]	306.0 [271.0, 341.0]	301.0 [283.0, 306.0]	315.0 [281.5, 341.5]	301.0 [268.0, 329.0]	301.0 [271.0, 334.0]	306.0 [268.0, 342.0]
Transferrin saturation, %	26.3 (14.4)	24.8 (13.0)	25.0 (14.0)	28.7 (11.9)	24.2 (14.6)	26.6 (12.8)	26.2 (14.2)	21.3 (9.2)
25(OH)D,[6] nmol/L	63.2 (14.5)	66.8 (13.8)	65.1 (14.4)	65.7 (13.6)	66.0 (15.6)	64.4 (13.1)	64.8 (14.1)	67.0 (15.0)
25(OH)D C3 epimer, nmol/L	3.0 (1.4)	2.8 (1.3)	3.0 (1.4)	2.6 (1.4)	2.9 (1.4)	2.9 (1.4)	2.8 (1.2)	3.6 (1.8)
Total 25(OH)D, nmol/L	66.2 (15.5)	69.6 (14.5)	68.1 (15.3)	68.4 (14.0)	68.9 (16.4)	67.3 (13.9)	67.5 (14.9)	70.7 (16.0)
Plasma								
Copper,[7] μg/dL	116.5 (20.5)	113.2 (28.7)	114.8 (25.1)	115.9 (26.7)	115.6 (20.8)	114.1 (28.4)	115.3 (25.1)	111.6 (26.2)
Adjusted zinc,[8] μg/dL	82.4 (11.9)	83.7 (15.6)	81.9 (12.5)	90.6 (19.4)	80.9 (11.3)	84.8 (15.6)	83.4 (14.0)	81.3 (13.4)
Adjusted retinol,[9] μg/dL	37.7 (12.9)	38.3 (15.5)	36.9 (12.9)	44.4 (20.2)	38.4 (17.6)	37.7 (11.0)	37.7 (14.0)	39.4 (15.8)
Whole blood								
Hemoglobin, g/dL	12.3 (1.3)	12.2 (1.4)	12.3 (1.2)	11.9 (1.9)	12.2 (1.6)	12.3 (1.1)	12.3 (1.4)	12.3 (1.1)

[1] Data are presented as arithmetic means (SDs) or medians [IQRs]; $n = 98$. *$P < 0.05$ for t test or Wilcoxon–Mann–Whitney test if distribution was normal or nonnormal, respectively. TIBC, total iron binding capacity; 25(OH)D, 25-hydroxyvitamin D.
[2] Response to the question, "Has a health professional ever told you that you have . . .?"; 1 response was "don't know" and is not included in the table.
[3] Response to the question, "Have you ever experienced night blindness?"
[4] Marker of inflammation; 11 α_1-acid glycoprotein values >100 mg/L.
[5] Adjustment using correction factors from Thurnham et al. (37).
[6] Marker of vitamin D status.
[7] $n = 97$ due to 1 missing value.
[8] Adjustment using correction factors from Mburu et al. (38).
[9] Marker of vitamin A status; adjustment using correction factors from Thurnham et al. (39).

status—by examining multiple biomarkers in blood (rather than relying on dietary intake), by accounting for inflammation, and by using robust lab methods [including measurement of several iron biomarkers and the C3 epimer of 25(OH)D]. We also assessed both deficiencies and insufficiencies. We were limited in resources (funds and the amount of blood collected) and were only able to assess 5 micronutrients; ideally, most essential vitamins and minerals would be examined, particularly others that cause anemia (e.g., folate and vitamin B-12). Yet, micronutrients are essential and have multiple roles in the body; and all of those we examined have been found to be important before and during pregnancy (48–50). Malaria is endemic in the study area, but we did not test for it in participants; thus, we could not examine associations with micronutrients, nor account for it in adjusting relevant biomarker concentrations. Of note, the Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia project found that accounting for malaria did not make a meaningful impact on the prevalence of iron deficiency (40).

The majority of women in this semi-urban area in Ghana were insufficient in ≥1 of 5 micronutrients assessed (iron, zinc, copper, vitamin A, and vitamin D), and more than one-fourth were deficient in at least 1 micronutrient. Although the true burden of deficiency in the population is expected to be much higher, because we measured only 5 of ∼30 essential or semi-essential vitamins and minerals, even the prevalence observed could have a large impact in this unique set of women expecting to become pregnant within the next 6 mo. Numerous studies have found that micronutrient deficiencies are high in women and children (5, 16, 51, 52), recognized as "hidden hunger" because it is more difficult to identify and measure compared with energy deficiency. However, quantifying concurrent deficiencies and their collective impact on health has not been given enough attention. Assessments are often limited by the complexity of collecting and measuring biomarker concentrations in a larger sample of blood. Moving forward, we strongly urge development of easier but accurate methods for assessing more biomarkers and the evaluation and reporting of concurrent deficiencies in all studies in which multiple micronutrients are assessed.

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