The prevalence of anemia and iron deficiency among pregnant Ghanaian women, a longitudinal study

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Abstract

Background

Gestational iron deficiency (ID) can be deleterious to mother and fetus. However, iron status is not routinely measured during pregnancy in Ghana. Therefore, the scope of ID in this population is unknown.

Objective

To determine the prevalence of anemia and ID across pregnancy in the Central Region of Ghana.

Methods

Women were recruited during their 1st trimester of pregnancy (< 13 weeks; n = 116) and followed through to their 2nd (n = 71) and 3rd (n = 71) trimesters. Data on socio-demographic variables, weekly intake of iron-rich foods and vitamin C-rich fruits were collected. Blood samples were drawn and the concentrations of hemoglobin (Hb), ferritin (Ft), serum iron (sFe), total iron binding capacity (TIBC), were measured; transferrin saturation (TSAT) was calculated. Repeated measures ANOVA was used to determine change in anemia and iron variables over time with groups categorized by 1st trimester iron status.

Results

Participants were 27.1 ± 5.2 years, on average. Prevalence of anemia (Hb <11.0 g/dL) was 37%, 63%, 58%; ID (Ft <15 μg/L) was 16%, 20%, 38%; and iron deficiency anemia (IDA; based on low Ft and Hb) was 6%, 12%, 25% in 1st, 2nd and 3rd trimesters, respectively. Significant changes in Hb, Ft and TIBC occurred across time. Iron status at 1st trimester had a significant effect on 2nd but not 3rd trimester iron status.
Conclusions
ID is prevalent in pregnant Ghanaian women, especially during the 3rd trimester. Anemia is a major public health problem during pregnancy in Ghana with a significant proportion due to factors other than ID.

Introduction
Iron deficiency (ID) is known to be the single most prevalent nutrient deficiency in the world, affecting about 2 billion people worldwide [1]. Women-of-reproductive age (WRA) and children are the most vulnerable to ID. The United Nation’s Children Fund (UNICEF) estimated that globally, 50,000 young women die each year in pregnancy and childbirth due to iron deficiency anemia (IDA), and about 40 million pregnant women suffer from ID [2]. In high-income countries, data exist on the prevalence of ID due to the assessment of specific iron status biomarkers (ferritin, transferrin saturation, total iron binding capacity and transferrin receptor) in pregnancy [3]. However, in many low- and middle-income countries, including Ghana, such assessments are not part of routine care and, therefore, data on the prevalence of ID during pregnancy are not available. Instead, ID is estimated using anemia prevalence (hemoglobin (Hb) status) as a proxy, when in fact about half of anemia is attributed to ID [4]. Data show that a wide range of ID [1, 4–7] and ID anemia (IDA) [1, 4, 6] exists among pregnant women irrespective of the country of origin. The World Health Organization (WHO) estimates that about 38% of pregnant women are anemic worldwide, with 18% in high-income countries and 35–75% in low- and middle-income countries. The rate of deficiency seems to increase as pregnancy progresses. The most recent Ghana Demographic and Health Survey (GDHS) estimates anemia prevalence to be 45% among pregnant women [8].

The demand for iron increases during pregnancy. This high demand is necessary for expansion of plasma volume, increase in red cell mass, growth of the fetal placental unit, and to fulfill the fetal iron requirements [9]. If maternal iron stores are severely depleted, this can lead to anemia, which may impair the oxygen delivery to the placenta and fetus, thereby interfering with intrauterine growth and leading to preterm delivery and low birth weight [4, 6]. The deficiency has also been associated with both maternal and infant mortality [10].

Maternal ID during pregnancy has further consequences for fetal brain function and development [11]. Iron accumulation in brain cells takes place early during fetal development [6]. As such, gestational ID may lead to irreversible damage to brain cells, causing poor child cognition, poor motor development, behavioral abnormalities [1, 6] and maternal emotional disturbances [12]. The aim of this study was therefore to assess the prevalence of ID and anemia among pregnant Ghanaian women in their 1st, 2nd and 3rd trimesters. We hypothesized that the prevalence of ID and anemia would increase through the course of pregnancy.

Methods
Study design
A longitudinal study was carried out from October 2017 to September 2018. Women who were <13 weeks of gestation were recruited when they reported for antenatal care at health care facilities in the Central Region of Ghana. Multistage sampling was adopted in selecting the health care facilities. Initial data on antenatal attendance throughout the year at the health facilities were obtained from the regional Ministry of Health office. Health facilities that had
previous antenatal attendances that were in-line with our research needs were purposefully selected. This yielded nine facilities. Simple random sampling was then used to select the health facilities used for the study. These facilities included the Moree Clinic, Cape Coast Teaching Hospital, the Cape Coast Metropolitan Hospital, Ewim Polyclinic, University of Cape Coast Hospital, Elmina Urban Health and Abura Dunkwa District Hospital. The nurses on duty informed prospective participants about the study and interested pregnant women were directed to the research team.

Screening and recruitment

A brief written screening form was used to determine eligibility, which included attendance at any of the seven selected prenatal clinics in Central Region of Ghana; aged between 18–38 years old at enrolment; <13 weeks gestation at enrolment (determined by last menstrual period or ultrasound scan); expecting a singleton pregnancy with no known congenital anomalies; and no known history of diabetes mellitus or hypertension. Eligible and interested participants completed the consent form (which was read to each participant, since some of the women had no formal education) and were recruited. Upon written informed consent (either providing a signature or a thumb print), socio-demographic characteristics, anthropometric and blood pressure assessments, and venous blood sample draw occurred immediately for the first trimester visit, unless the participant requested to come back at a later date. Four trained field assistants carried out data collection. After the first visit was completed, each participant was provided with a date for her 2nd trimester visit. Participants were then followed into their 2nd (13–27 weeks) and 3rd (28–36 weeks) trimesters. At each health facility, a nurse was recruited to coordinate activities between patients and the trained data collectors. At the end of the first two visits, each woman received a bar of soap plus transportation cost as incentive, and at the end of the third visit, each woman received a baby onesie plus transportation cost.

Data collection procedure

Samsung Galaxy tablets were used to collect socio-demographic information including age, marital status, parity, education level, income and employment status. Dietary intake of vegetables, fruits and iron-rich foods, during the week prior to data collection, were also assessed at each visit.

Blood draws. Approximately 4 mL of blood were taken from the participant at each of the three trimesters during pregnancy by a trained phlebotomist at each health facility. Hb levels were determined on the spot via Hemocue (HB201; HemoCue America, Brea, CA, USA). The blood samples were stored on ice and delivered to the Cape Coast Teaching Hospital within 30 minutes of collection. Blood samples were centrifuged and serum aliquoted by a laboratory technician at the Cape Coast Teaching Hospital and subsequently stored in a -80°C freezer prior to shipping to The Pennsylvania State University, USA, where iron status biomarkers (serum iron (sFe), total iron binding capacity (TIBC) and serum ferritin (Ft)) and inflammatory markers (alpha-1-acid glycoprotein (AGP) and c-reactive protein (CRP)) were determined in Dr. Murray-Kolb’s laboratory. Ft was determined via ELISA (Ramco Laboratories TX, USA), calibrated against WHO standards. sFe and TIBC were determined using colorimetric methods [13]. Transferrin saturation (TSAT) was calculated as (sFe/TIBC)×100. AGP and CRP were measured using radial immunodiffusion tests (Kent Laboratories Inc., Bellingham, WA, USA) and used to adjust Ft concentrations when inflammation was present. Ft values reported have therefore been adjusted for inflammation based on Thurnham criteria [14].

Follow-up visits for 2nd and 3rd trimesters. During the 2nd (13–27 weeks) and 3rd (28–36 weeks) trimester visits, all assessments, measurements and blood sample collection were
repeated as described above, with the exception of socio-economic status (SES) assessment (which was only administered at the first visit).

Ethical approval
Ethical approval was obtained from the ethical review board of the Ghana Health Service Ethical Review Committee, University of Cape Coast Institutional Review Board, Cape Coast teaching Hospital Ethical Review Committee and The Pennsylvania State University Institutional Review Board.

Statistical methods
Statistical Analysis Software (SAS) version 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for data analysis. Univariate analyses were run for all variables with the appropriate transformations applied to normalize all non-normal variables. The prevalence of ID was determined at each trimester. Proportions were presented for all sociodemographic (SES, marital status, age, years of schooling, income, parity) variables and iron supplement intake variables, and differences between the proportions of pregnant women who were iron deficient across the three trimesters were determined. One-way ANOVA and repeated measures ANOVA were used to determine significant differences between iron biomarkers over time and for change in iron variables over time based on 1st trimester iron status, respectively. Simple regression models were used to determine if gestational age predicted iron status, adjusting for prenatal supplement intake.

Results
Sample population
Two hundred and twelve pregnant women were screened, out of which 154 were eligible (Fig 1). Thirty-five women refused participation so a total of 119 participants were recruited in their first trimester. Out of those recruited, 46 pregnant women dropped out of the study due to reasons such as miscarriage, spouse refusal, and unreachable (recipient out of coverage area and unanswered phone calls). Seventy-three pregnant women were followed into their 2nd trimester. In the 3rd trimester, 72 pregnant women were followed with 15 pregnant women dropping out between 2nd and 3rd trimesters due to reasons such as relocation, delivery and refusal to continue participation. Fourteen pregnant women from their 1st trimester who were absent during the 2nd trimester visit came back for the 3rd trimester visit. The number of participants with complete socio-demographic and intake data were 116, 71 and 71, while 111, 68 and 65 participants provided a blood sample for 1st, 2nd and 3rd trimesters, respectively. After removal of outliers and missing blood concentrations (not enough blood to measure every biomarker), 109, 65 and 60 participants had values for all iron biomarkers for 1st, 2nd and 3rd trimesters, respectively (Fig 1).

Baseline characteristics of pregnant women
The baseline socio-demographic characteristics of the pregnant women are shown in Table 1. The majority of the women (56.9%) were recruited from the Komenda Edina Eguafio Abirem (KEEA) District, specifically from Elmina Urban Health Center, with the least recruited from the Abura Asebe Kwamankese District (12.9%). The average age of participants was 27 years with 26% having BMI in the overweight range and 11% classified as obese (Table 1). About 68% of participants were married with 80% living with their husbands/partners or co-existing. About 17% of these women were heads of their households, meaning they were the sole
breadwinners of their household. About 8% of the women had no formal education, while 45% had up to a middle school education, with only 10% having a university degree. In terms of income, 79% of these women had an income-generating activity, and the majority of these women (84%) earned between 500–1000 Ghana cedis per month (equivalent to US $125-
Table 1. Socio-demographic characteristics of pregnant Ghanaian women in their first trimester (n = 116).

| Sociodemographic variables | n   | %    |
|----------------------------|-----|------|
| Age (yrs)                  | 116 (18–38) | 27.1 (5.2) |
| BMI (kg/m²)                | 116 (16.2–40.2) | 24.6 (4.8) |
| Underweight (<18.5)        | 6   | 5.2  |
| Normal (18.5–24.9)         | 67  | 57.8 |
| Overweight (25.0–29.9)     | 30  | 25.9 |
| Obese (≥30.0)              | 13  | 11.2 |
| Marital Status             |     |      |
| Married                    | 79  | 68.1 |
| Not Married                | 37  | 31.9 |
| Lives with a partner/husband |     |      |
| Yes                        | 93  | 80.2 |
| No                         | 23  | 19.8 |
| Head of household          |     |      |
| Yes                        | 23  | 17.2 |
| No                         | 93  | 82.8 |
| Educational Level          |     |      |
| No school                  | 9   | 7.8  |
| Primary                    | 17  | 14.7 |
| Middle                     | 52  | 44.8 |
| Secondary                  | 26  | 22.4 |
| University                 | 12  | 10.3 |
| Years of schooling         |     |      |
| 0–6                        | 12  | 11.2 |
| 7–9                        | 56  | 52.4 |
| >9                         | 39  | 36.5 |
| Income generating activity |     |      |
| Yes                        | 92  | 79.3 |
| No                         | 24  | 20.7 |
| Income level (Ghana Cedis) |     |      |
| GH 501–1000                | 97  | 83.6 |
| GH 1001–2000               | 14  | 12.1 |
| GH 2001–3000               | 3   | 2.6  |
| GH 3001–5000               | 2   | 1.7  |
| Valid health insurance     |     |      |
| Yes                        | 75  | 74.3 |
| No                         | 26  | 25.7 |
| Children 17 yrs and below in household |     |      |
| 0                          | 40  | 34.5 |
| 1–4                        | 66  | 56.9 |
| >4                         | 10  | 8.6  |
| Children <5 yrs in household |     |      |
| Yes                        | 55  | 47.4 |
| No                         | 61  | 52.6 |
| Number of children <5 yrs  |     |      |
| 1                          | 32  | 58.2 |
| 2                          | 20  | 36.4 |
| >2                         | 3   | 5.5  |

(Continued)
The majority (74%) of these women had national health insurance. Forty-seven percent of participants had children <5 years of age in their households, with 36% having two children <5 years in the household. The majority of participants (70%) had between 1–5 children, while 30% had no child prior to their current pregnancy. About 5% of the pregnant women had sickle cell traits.

Prevalence of iron deficiency and inflammation

The prevalence of anemia (Hb <11.0 g/dL) was 37%, 63%, 58%; ID (Ft <15 μg/L) was 16%, 20%, 38%; ID (TIBC ≥ 400 μg/L) was 19%, 29% and 40%; ID (TSAT <16%) was 12%, 9% and 17%; iron deficiency anemia (IDA) based on Ft <15 μg/L and Hb <11.0 g/dL was 6%, 12% and 25% for 1st, 2nd and 3rd trimesters, respectively (Table 2). About 30%, 22% and 32% of pregnant women had inflammation based on CRP (>5.0 mg/L) while 29%, 6% and 2% had inflammation based on AGP (>1.0 mg/L) for 1st, 2nd and 3rd trimesters, respectively.

Change in iron biomarkers over time

Significant changes occurred in Hb, Ft and TIBC across time (Table 2). No significant change was found for sFe or TSAT over time. The pattern of change varied depending on the iron biomarker in question. Women showed significantly higher Hb concentrations in their 1st trimester (11.1 ± 0.1 g/dL) compared with 2nd (10.6 ± 0.2 g/dL) and 3rd (10.5 ± 0.2 g/dL) trimesters; 2nd and 3rd trimester Hb concentrations did not differ. Like Hb, Ft values decreased over time. Pregnant women in their 1st trimester showed significantly higher Ft concentrations (84.9 ± 5.6 μg/L) than pregnant women in their 2nd (40.8 ± 7.2 μg/L) and 3rd (27.6 ± 7.5 μg/L) trimesters, even after adjusting for inflammation. There was no significant difference in Ft concentrations between 2nd and 3rd trimesters. Consistent with iron status decreasing over time when measured by Ft, there was an increase in TIBC over time. Women in their 1st trimester showed a significantly lower TIBC concentration (352.2 ± 8.1 μg/dL) than women in their 3rd trimester (387.1 ± 10.7 μg/dL). Even though trends increased over time, there was no significant difference in TIBC concentration between 1st and 2nd trimesters nor between 2nd and 3rd trimesters. In a linear regression model with gestational age as a predictor of iron biomarker (Table 3), gestational age significantly predicted Ft and TIBC concentrations after adjusting for the number of prenatal supplements consumed. A unit increase in gestational age was associated with a decrease in Ft by 3.73 μg/L (p = <0.001) and an increase in TIBC by 1.96 μg/dL (p = 0.041). Gestational age was not a significant predictor of Hb, sFe or TSAT after adjusting for the number of prenatal supplements consumed.

Table 1. (Continued)

| Sociodemographic variables | n | % |
|---------------------------|---|---|
| Parity                    |   |   |
| 0                         | 35| 30.2|
| 1–5                       | 81| 69.8|
| Sickle cell trait         |   |   |
| Yes                       | 6 | 5.2 |
| No                        | 107| 92.2|
| Unknown                   | 3 | 2.6 |

*n (range),

*mean (SD)

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$250). The majority (74%) of these women had national health insurance. Forty-seven percent of participants had children <5 years of age in their households, with 36% having two children <5 years in the household. The majority of participants (70%) had between 1–5 children, while 30% had no child prior to their current pregnancy. About 5% of the pregnant women had sickle cell traits.
Change in iron status over time depending on iron status at 1st trimester

There was significant interaction between all iron biomarkers (Hb, Ft, sFe, TIBC, TSAT) and time categorized by 1st trimester iron status (S1 Table, Fig 2). Pregnant women who were non-anemic at 1st trimester experienced a significant decrease in Hb concentrations by the 2nd trimester ($\beta = -1.22$, 95% CI: [-1.63, -0.81]), with a further decrease by the 3rd trimester ($\beta = -1.58$, 95% CI: [-1.99, -1.18]). However, women who started pregnancy anemic experienced a
gradual but non-significant increase in Hb concentration by the 2nd trimester and a significant increase by the 3rd trimester ($\beta = 1.18$, 95% CI: [0.41, 1.95]) compared with the 1st trimester Hb concentrations. At the 3rd trimester, Hb concentrations did not differ between women who were anemic and those who were non-anemic at 1st trimester (Fig 2). For Ft levels, pregnant women who were sufficient in Ft at 1st trimester had a significantly decreased Ft concentration by the 2nd trimester ($\beta = -57.02$, 95% CI: [-78.98, -35.06]), with a further decrease by the 3rd trimester ($\beta = -67.40$, 95% CI: [-86.77, -48.03]). On the other hand, pregnant women who had low Ft at 1st trimester had an increase in Ft concentration by the 2nd trimester, though not significant, and a significant increase by the 3rd trimester ($\beta = 18.04$, 95% CI: [4.99, 31.09]); at the 3rd trimester, ferritin levels were comparable between pregnant women who were Ft sufficient and those who were deficient in trimester one (Fig 2). For sFe, pregnant women who were deficient in the 1st trimester increased sFe concentrations significantly across trimesters while those who were sufficient in sFe at 1st trimester did not experience a change in their levels across trimesters. At the 3rd trimester, women who were sFe deficient and those who were sufficient in the 1st trimester had similar sFe levels. A similar trend was observed for TSAT. For TIBC, pregnant women with high levels in the 1st trimester decreased their TIBC levels by the 2nd trimester ($\beta = -97.87$, 95% CI: [-181.28, -14.45]), while sufficient women increased significantly in TIBC by the 2nd ($\beta = 37.92$, 95% CI: [0.86, 74.98]) and 3rd trimesters ($\beta = 46.53$, 95% CI: [8.68, 84.40]).

**Discussion**

In Ghana, not all biomarkers of iron status are routinely assessed during pregnancy and therefore the prevalence of iron deficiency in this population is unknown. We found that the prevalence of ID increased as pregnancy progressed with a high prevalence in the 3rd trimester, as hypothesized. We found a significant time effect for Ft and TIBC concentrations, reflecting a pattern of ID which is commonly observed during pregnancy. As iron stores deplete, the amount of transferrin which is available to bind iron increases. Ft levels significantly decreased with gestational age while TIBC significantly increased with gestational age, as expected, during pregnancy.

A limited number of studies have measured iron status during pregnancy in Ghana with one reporting a prevalence rate of iron deficiency ranging from 5–46%, depending on the definition applied [15], while another reported a rate of 11% for women ≤24 weeks and 20% for women ≥36 weeks pregnant, with an overall prevalence of 16% during pregnancy [16]. We observed prevalence rates of 16%, 20% and 38% in the 1st, 2nd and 3rd trimesters, respectively. The major difference between these previously published studies and ours is that the previous studies were cross sectional while our study was longitudinal. Additionally, Mockenaupt...
et al. [18] did not report prevalence rates by trimester and Engmann et al. [19] dichotomized gestational age into either ≤24 weeks or ≥36 weeks whereas we reported prevalence rates for 1st (<13 weeks), 2nd (13–27 weeks) and 3rd (28–36 weeks) trimesters. Furthermore, Engmann et al. [19] used a population of women from the city and Mockenhaupt et al. [18] used a rural...
population; our population was predominately semi-urban. These differences may contribute to the various prevalence rates observed in our study compared to previous studies. A third study from Ghana which occurred in a comparable setting to the Central Region found the prevalence of ID to be 14%, 23% and 26% in the 1st, 2nd and 3rd trimesters, respectively [17].

Several measures of iron status were used, as in our study; however, the authors did not indicate the definition used to determine ID. Not only were cut-offs not reported, but, whether ID was based on one or multiple iron biomarkers was not indicated. Their rates in the 1st and 2nd trimesters were comparable to our 1st and 2nd trimester rates (which were based on Ft<15 μg/L) but our 3rd trimester ID prevalence rate was higher (38%) than their 3rd trimester prevalence of 26% [17]. As with the other two previous studies in Ghana, this study was cross sectional. Previous studies conducted in neighboring countries have reported higher ID rates during pregnancy. A study in Nigeria reported an ID prevalence rate of 48% [18], while a study in Malawi reported a prevalence rate of 32% [19] throughout pregnancy. Both studies were cross sectional, with the Nigerian study recruiting women at term while the Malawi study recruited only anemic pregnant women, thus excluding women who may have been iron deficient but not anemic.

When we compared the prevalence rates of ID found in our study to those in a high-income country such as the USA, we found a comparable pattern. Miller used the US NHANES 1999–2006 data and found an ID prevalence rate of 25.4% during pregnancy [20], while Mei et al. found ID prevalence of 7.3%, 23.7% and 29.2% for 1st, 2nd and 3rd trimesters, respectively [21] using a Ft cut-off of <12 μg/L. If we use the same cutoff of 12 μg/L for our population, we see a higher prevalence in the 1st trimester (12%), a slightly lower prevalence rate in the 2nd trimester (18%), and a comparable prevalence rate in the 3rd trimester (30%). This suggests that ID prevalence among pregnant women in Ghana is comparable to rates observed in the US but lower when compared with rates from neighboring low- and middle-income countries. One likely explanation is the use of iron supplements, especially during the 2nd and 3rd trimesters. This is the standard of care in Ghana where pregnant women are given 30–60 mg of iron daily from the 2nd trimester until delivery. If a woman is found to be anemic before this time point, iron supplements are prescribed upon the diagnosis of anemia. In our population, 23% took iron supplements in the 1st trimester, while 90% and 82% took iron supplements in the 2nd and 3rd trimesters, respectively. Another possible explanation for prevalence rates that are lower than neighboring countries could be the high consumption of meat and fish in this population [22]. Our population is located along the coast with fishing as the main source of employment and fish as the main source of protein in these communities [23]. In our study, we found that the frequency of consuming red meat increased throughout pregnancy and that most of the women regularly consumed fruits with a high vitamin C content. Consumption of meat and fish along with foods containing a high vitamin C content likely reflects a diet with higher iron bioavailability compared to the diets of neighboring countries.

Despite the fact that these women were given iron supplements as routine standard of care, Ft levels decreased across pregnancy. Other iron biomarkers such as TSAT and serum iron did not change significantly over time, perhaps as a result of the iron supplements given as standard of care. The rate of anemia was high among the women studied. A rate of 37% in the 1st trimester is regarded as a public health problem. Higher rates were observed in the 2nd (63%) and 3rd (58%) trimesters, both classified as severe public health problems.

The prevalence of IDA was 6%, 12% and 25% for 1st, 2nd and 3rd trimesters, respectively. Using the common assumption that half of anemia is due to iron deficiency [4], we would have expected rates of approximately 19%, 32%, and 29% for the 1st, 2nd, and 3rd trimesters, respectively. Our lower than expected rates are an indication that much of the anemia in this population is due to factors other than iron deficiency. Although these rates are lower than
those reported in several other countries [24], they are comparable to rates reported in other studies conducted in Ghana [19, 20]. These findings challenge the WHO estimation that half of all anemic cases can be attributed to ID and remind us that careful examination regarding the etiology of the anemia is warranted, before ID is assumed and supplements are dispensed. This is especially important in low- and middle-income countries where the etiology of anemia is complex and the risk for poor outcomes of iron supplementation as a result of underlying infections could be significant. In this population, we cannot rule out other causes of anemia such as hemoglobinopathies, nutritional deficiencies (such as folate, vitamin B12 and vitamin A), infectious diseases (e.g. tuberculosis and HIV/AIDS), parasitic infestations (e.g. hook worm) and malaria [25]. As such, before one begins to treat anemia, it is important to consider the various causes and to determine an appropriate diagnosis before supplementation is started.

When we examined changes in iron biomarkers over pregnancy, based on iron status in the first trimester, we found a significant effect of iron status at first trimester on 2nd trimester iron concentrations but not on 3rd trimester concentrations. Generally, pregnant women who were iron sufficient at 1st trimester had a drop in iron concentrations but still maintained a higher iron status in the 2nd trimester than those who were iron deficient in the 1st trimester. However, by their 3rd trimester, both those who started pregnancy deficient and those who started pregnancy sufficient in iron had comparable iron status. This likely reflects the physiology of pregnancy in the 3rd trimester where maternal iron is quickly mobilized in order to meet the fetal transfer demands, and the provision of iron supplements during the 1st trimester to women diagnosed with anemia but not given to all others until the 2nd trimester of pregnancy.

The strengths of our study include the longitudinal design with the assessment of iron status at three time points, using multiple iron biomarkers during pregnancy and the fact that the population studied included individuals whose iron status is not routinely assessed during pregnancy, therefore adding needed knowledge to the literature. The main study limitation was the high dropout rate (37.8%) observed between 1st and 2nd trimesters.

**Conclusion**

In Ghana, ID is prevalent during pregnancy, with the highest rates seen in the 3rd trimester. Additionally, anemia remains a major public health problem during pregnancy in Ghana, and a significant proportion of anemia in this population is attributable to causes other than ID. Measures must therefore be put in place for thorough examination of anemia in pregnant women which should include assessment of iron biomarkers and not just Hb. This will help determine the cause of anemia before supplementation is started, which is especially important in countries like Ghana, where there are many potential causes of anemia.

**Supporting information**

**S1 Data.**
(CSV)

**S2 Data.**
(CSV)

**S1 Table.** Change in iron status over time categorized by 1st trimester iron status in Ghanaian women.
(DOCX)
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References

1. Beard J. Iron deficiency alters brain development and functioning. J Nutr. 2003; 133:1468S–1472S. https://doi.org/10.1093/jn/133.5.1468S PMID: 12730445

2. Osungbade KO, Oladunjaye AO. Preventive treatments of iron deficiency anaemia in pregnancy: A review of their effectiveness and implications for health system strengthening. J Pregnancy. 2012; 2012:e454601. https://doi.org/10.1155/2012/454601 PMID: 22848829

3. Park CY, Eicher-Miller HA. Iron deficiency is associated with food insecurity in pregnant females in the United States: National Health and Nutrition Examination Survey 1999–2010. J Acad Nutr Diet. 2014; 114:1967–73. https://doi.org/10.1016/j.jand.2014.04.025 PMID: 24953790

4. Allen LH. Anemia and iron deficiency: Effects on pregnancy outcome. Am J Clin Nutr. 2000; 71:1280s–4s. https://doi.org/10.1093/ajcn/71.5.1280s PMID: 10799402

5. Scholl TO, Reilly T. Anemia, iron and pregnancy outcome. J Nutr. 2000; 130:443S–447S. https://doi.org/10.1093/jn/130.2.443S PMID: 10721924

6. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006; 64:S34–91. https://doi.org/10.1301/nr.2006.may.s34-s43 PMID: 16770951

7. Stoltzfus RJ, Dreyfuss ML. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: ILSI Pr.; 1999.

8. Ghana Demographic and Health Survey. ICF International, Rockville, Maryland, USA; 2014.

9. Scholl TO. Iron status during pregnancy: Setting the stage for mother and infant. Am J Clin Nutr. 2005; 81:1218S–1222S. https://doi.org/10.1093/ajcn/81.5.1218S PMID: 15883455

10. Stoltzfus RJ. Iron deficiency: Global prevalence and consequences. Food Nutr Bull. 2003; 24:S99–103. https://doi.org/10.1177/15648265030244S206 PMID: 17016951

11. Beard JL, Connor JR. Iron status and neural functioning. Annu Rev Nutr. 2003; 23:41–58. https://doi.org/10.1146/annurev.nutr.23.020102.075739 PMID: 12704220

12. Murray-Kolb LE. Iron status and neuropsychological consequences in women of reproductive age: What do we know and where are we headed? J Nutr. 2011; 141:747S–755S. https://doi.org/10.3945/jn.110.130658 PMID: 21346109
13. Huebers HA, Eng MJ, Josephson BM, Ekpoorn N, Rettmer RL, Labbé RF, et al. Plasma iron and transferrin iron-binding capacity evaluated by colorimetric and immunoprecipitation methods. Clin Chem. Oxford Academic; 1987; 33:273–7. PMID: 3542299

14. Thurnham McCabe LD, Haldar S, Wierenga FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr. 2010; 92:546–55. https://doi.org/10.3945/ajcn.2010.29284 PMID: 20610634

15. Mockenhaupt FP, Rong B, Günther M, Beck S, Till H, Kohne E, et al. Anaemia in pregnant Ghanaian women: importance of malaria, iron deficiency, and haemoglobinopathies. Trans R Soc Trop Med Hyg. 2000; 94:477–83. https://doi.org/10.1016/s0035-9203(00)90057-9 PMID: 11132370

16. Engmann C, Adanu R, Lu T-S, Bose C, Lozoff B. Anemia and iron deficiency in pregnant Ghanaian women from urban areas. Int J Gynecol Obstet. 2008; 101:62–6. https://doi.org/10.1016/j.ijgo.2007.09.032 PMID: 18068171

17. Obirikorang C, Fondjo L, Adomako S, Bio F, Acheampong I. Iron deficiency among pregnant women attending antenatal clinic at the KNUST Hospital, Kumasi, Ghana. Br J Med Med Res. 2015; 6:823–32.

18. Daouda H, Galan P, Prual A, Sekou H, Hercberg S. Iron status in Nigerian mothers and their newborns. Int J Vitam Nutr Res Int Z Vitam- Ernahrungsfor schung J Int Vitaminol Nutr. 1991; 61:46–50. PMID: 1856046

19. Van den Broek NR, Letsky EA. Etiology of anemia in pregnancy in south Malawi. Am J Clin Nutr. 2000; 72:247S–256S. https://doi.org/10.1093/ajcn/72.1.247S PMID: 10871590

20. Miller EM. Iron status and reproduction in US women: National Health and Nutrition Examination Survey, 1999–2006. Collins JF, editor. PLoS ONE. 2014; 9:e112216. https://doi.org/10.1371/journal.pone.0112216 PMID: 25375360

21. Mei Z, Cogswell ME, Looker AC, Pfeiffer CM, Cusick SE, Lacher DA, et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999–2006. Am J Clin Nutr. 2011; 93:1312–20. https://doi.org/10.3945/ajcn.110.007195 PMID: 21430118

22. FAO Fisheries & Aquaculture—Fishery and Aquaculture Country Profiles—The Republic of Ghana [Internet]. [cited 2019 May 14]. http://www.fao.org/fishery/facp/GHA/en

23. Heinbuch U. Animal protein sources for rural and urban populations in Ghana. Programme Integr Dev Artis Fish West Afr 25 P Annex IDAF/WP/58. 1994; 39.

24. Alper BS, Kimber R, Reddy AK. Using ferritin levels to determine iron-deficiency anemia in pregnancy. J Fam Pract. 2000; 49:829–32. PMID: 11032208

25. SPRING. Ghana: Landscape analysis of anemia and anemia programming. Arlington, VA: Strengthening Partnerships, Results, and Innovations in Nutrition Globally (SPRING) project. 2016;64.