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Original article

Malaria early in the first pregnancy: Potential impact of iron status

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SUMMARY

Background & aims: Low iron stores may protect from malaria infection, therefore improving iron stores in early pregnancy in line with current recommendations could increase malaria susceptibility. To test this hypothesis we compared iron biomarkers and red cell indices in nulliparae and primigravidae who participated in a randomized controlled trial of long-term weekly iron supplementation.

Methods: Cross-sectional and longitudinal data analysis from a randomized controlled trial of long-term weekly iron supplementation in rural Burkina Faso. Malaria parasitaemia was monitored and biomarkers and red cell indices measured at study end-points: plasma ferritin, transferrin receptor (sTfR), zinc protoporphyrin, hepcidin, sTfR/$\log_{10}$ ferritin ratio, body iron, haemoglobin, red cell distribution width; mean corpuscular haemoglobin concentration/volume, and C-reactive protein. Correlation coefficients between biomarkers and red cell indices were determined. A regression correction approach based on ferritin was used to estimate iron body stores, allowing for inflammation. Body iron differences were compared between nulliparae and primigravidae, and the association determined of iron biomarkers and body iron stores with malaria.

Results: Iron and haematological indices of 972 nulliparae (mean age 16.5 years) and 314 primigravidae (median gestation 18 weeks) were available. Malaria prevalence was 54.0% in primigravidae and 41.8% in nulliparae (relative risk 1.28, 95% CI 1.13−1.45, $P < 0.001$), anaemia prevalence 69.7% and 43.4% ($P < 0.001$), and iron deficient erythropoiesis (low body iron) 8.0% and 11.7% ($P = 0.088$) respectively. Unlike other biomarkers the sTfR/$\log_{10}$ ferritin ratio showed no correlation with inflammation as measured by CRP. Most biomarkers indicated reduced iron deficiency in early pregnancy, with the exception of haemoglobin. Body iron increased by 0.6−1.2 mg/kg in early gestation, did not differ by malaria status in nulliparae, but was higher in primigravidae with malaria (6.5 mg/kg versus 5.0 mg/kg; relative risk 1.53, 95% CI 0.67−2.38, $P < 0.001$).

Conclusion: In primigravidae, early pregnancy haemoglobin was not a good indicator of requirement for iron supplementation, which could be detrimental given the association of better iron status with increased malaria infection.

List of abbreviations: WHO, World Health Organisation; ANC1, First scheduled antenatal survey in primigravidae; FIN, End assessment survey in nulliparae; sTfR, Serum transferrin receptor; CRP, C-reactive protein; AGP, Alpha-1-acid glycoprotein; ZnPP, Zinc protoporphyrin; MCV, Mean corpuscular volume; MCHC, Mean corpuscular haemoglobin concentration; RDW, Red cell distribution width; BIS, Body iron stores; IQR, Inter-quartile range; SD, Standard deviation; LTFU, Loss to follow-up; *P.falciparum*, Plasmodium falciparum.

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Trial registration: clinicaltrials.gov:NCT01210040. Until placed in a public repository, data relating to the current study can be requested from the corresponding author and will be made available following an end user data agreement and sponsor approval.

1. Introduction

We describe iron and haematological biomarkers in young women (non-pregnant and pregnant) in Burkina Faso. There is a growing body of evidence showing that low iron stores may protect from malaria infection in pregnancy [1]. Given that malaria parasitaemia prevalence is highest in the first pregnancy [2], and that most primigravidae are adolescents, studies in this population group in malaria endemic areas are of primary importance. Evidence for or against the hypothesis that iron status early in pregnancy affects malaria risk is required because the present approach to control iron deficiency is to identify areas where anaemia prevalence is above ≥40% and to provide iron supplements to all pregnant and non-pregnant women of reproductive age [3]. One aim of the policy is to improve iron status before child-bearing begins, so testing the hypothesis requires a homogenous population of young non-pregnant and pregnant women. This study was conducted in West Africa where iron deficiency affects up to a third of women attending first antenatal visits [4], and up to 20% of non-pregnant women of reproductive age [5]. Without malaria control measures during pregnancy, 45% of 32 million pregnancies in malaria-endemic sub-Saharan Africa are exposed to *Plasmodium falciparum* malaria [6].

A limitation in estimating iron deficiency was interpretation of the various iron biomarkers which may differ between non-pregnant and pregnant women due to physiological and immunological changes in pregnancy [7], as well as the effects of inflammation on some iron indices. This is a significant problem in African countries with a high prevalence of malaria and other infections. Adjustment methods to correct for inflammation have been proposed, based primarily on analyses of pre-school children in cross-sectional studies from several countries [8–11]. Less information is available using adjusted estimates for iron deficiency in adolescents or young pregnant women [12,13], and most reports assessing inflammation are in older parous women from non-malaria endemic areas [14].

The objectives of the present study were firstly: to compare prevalence estimates for iron deficiency using an extensive series of iron-related biomarkers in nulliparae and primigravidae, as previous studies have based their conclusions on selected biomarkers; secondly to assess correlation between these biomarkers and red cell indices; thirdly to compare iron status early in pregnancy with that in non-pregnant nulliparae from the same population; fourthly, to test the hypothesis that iron status early in pregnancy affects malaria risk. This was an analysis of participants enrolled in a large periconceptional, controlled randomized trial investigating safety of long-term weekly iron supplementation in a rural malaria endemic in which young women were followed from before pregnancy into their first pregnancy. As there were no significant differences in iron biomarker profiles between arms at trial end-points [15], data from both trial arms were pooled for the present analysis.

2. Materials and methods

The protocol was approved by ethical review boards at collaborating centres and registered with clinicaltrials.gov:NCT01210040 (supplementary file 1). The study was undertaken between April 2011 and January 2014 and was located within the Health Demographic Surveillance System of the Clinical Research Unit in Nanoro covering a population of 63,000 individuals (altitude 300 metres), [16]. Malaria is hyperendemic with seasonal transmission [17], and HIV prevalence is low (<2%) [18]. Datasets comprised primigravidae at first antenatal visit (ANC1; n = 315), and nulliparae at end assessment (n = 899). The participant flow diagram is shown in Fig. 1. Individual/guardian written consents were obtained from participants at recruitment, with re-consent at entry to the pregnancy cohort (supplementary file 1).

2.1. Main trial design

We recruited young, nulliparous and non-pregnant women aged 15–24 years from thirty rural villages. Participant characteristics have been previously reported [15]. At enrolment diets were assessed by requesting information on daily frequency of consumption of meat, fish, eggs, rice, vegetables (other than leaves), and fresh fruit (mango, guava) in the previous week. A morning blood sample was requested for sera storage (−80 °C) for biomarker assays. Women were randomised to receive a capsule containing ferrous gluconate (containing 60 mg elemental iron) and folic acid (2.8 mg), or an identical capsule containing folic acid alone (2.8 mg), [19]. Weekly supplements continued for 18 months, when women who remained non-pregnant were referred for an end assessment (FIN). Those who became pregnant during the 18 months follow-up entered the pregnant cohort and were screened at a scheduled first study antenatal visit (ANC1). Adherence to iron supplement was assessed by directly observed ingestion at the weekly field worker visit (number of tablets received/number of weeks in the study starting from first weekly visit to ANC1, or end assessment) x 100. Median adherence has been reported and was 80% in pregnant women attending at ANC1, and 84% in non-pregnant women at their end assessment (Gies et al., 2018). The longitudinal group comprises women seen at end assessment who became pregnant and were then also screened at ANC1. Gestational age was assessed by ultrasound at ANC1, when weekly iron was replaced by routine daily antenatal iron and folic acid supplementation (60 mg elemental iron, 400 μg folic acid). A blood sample was collected and a malaria smear prepared at the primary endpoint for the main trial (ANC1), and at end assessment after 18 months follow-up (FIN) for non-pregnant women (supplementary file 1). At enrolment, ANC1, and end assessment duplicate measurements of height (nearest mm, Minimeter, Raven Equipment Ltd, Essex, UK), and weight (nearest 100 gms, SECA scale) were measured, and a history of current signs and symptoms of illness checked.

2.2. Laboratory procedures

Blood was transported to the research laboratory and within 3 h, centrifuged, aliquoted and stored at −80 °C. Haemoglobin was measured (Sysmex automated analyser) on fresh whole blood. Ferritin and serum transferrin receptor (sTfR) were measured using duplicate sampling by ELISA (Spectro Ferritin S-22 and TFC 94

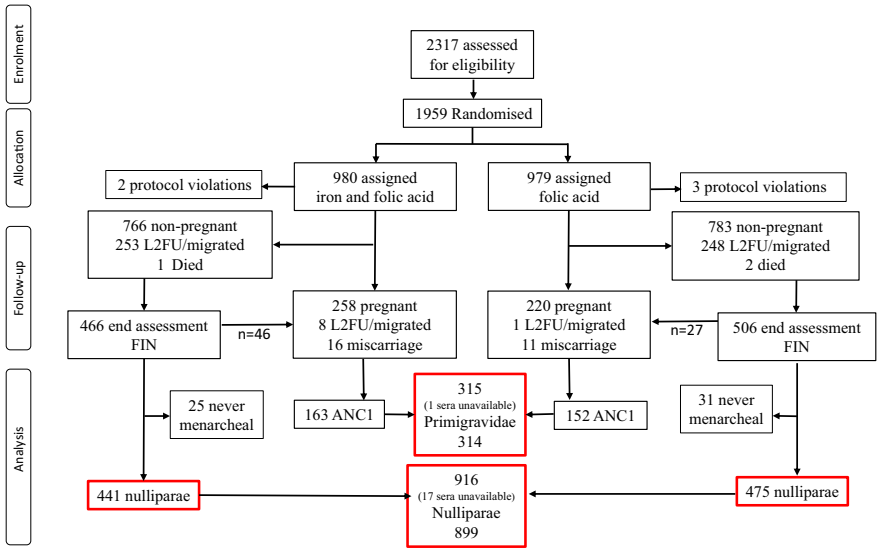

Fig. 1. Participant Flow diagram. 46 (iron) and 27 (control) women were identified as in early pregnancy at end assessment (FIN), or became pregnant within a few months following this assessment, and were then also screened at ANC1. These are totalled in the pregnant cohort in the Figure. 57 were subsequently screened at ANC1 and are considered in the separate longitudinal analysis. LTFU indicates loss to follow-up.

Transferrin Receptor, RAMCO Laboratories Inc, Texas) at the Research Laboratory in Nanoro; C-reactive protein (CRP) by ELISA (EU59131IBL International, GmbH, Hamburg); whole blood zinc protoporphyrin (ZnPP) by fluorometer (Aviv Biomedical, Lakewood, NJ). Intra-assay CVs were all <10%. Serum hepcidin was measured by competitive ELISA assay as previously described [20]. Red cell counts, haemoglobin, mean corpuscular volume (MCV) haemoglobin concentration (MCHC), and red cell distribution width (RDW), were measured using a Sysmex automated analyser. Body iron stores (BIS) (mg/kg) were calculated using the equation derived by Cook et al.: body iron (mg/kg) = − [$\log_{10}$ (1000 × sTfR/ferritin) − 2.8229]/0.1207 [21].

Whole blood for malaria films was stained with Giemsa and read independently by two qualified microscopists and data from each entered in separate files. For discrepant findings (positive/negative; > two-fold difference for parasite densities ≥400/μl; > log10 if < 400/μl), a third independent reading was made, with the mean of the two closest observations accepted as the true value. Parasite density was calculated by counting the number of asexual parasites per 200 white blood cells (WBC) in the thick blood film by light microscopy at 100× magnification. The parasite density per μl was calculated assuming a white cell count of 8000 mm^3.

2.3. Definitions of iron deficiency and iron deficient erythropoiesis

(1) Unadjusted ferritin <15 μg/l [22], with use of higher cut-offs allowing for effects of inflammation (<30 μg/l and <70 μg/l) [23].
(2) Adjusted ferritin based on the internal regression correction approach described by Namaste et al. [10].
(3) Ratio of sTfR (mg/l) to $\log_{10}$ ferritin (μg/l) > 5.6, which assesses both stored and functional iron and is possibly less affected by inflammation. The ratio derives from the cut-offs sTfR >8.3 μg/ml and ferritin <30 μg/l. This cut-off >5.6 best predicted iron deficient bone marrow stores (sensitivity 74%, specificity 73%, accuracy 73%), using the same manufacturer's assay for sTfR (RAMCO) as in the present study [24], in an area of high malaria transmission. The ferritin cut-off used in the ratio of <30 μg/l had a sensitivity 90% and specificity 85.1% in identifying pregnant Malawian women with absent bone marrow iron stores [25].
(4) sTfR concentration of >8.3 μg/ml [9].
(5) Zinc protoporphyrin cut-off of >70 μmol/mol heme defined iron-deficient erythropoiesis following conventional usage [26], or >85 μmol/mol heme based on a sensitivity analysis [27].
(6) Body iron stores calculated using an internal regression correction approach for ferritin allowing for inflammation as described by Mei et al. [11]. Iron deficiency was estimated using specific biomarker definitions, as well as adjusted ferritin estimates allowing for inflammation (10). A combined (non-pregnant and pregnant) internal regression slope log (Ferritin) against log (CRP) estimate was used for ferritin correction, adjusting ferritin levels where CRP exceeded a reference level of the 10th centile to that reference level. A sensitivity analysis utilised separate regression adjustments for the pregnant and non-pregnant women. Low body iron was <0 mg/kg.
(7) Red cell indices cut-offs: red cell distribution width, RDW <14.5% [28]; mean corpuscular haemoglobin concentration, MCHC < 32 g/dl; mean corpuscular volume, MCV < 75 fL [29].

The median hepcidin reference level of serum/plasma used was for a healthy non-malarious Dutch population [30]. The hepcidin 95% reference range for women 18–24 yrs age from this population was: median 2.6 nM; 2.5th percentile 0.7 nm; and 97.5th percentile 10.5 nM. The CRP cut-off point of <10 μg/ml was used to indicate inflammation which allowed comparison with non-pregnant and pregnant women from a non-malarious region (Mexico) [31].

2.4. Statistical analysis

The sample size was predicated by the main trial design and was based on the primary outcome of *P.falciparum* malaria at ANC1 [15]. Trial arms were pooled. Women with uncertain pregnancy status at end assessment were excluded from the non-pregnant cohort. Of women first identified as in very early pregnancy at FIN, or within 6 months of end assessment, 57 were subsequently screened at ANC1 and are considered in a separate longitudinal analysis.

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Variables with non-Gaussian distributions were log transformed and geometric means reported. Mann–Whitney U-tests and Fisher's Exact tests were used to compare biomarker levels between primigravidae and nulliparae in the full sample. Paired Wilcoxon and McNemar tests were used to compare pre-pregnant and pregnant assessments in the longitudinal subset. Associations between iron markers were explored using linear regression and summarized with Pearson's correlation coefficients (r). Linear regression models of the association between pairs of markers (with interaction term) were used to test the hypothesis that the relationship between the two variables differs between pregnant and non-pregnant women. Malaria effects were tested using linear or logistic regression models within each cohort and the difference in malaria effects tested using a malaria by cohort interaction test. Cumulative frequency distributions of body iron stores were calculated which used combined, or separate internal regression slope correction estimates for ferritin in primigravidae or nulliparae. Statistical analyses were performed in R version 3.3 [32].

2.5. Ethics approval and consent

The clinical protocol was approved by the Liverpool School of Tropical Medicine, UK, Research Ethics Committee (LSTM/REC Research Protocol 10.55); the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, Belgium (IRB/AB/AC/016); the Antwerp University Hospital Ethics Committee (EC/UZA); in Burkina Faso the Institutional Ethics Committee of Centre Muraz (Comité d'Ethique Institutionnel du Centre Muraz, and the National Ethics Committee (Comité Ethique pour la Recherche en Santé, CERS. Ref 015-2010/CE-CM). The work described was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Prior to enrolment the study team visited each village to inform village elders and senior women about trial objectives and for permission to invite young women to take part. Informed consent with right to withdraw (signature or thumb print) was granted by each participant, or by her appointed guardian if a minor or married at recruitment, and repeated later by participants continuing to be followed in the pregnant cohort. Participants provided written informed consent for publication of research results.

Trial registration number NCT01210040. Registered with Clinicaltrials.gov on 27 September 2010.

3. Results

3.1. Participant characteristics

At enrolment median age (IQR) was 16.5 (15-18) years for women who became pregnant, and 16.0 (15-17) years for the non-pregnant cohort. The median (range) period of weekly iron and folic acid, or folic acid supplementation before ANC1 among women who became pregnant was 58 weeks (7–117 weeks) and before FIN 83 (51-117) weeks. For this analysis, 899 non-pregnant samples collected at FIN were available for iron biomarkers and malaria, and for 315 primigravidae who attended ANC1. The proportion of women at enrolment reporting consumption of meat and/or fish in the previous week was 75%, eggs 7%, vegetables 7%, fresh fruit 80%, rice 83%, and bread 44%. Mean weight, height and body mass index were 49.2 ± 6.9 kg, 159 ± 6 cms, and 19.4 ± 2.1 kg/m^2 at enrolment, and 32.5% had a BMI less than 18.5 kg/m^2. Mean weight at ANC1 was 54.9 ± 6.2 kg, and at FIN, 53.1 ± 6.5 kg. The proportion of pregnant women reporting nausea and vomiting at the time of ANC1 was 4.1%.

Table 1 shows cross-sectional iron biomarker and red cell indices for both groups of women. Primigravidae were more anaemic (<11 g/dl) than nulliparae (69.7% versus 43.4%, RR 1.6 95% CI: 1.4:1.7, P < 0.001). 73 were identified at, or shortly after FIN, as in very early pregnancy and were excluded from the non-pregnant data set. These formed the basis for a discrete longitudinal sample of women if assessed both at FIN and again in early pregnancy at ANC1.

3.2. Prevalence of iron deficiency and iron deficient erythropoiesis using different biomarker profiles

The combined regression slope estimate for log ferritin against log CRP for primigravidae and nulliparae, using the correction method recommended by Namaste et al. [9], was 0.171 ± 0.014 (SE). Based on this regression correction, adjusted ferritin <15 μg/l was 12.8% for primigravid and 15.9% for nulliparous women (P = 0.20). This compares with 11.9% and 20.4% respectively using the sTfR/log ferritin ratio >5.6 (P < 0.001). Using regression correction, low body iron was 8.0% in primigravid and 11.7% in nulliparous women (P = 0.088). Low RDW percentage values also indicated lower prevalence of iron deficient erythropoiesis in pregnancy (P < 0.001) as did MCV, with a lower proportion of primigravidae having microcytosis (11.1% versus 20.2%; (P < 0.001). Hepcidin concentration did not differ significantly between the two groups. Biomarkers and red cell indices for the longitudinal cohort are summarised in Table 2. These are comparable to estimates for the cross-sectional sample.

3.3. Correlations between iron biomarkers and red cell indices

Table 3 summarises the correlation matrix for iron and red cell indices for nulliparae and primigravidae. P values for each cell are provided in Table S1 in supplementary file 1. All coefficients were lower for primigravidae than nulliparae, except those for CRP. Correlations are provided for both adjusted and unadjusted ferritin. Unadjusted ferritin correlated positively with haemoglobin concentration in nulliparae (r = 0.34, P < 0.0001) but negatively in primigravidae (r = −0.26, P < 0.001). This difference was highly significant (P < 0.0001) and remained in malaria positive (P < 0.0001) and negative sub-groups (P < 0.0001) (Fig. 2). With adjusted ferritin, correlations with haemoglobin in nulliparae and primigravidae were 0.36 (P < 0.0001) and − 0.13 (P < 0.022) respectively.

In both groups of women the sTfR/log ferritin ratio negatively correlated with haemoglobin and hepcidin, and positively with ZnPP (all P < 0.0001) (Fig. 2, Table 3 and supplementary Table S1). Adjusted BIS positively correlated with haemoglobin in nulliparae (P < 0.001), but not primigravidae (P = 0.46) (Fig. 2). The linear regression model comparing slopes demonstrated that most differed significantly between primigravid and nulliparous women (see supplementary file 1, Table S2).

3.4. Iron status early in pregnancy and in non-pregnant nulliparae

In the cross-sectional analysis mean adjusted BIS was 4.6 mg/kg at FIN and 5.8 mg/kg at ANC1 (P < 0.001). In the longitudinal cohort mean adjusted BIS was 5.7 mg/kg at FIN and increased to 6.3 mg/kg at ANC1 (P = 0.38).

3.5. Association of malaria and iron biomarkers (pregnant women Table 4; non-pregnant women Table 5)

At ANC1 median (IQR) gestational age was 18 (14-22) weeks. *P.falciparum* parasitemia prevalence was 54.0% at ANC1 and 41.8% at FIN (relative risk, RR, 1.29, 95%CI 1.13–1.45, P < 0.001), and pregnant women had higher parasite densities (P < 0.001). Malaria in

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Table 1
Cross-sectional biomarkers of nulliparae at FIN and primigravidae at ANC1.

Variable	FIN	ANC1	P value[a]
GM ferritin, μg/l, [95%CI] (n)	49.9 [47.2:52.9] (895)	89.5 [80.1:100.0] (313)	<0.001
GM adjusted ferritin, μg/l, [95%CI] (n)[b]	34.5 [32.6:36.5] (895)	45.4 [40.9:50.4] (313)	<0.001
Ferritin <15 μg/l, n/N (%)	78/895 (8.7)	17/313 (5.4)	0.067
Adjusted ferritin <15 μg/l, n/N (%)[b]	142/895 (15.9)	40/313 (12.8)	0.20
Ferritin <30 μg/l, n/N (%)	249/895 (27.8)	50/31 (16.0)	<0.001
Adjusted ferritin <30 μg/l, n/N (%)[b]	374/895 (41.8)	95/313 (30.4)	<0.001
GM sTfR μg/ml, [95%CI] (n)	6.5 [6.3:6.6] (892)	5.9 [5.7:6.2] (313)	0.002
sTfR > 8.3 μg/ml, n/N (%)	203/892 (22.8%)	62/313 (19.8%)	0.30
Mean sTfR/$\log_{10}$ ferritin, [95%CI] (n)	3.9 [3.8:4.1] (892)	3.1 [3.0:3.3] (312)	<0.001
sTfR/$\log_{10}$ ferritin >5.6, n/N (%)	182/892 (20.4)	37/312 (11.9)	<0.001
Mean adjusted BIS, mg/kg [95%CI] (n)[b]	4.6 [4.3:4.8] (892)	5.8 [5.4:6.3] (312)	<0.001
Low adjusted BIS <0 mg/kg, n/N (%)[b]	104/892 (11.7)	25/312 (8.0)	0.088
GM CRP, mg/l, [95%CI] (n)	0.5 [0.5:0.6] (893)	3.8 [3.1:4.5] (311)	<0.001
CRP >10 mg/l, n/N (%)	25/893 (2.8%)	101/311 (32.5%)	<0.001
CRP >5 mg/l, n/N (%)	62/893 (6.9%)	152/311 (48.9%)	<0.001
GM ZnPP, μmol/molHeme, [95%CI] (n)	100.8 [98.3:103.4] (897)	105.8 [102.0:109.7] (285)	<0.001
ZnPP >70 μmol/molHeme,n/N (%)	790/897 (88.1)	272/285 (95.4)	<0.001
ZnPP >85 μmol/molHeme,n/N (%)	569/897 (63.4)	222/285 (77.9)	<0.001
Mean hepcidin, nmol/l, [95%CI] (n)	2.8 [2.6:3.0] (892)	2.9 [2.5:3.4] (311)	0.43
Hepcidin <0.7 nmol/l, % n/N (%)	131/892 (14.7)	58/311 (18.6)	0.10
Mean Hb, g/dl [95%CI] (n)	12.3 [12.2:12.3] (898)	10.2 [10.0:10.3] (314)	<0.001
Hb < 11 g/d, or <12 g/dL, n/N (%)[c]	389/896 (43.4)	219/314 (69.7)	<0.001
Mean RDW, [95%CI] (n)	13.9 [13.8:14.0] (887)	14.8 [14.6:15.0] (309)	<0.001
RDW <14.5, n/N (%)	670/887 (75.5)	148/309 (47.9)	<0.001
Mean MCHC, g/dL, [95%CI] (n)	34.4 [34.3:34.5] (894)	33.6 [33.4:33.8] (314)	<0.001
MCHC <32, g/dL, n/N (%)	40/894 (4.5)	73/314 (23.2)	<0.001
Mean MCV, fL, [95%CI] (n)	80.1 [79.6:80.5] (894)	85.8 [84.8:86.7] (314)	<0.001
MCV <75, n/N (%)	181/894 (20.2)	35/314 (11.1)	<0.001
Malaria prevalence, n/N (%)[d]	376/899 (41.8)	170/315 (54.0)	<0.001
Parasite density (n)	298.3 [259.9:342.4] (376)	2116 [1720.4: 2604] (170)	<0.001

GM: geometric mean; BIS: body iron store; sTfR: serum transferrin receptor; ZnPP, whole blood zinc protoporphyrin; Hb: hemoglobin; RDW: red cell distribution width; CRP: C-reactive protein; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration.
[a] Mann-Whitney U test or Fisher's Exact test comparing pregnant and non-pregnant women.
[b] Adjusted by internal regression correction using common slope for non-pregnant and pregnant women (see methods).
[c] Hb <11 g/dl pregnant; <12 g/dl non-pregnant; 56 non-pregnant women were pre-menarcheal.
[d] Blood slide positive.

pregnancy was associated with iron deficiency as defined by any of the iron biomarkers assessed. Primigravidae with malaria were less likely than those without to have adjusted ferritin levels <15 μg/l, <30 μg/l, or <70 μg/l (all $P < 0.001$), or iron deficient erythropoiesis based on the sTfR/log ferritin ratio > 5.6 ($P = 0.014$) (Table 4). Mean BIS was higher with malaria (6.5 mg/kg versus 5.0 mg/kg; risk difference 1.53, 95%CI 0.67:2.38, $P < 0.001$), and primigravidae were less likely to have BIS less than 0 mg/kg (RR, 0.21, 0.08:0.55, $P < 0.001$). Raised ZnPP (>85 μmol/molHeme) was more frequent with malaria (1.30, 1.14:1.48. $P < 0.001$), and hepcidin concentration was higher ($P = 0.002$). Primigravidae with malaria were also more anaemic (84.1% versus 52.8%, $P < 0.001$), but less likely to have a low RDW index ($P = 0.004$). For all iron biomarker and red cell indices listed in Tables 4 and 5, except sTfR and MCV, values in primigravidae differed significantly from those in nulliparae.

Nulliparae with malaria showed no significant differences compared to those without malaria in prevalence of adjusted ferritin cut-off values, a raised sTfR/log ferritin ratio, or mean or low BIS estimates (Table 5). Raised ZnPP was more frequent in nulliparae with malaria (relative risk 1.20, 1.08:1.32. $P < 0.001$), who also had higher hepcidin ($P = 0.077$) and sTfR ($P < 0.001$) concentrations. Anaemia prevalence was higher in nulliparae with malaria ($P = 0.005$).

Cumulative frequency distributions of BIS were examined by malaria category using adjusted ferritin estimates (Fig. 3). These were unimodal in primigravidae and nulliparae but shifted to the right in primigravidae with malaria ($P < 0.001$). Published BIS distributions in non-pregnant, or pregnant women in the first trimester, assessed in the US National Health and Nutrition Examination Survey (NHANES) from 1999 to 2006 [21,33], were plotted in Fig. 3 for comparison. Non-pregnant US women aged between 20 and 45 years had only marginally better iron status than Burkinabé women. Pregnant US women in the first trimester (all parities) had comparable BIS distribution to Burkinabé primigravidae without malaria (mean gestational age 18 weeks). Cumulative distributions are also shown in Fig. 3 using ferritin correction based on a pregnancy-specific regression slope estimate. This decreased the malaria difference and brought the slopes closer to the US data, but the malaria difference remained significant ($P = 0.008$).

CRP concentrations did not correlate with the sTfR/log ferritin ratio in either primigravidae ($r = 0.005$) or nulliparae ($r = -0.001$) (Table 3). CRP values ≥ 5 μg/ml were more frequent in pregnancy (48.9% versus 6.9%; RR 7.0, 95%CI: 5.4:9.2, $P < 0.001$) (Table 1). CRP concentrations were significantly increased with malaria in both groups, although the effect of malaria was greater in primigravidae (Tables 4 and 5).

4. Discussion

Nulliparous and primigravid participants were comparable in terms of age, rural residence, malaria exposure, and low HIV prevalence (<2%). The sample size allowed a detailed analysis, and the longitudinal data, albeit with a smaller sample size, confirmed the cross-sectional data. Iron deficiency prevalence was low, probably because these young women were recently menarcheal and had not experienced cumulative iron loss from menstruation or repeat pregnancies. Prevalence estimates were lower based on adjusted ferritin biomarkers rather than red cell indices (MCHC, RDW) or ZnPP, which is not adjusted for inflammation. Based on a

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Table 2
Longitudinal biomarkers in 57 women screened at both FIN and ANC1.

Variable	FIN	ANC1	P value[a]
GM ferritin, µg/l, [95%CI]	68.3 [54.2:86.0]	86.9 [67.7:111.7]	0.072
GM adjusted ferritin, µg/l, [95%CI][b]	45.5 [36.9:56.2]	45.9 [36.2:58.3]	0.95
Ferritin <15 µg/l, n/N (%)	4/57 (7.0)	5/57 (8.8%)	1
Adjusted ferritin <15 µg/l, n/N (%)[b]	7/57 (12.3)	7/57 (12.3)	1
Ferritin <30 µg/l, n/N (%)	10/57 (17.5)	8/57 (14.0)	0.72
Adjusted ferritin <30 µg/l, n/N (%)[b]	15/57 (26.3)	14/57 (24.6)	1
GM sTfR µg/ml, [95%CI]	6.2 [5.6:6.8]	5.3 [4.8:5.9]	0.055
sTfR > 8.3 µg/ml, n/N (%)	9/57 (15.8)	6/57 (10.5)	0.50
Mean sTfR/$\log_{10}$ ferritin, [95%CI]	3.5 [3.0:3.9]	2.8 [2.5:3.2]	0.048
sTfR/$\log_{10}$ ferritin >5.6, n/N (%)	9/57 (15.8)	5/57 (8.8)	0.22
Mean adjusted BIS, mg/kg [95%CI][b]	5.7 [4.8:6.6]	6.3 [5.3:7.3]	0.38
Low adjusted BIS < 0 mg/kg,n/N (%)[b]	3/57 (5.3)	6/57 (10.5)	0.37
GM CRP, mg/l, [95%CI] (n)	0.7 [0.4:1.1] (56)	3.0 [1.9:4.6] (56)	<0.001
CRP >10 mg/l, n/N (%)	3/57 (5.3)	12/56 (21.4)	0.016
CRP >5 mg/l, n/N (%)	5/57 (8.8)	22/56 (39.3)	<0.001
GM ZnPP, µmol/molHeme, [95%CI]	100.2 [91.7:109.4]	107.9 [101.0:115.3]	0.059
ZnPP >70 µmol/molHeme,n/N (%)	53/57 (93.0)	56/57 (98.2)	0.37
ZnPP >85 µmol/molHeme,n/N (%)	36/57 (63.2)	47/57 (82.5)	0.003
Mean Hepcidin, nmol/l, [95%CI] (n)	3.9 [2.8:5.5] (56)	3.5 [2.6:4.8] (56)	0.61
Hepcidin <0.7 nmol/l, % n/N (%)	7/56 (12.5)	8/57 (14.0)	1
Mean Hb, g/dl [95%CI]	11.9 [11.7:12.2]	10.4 [10.1:10.7]	<0.001
Hb < 11 g/d, or <12 g/dL, n/N[c] (%)	25/57 (43.9)	37/57 (64.9)	0.031
Mean RDW, [95%CI]	13.6 [13.3:13.9]	14.1 [13.7:14.5]	0.13
RDW <14.5, n/N (%)	42/54 (77.8)	39/57 (68.4)	0.18
Mean MCHC, g/dL, [95%CI]	34.8 [34.4:35.2]	34.9 [34.5:35.3]	0.93
MCHC <32, g/dL, n/N (%)	2/57 (3.5)	1/57 (1.8)	1
Mean MCV, fL, [95%CI]	79.0 [77.2:80.8]	82.6 [80.7:84.5]	0.011
MCV <75, n/N (%)	16/57 (28.1)	7/57 (12.3)	0.008
Malaria prevalence, n/N (%)[d]	23/57 (40.4)	29/57 (50.9)	0.33
Parasite density (n)	320 [170:600]	1594 [1106:2296]	<0.001

GM: geometric mean; BIS: body iron store; sTfR: serum transferrin receptor; ZnPP, whole blood zinc protoporphyrin; Hb: hemoglobin; RDW: red cell distribution width; CRP: C-reactive protein; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration.
[a] Paired Wilcoxon test or McNemar's test comparing pregnant and non-pregnant women.
[b] Adjusted by internal regression correction using common slope for non-pregnant and pregnant women (see methods).
[c] Hb <11 g/dl pregnant; <12 g/dl non-pregnant.
[d] Blood slide positive.

Table 3
Biomarker correlation coefficients in primigravidae and nulliparae.

	Ferritin*	Adjusted ferritin*	sTfR*	ZnPP*	Hepcidin*	sTfR/$\log_{10}$Fer*	Hb	RDW	MCHC	MCV	Adjusted BIS
Primigravidae at ANC1											
Ferritin*	1										
sTfR*	−0.052	−0.196	1								
ZnPP*	0.018	−0.069	0.502	1							
Hepcidin*	0.628	0.633	−0.302	−0.226	1						
sTfR/$\log_{10}$Fer*	−0.530	−0.564	0.871	0.442	−0.560	1					
Hb	−0.259	−0.130	−0.403	−0.606	0.127	−0.230	1				
RDW	−0.015	−0.060	0.343	0.481	−0.185	0.306	−0.379	1			
MCHC	−0.003	0.054	−0.239	−0.129	0.103	−0.211	0.343	−0.393	1		
MCV	0.247	0.277	0.139	−0.028	0.016	−0.003	−0.154	−0.078	−0.336	1	
Adjusted BIS	0.863	0.924	−0.488	−0.256	0.675	−0.832	0.042	−0.187	0.142	0.144	1
CRP*	0.372	0.096	0.208	0.307	0.125	0.005	−0.490	0.156	−0.199	0.137	0.003
Nulliparae at FIN											
Ferritin*	1										
sTfR*	−0.303	−0.338	1								
ZnPP*	−0.388	−0.429	0.632	1							
Hepcidin*	0.656	0.629	−0.348	−0.420	1						
sTfR/$\log_{10}$Fer*	−0.679	−0.688	0.901	0.673	−0.561	1					
Hb	0.340	0.364	−0.483	−0.652	0.348	−0.538	1				
RDW	−0.318	−0.335	0.431	0.578	−0.333	0.482	−0.474	1			
MCHC	0.331	0.353	−0.336	−0.455	0.295	−0.417	0.594	−0.267	1		
MCV	0.269	0.287	−0.306	−0.417	0.212	−0.364	0.398	−0.577	0.083	1	
Adjusted BIS	0.892	0.937	−0.644	−0.543	0.640	−0.892	0.474	−0.432	0.411	0.347	1
CRP*	0.183	−0.099	0.103	0.123	0.129	−0.001	−0.068	0.042	−0.063	−0.053	−0.119

sTfR: serum transferrin receptor sTfR/$\log_{10}$Fer: sTfR/$\log_{10}$ferritin ratio; ZnPP: whole blood zinc protoporphyrin; Hb:hemoglobin; RDW: Red cell distribution width; CRP: C-reactive protein; MCV = mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; BIS: body iron stores.
Asterisk: log $_{10}$ transformed. Adjusted ferritin based on internal regression correction allowing for inflammation based on CRP.
Cell sample sizes for pregnant women vary from 282 to 314 and for non-pregnant women from 882 to 897. P values for individual cell correlations are shown in Table S1 supplementary file. P values for a comparison of the relationships between biomarkers in pregnant and non-pregnant women in Table 3 are shown in Table S2 supplementary file.

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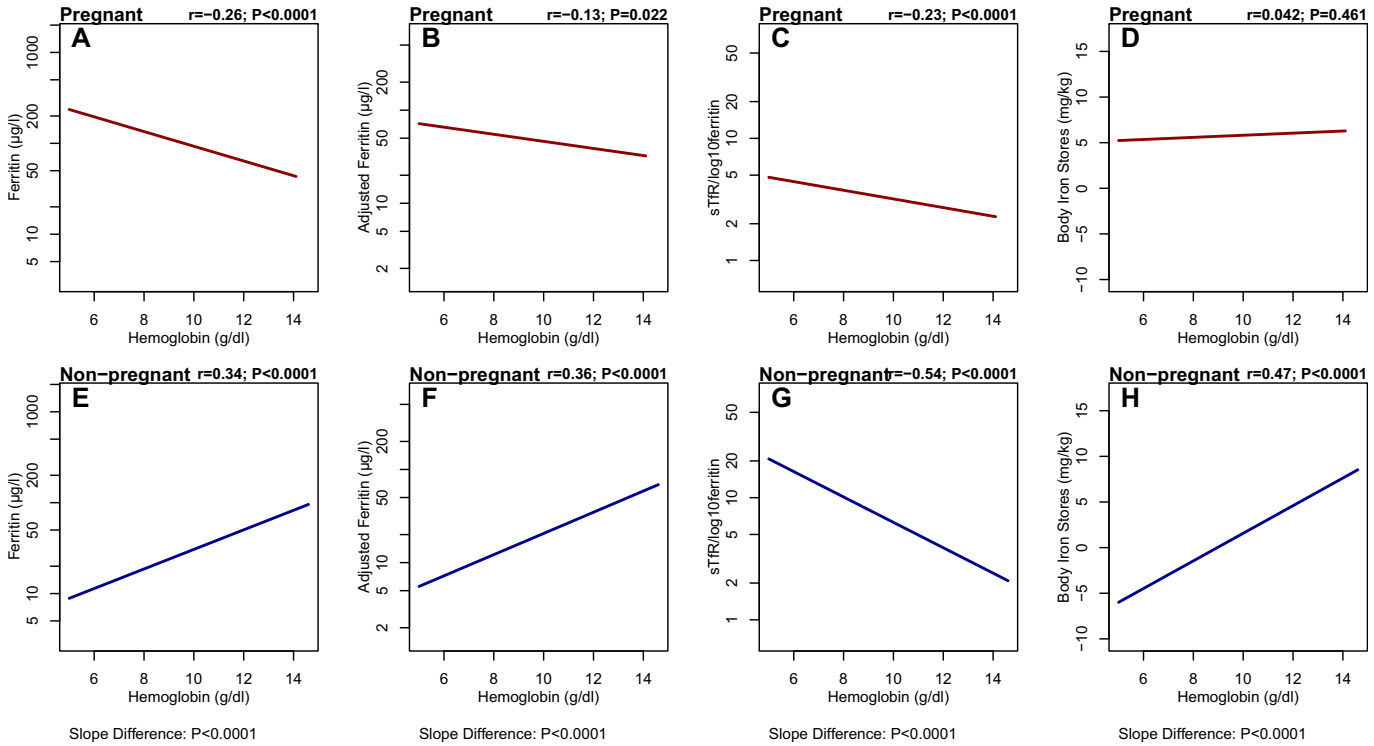

Fig. 2. Correlations for unadjusted and adjusted serum ferritin, sTfR/log$_{10}$ferritin ratio, and adjusted body iron stores with haemoglobin in pregnant and non-pregnant women. Panel key. For primigravidae, A: Unadjusted ferritin; B: adjusted ferritin; C: sTfR/log$_{10}$ferritin; D: Body iron stores. For nulliparae, E Unadjusted ferritin; F: adjusted ferritin; G: sTfR/log$_{10}$ferritin; H: Body iron stores. Difference in the relationships between pregnant and non-pregnant women were highly significant ($P < 0.0001$) (supplementary file) and remained in the malaria ($P < 0.0001$) and non-malaria ($P < 0.0001$) sub-groups for each comparison. Open circles represent non-malaria cases; closed circles malaria blood slide positive cases.

tissue iron deficit of minus 4 mg/kg indicating anaemia [34], iron deficiency anaemia was uncommon. Although other nutritional deficiencies may be contributory, the high anaemia prevalence, especially in early pregnancy, was probably primarily malaria-related as malaria causes haemolysis and dyserythropoiesis. Correlation coefficients between iron biomarkers were lower in primigravidae than nulliparae. The positive ferritin-haemoglobin coefficient in nulliparae is consistent with reduced iron stores causing anaemia [35]. The negative coefficient in primigravidae probably arises because of chronic malarial anemia resulting from their higher pregnancy-related *P.falciparum* infection risk and parasite densities [2]. As reported in parasitaemic Ghanaian pregnant women [36], primigravidae with malaria had high mean hepcidin levels consistent with their high malaria burden [37]. In non-pregnant women, mean hepcidin was also higher with malaria, although this difference did not reach significance. With infection, hepcidin would be expected to rise to reduce iron absorption [38].

As previous studies have mostly used only one or two iron biomarkers, our measurement were based on an extensive array of biomarkers which allowed comparison of functional iron deficit, iron stores, and erythropoiesis. We adjusted ferritin using internal regression correction based on CRP, which would be appropriate if the effects of malaria on iron measurements (as opposed to true iron status) were wholly mediated through inflammation, although direct effects are plausible. A strength of this analysis is that the sTfR assay used corresponded to that employed in developing the BIS model [39], and the sTfR/log ferritin ratio cut-off (>5.6) was also derived using this same RAMCO assay [24]. Prevalence estimates of iron deficiency in both nulliparae and primigravidae in this study were 3%–11% higher using the ratio compared to the adjusted BIS estimates. We did not have information on AGP responses and were unable to include this correction in the linear regression model. We did not adjust sTfR as the difference in prevalence of iron deficient erythropoiesis when adjusting sTfR with the use of CRP seems minimal [9]. We found the correlation of the sTfR/log ferritin ratio (>5.6) with CRP in primigravidae or nulliparae was almost zero, indicating that sTfR and ferritin concentrations which derive from the ratio were increased to an equivalent extent with CRP-related inflammation, and thus cancel out. The mean ratio did not differ by malaria sub-group in nulliparae, which is also reported from Côte d'Ivoire [12]. For these reasons we conclude the ratio may be preferable for identifying iron deficiency in women exposed to malaria and with chronic inflammation [40], especially if CRP or AGP cannot be measured [41]. Use of the ratio in pregnancy may also obviate the problem of blood volume dilution which would influence sTfR and ferritin concentrations.

Early first trimester iron requirements are lower than before pregnancy due to menses cessation and suppression of erythropoiesis such that iron stores may actually increase [42], facilitated by reduced hepcidin [43] and increased iron absorption [44]. This precedes red cell mass expansion when fetal iron requirements are increasing. In healthy primigravidae first trimester serum ferritin concentrations increase from non-pregnant levels [45]. We found that primigravidae, with or without malaria, had higher mean BIS than nulliparae. Mean adjusted BIS estimates at ANC1 (5.8 mg/kg, 95% CI 5.4:6.3 mg/kg) were within the range reported for first trimester US women in the NHANES survey (6.28 mg/kg, 95% CI 5.7:6.9 mg/kg) (31), and those at FIN (4.6 mg/kg, 95% CI 4.3:4.8) within the range for non-pregnant Vietnamese women (4.7 mg/kg) [46]. The 1.2 mg/kg higher BIS value in primigravidae compared to nulliparae is equivalent to a 0.55 mg/day gain for a 55 kg women

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Table 4
Iron biomarkers, red cell indices and CRP at ANC1 in pregnant women negative or positive for malaria parasitemia.

Biomarker	Pregnant				P_{int}[b]
	Negative	Positive	RR [95%CI][a]	P	
GM ferritin, μg/l, (n)	55.4 [47.0:65.3] (144)	134.6 [119.1:152.1] (169)	0.89 [0.68:1.09]	<0.001	<0.001
GM adjusted ferritin <15, μg/l, (n)[c]	33.7 [28.5:39.8] (144)	58.6 [52.0:66.1] (169)	0.55 [0.35:0.76]	<0.001	<0.001
Ferritin <30, μg/l, n/N (%)	44/144 (30.6)	6/169 (3.6)	0.12 [0.05:0.27]	<0.001	<0.001
Adjusted ferritin <30 μg/l, n/N (%)	65/144 (45.1)	30/169 (17.8)	0.39 [0.27:0.57]	<0.001	<0.001
Ferritin <70, μg/l, n/N (%)	88/144 (61.1)	26/169 (15.4)	0.25 [0.17:0.37]	<0.001	<0.001
Adjusted ferritin <70 μg/l, n/N (%)[c]	105/144 (72.9)	98/169 (58.0)	0.80 [0.68:0.94]	<0.001	<0.001
GM sTfR μg/ml, (n)	5.5 [5.2:5.9] (143)	6.3 [6.0:6.7] (170)	0.13 [0.04:0.22]	0.005	0.475
sTfR >8.3, μg/ml, n/N (%)	22/143 (15.4)	40/170 (23.5)	1.53 (0.96:2.45)	0.087	0.99
GM sTfR/log ferritin ratio,(n)	3.3 [3.0:3.6] (143)	3.0 [2.8:3.2] (169)	−0.08 [−0.20: 0.03]	0.14	0.041
sTfR/log ferritin >5.6, n/N (%)	24/143 (16.8)	13/169 (7.7)	0.46 [0.24:0.87]	0.014	0.007
Mean adjusted BIS, mg/kg (n)[c]	5.0 [4.3:5.7] (143)	6.5 [6.0:7.0] (169)	1.53 [0.67:2.38]	<0.001	<0.001
Low adjusted body iron <0 mg/kg, n/N (%)[c]	20/143 (14.0)	5/169 (3.0)	0.21 [0.08:0.55]	<0.001	<0.001
GM CRP, mg/l, (n)	1.3 [1.0:1.7] (142)	9.2 [7.8:10.9] (169)	1.97 [1.66:2.29]	<0.001	<0.001
CRP <10, mg/l, n/N (%)	126/142 (88.7)	84/169 (49.7)	0.56 [0.48:0.66]	<0.001	0.078
CRP <5, mg/l, n/N (%)	113/142 (79.6)	46/169 (27.2)	0.34 [0.26:0.44]	<0.001	<0.001
GM ZnPP, μmol/molHeme, (n)	95.8 [91.5:100.2] (135)	115.7 [110:122] (150)	0.19 [0.12:0.26)	<0.001	0.003
ZnPP >70 n/N, (%)	124/135 (91.9)	148/150 (98.7)	1.07 [1.02:1.13]	0.008	0.038
ZnPP >85 n/N, (%)	91/135 (67.4)	131/150 (87.3)	1.30 [1.14:1.48]	<0.001	0.036
GM hepcidin, nmol/l, (n)	2.3 [1.8:2.9] (143)	3.6 [3.0:4.4] (168)	0.45 (0.16:0.74)	0.002	0.049
Hepcidin <0.7, n/N (%)	38/143 (26.6)	20/168 (11.9)	0.45 [0.27:0.73]	0.001	0.071
Mean Hb, g/dl, (n)	10.8 [10.6:11.0] (144)	9.7 [9.5: 9.9] (170)	−1.14 [−1.42:−0.86]	<0.001	<0.001
Hb < 11 g/dl, (%)	76/144 (52.8)	143/170 (84.1)	1.59 [135:1.88]	<0.001	<0.001
Mean RDW, % (n)	14.4 [14.2:14.7] (143)	15.1 [14.8:15.5] (166)	0.73 [0.33:1.13]	<0.001	0.028
RDW <14.5, n/N (%)	81/143 (56.6)	67/166 (40.4)	0.71 [0.56:0.90]	0.004	0.247
Mean MCHC, g/dl (n)	33.9 [33.5:34.2] (144)	33.3 [33.0:33.7] (170)	−0.54 [−0.99:−0.09]	0.022	0.021
MCHC <32, n/N (%)	31/144 (21.5)	42/170 (24.7)	1.15 [0.76:1.73]	0.592	0.166
Mean MCV, fl (n)	85.4 [84.0:86.8] (144)	86.1 [84.7:87.4] (170)	0.63 [−1.28: 2.55]	0.516	0.236
MCV <75, n/N (%)	16/144 (11.1)	19/170 (11.2)	1.01 [0.54:1.88]	1	0.568

GM: geometric mean; BIS: body iron store; Square parenthesis: 95% confidence interval.
[a] Risk-ratio associated with malaria for dichotomous outcomes or difference in (log-transformed) for continuous outcomes.
[b] Interaction test comparing the magnitude of the malaria effect between the pregnant and non-pregnant cohorts.
[c] Adjusted with internal regression correction using common slope for non-pregnant and pregnant women.

over a 4 month period from conception. The longitudinal study confirmed an increase in BIS in early pregnancy, although the gain was smaller (0.6 mg/kg). Average menstrual loss of iron is estimated at 0.53 mg/day [47], which compares with the early gestational gain in body iron in primigravidae in the present study.

The importance of these observations relates to the hypothesis that iron status may affect susceptibility to malaria in pregnancy. This is consistent with the observation in sub-Saharan Africa that highest prevalence of *P. falciparum* malaria in primigravidae occurs during early pregnancy at 13–16 weeks gestation [2], when mean BIS increases, and with our observation of higher mean BIS in malaria-positive primigravidae. Higher mean BIS has also been reported with malaria in early in pregnancy in a Congolese study [48]. Primigravidae with a raised sTfR/log ferritin ratio (>5.6) also had less malaria, suggesting iron deficiency was associated with lower malaria risk. Other contributory explanations for the altered susceptibility of pregnant women to malaria include hormonal and immunological changes [49], and lack of immunity to pregnancy-specific *P.falciparum* parasites that sequester in the placenta [50].

All participants received weekly folic acid supplementation following WHO guidelines for prevention of neural tube defects with a weekly dose of 2.8 mg, equivalent to seven times the daily recommended dose of 400 μg [51]. High daily doses (5 mg) can increase malaria risk [52], which would be less likely with the much lower weekly dose used in this study. It is unclear whether this folate supplementation was beneficial in reducing anaemia prevalence as both trial arms received the folate supplement for ethical reasons. Folate deficiency anaemia can occur with chronic malaria infection which increases haemolysis and contributes to altered folate homeostasis [53]. The dietary pattern of participants at enrolment suggested moderate folate intake, but seasonal factors could influence folate intake and requirements over an 18 month period. Folate deficiency might increase MCV values, although when associated with iron deficiency the anaemia may be only mildly macrocytic, or even microcytic [54].

Limitations of this study include use of CRP alone as an infection marker which may have resulted in some misclassification of inflammation status – also because CRP normally rises in early pregnancy [55]. We were not able to determine sub-microscopic malaria infections which may lead to underestimation of malaria prevalence. Women with serious illnesses including sickle cell disease were excluded from the trial although participants were not screened for haemoglobinopathies or other genetic disorders prevalent in sub-Saharan Africa which may influence iron metabolism, relate to anaemia or influence susceptibility to malaria. There is limited experience on the accuracy and sensitivity of the Cook equation in calculating BIS in pregnant women. Their initial study did not include pregnant women, but they subsequently demonstrated the model's utility in an iron supplementation trial in Jamaican pregnant women [56]. Other reports on its use in pregnancy are also mostly from non-malaria endemic areas [33]. Our analysis showed that in non-pregnant women anaemia prevalence increased exponentially with lower BIS, suggesting BIS related to the severity of iron deficiency anaemia. This was not replicated in pregnant women (Fig. 2) presumably because pregnant women experienced more inflammation and chronic malarial anaemia than non-pregnant. Hence we cannot rule out that BIS estimates in pregnancy have lower sensitivity for detection of iron deficiency. Adjusting ferritin concentration for inflammation using regression as in this study, in theory should improve sensitivity of BIS in pregnant women in malaria endemic areas.

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Table 5
Iron biomarkers, red cell indices and CRP at FIN in non-pregnant women negative or positive for malaria parasitemia.

Biomarker	Non-pregnant			
	Negative	Positive	RR [95%CI][a]	P
GM ferritin, μg/l, (n)	47.3 [43.9:51.0] (521)	53.8 [49.3:58.7] (374)	0.13 [0.01:0.24]	0.028
GM adjusted ferritin <15, μg/l, (n)[b]	34.1 [31.7:36.8] (521)	35.1 [32.2:38.2] (374)	0.03 [−0.09:0.14]	0.644
Ferritin <30, μg/l, n/N (%)	154/521 (29.6)	95/374 (25.4)	0.86 [0.69:1.07]	0.17
Adjusted ferritin <30 μg/l, n/N (%)	221/521 (42.4)	153/374 (40.9)	0.96 [0.82:1.13]	0.68
Ferritin <70, μg/l, n/N (%)	343/521 (65.8)	223/374 (59.6)	0.91 [0.82:1.00]	0.058
Adjusted ferritin <70 μg/l, n/N (%)[b]	408/521 (78.3)	293/374 (78.3)	1.00 [0.93:1.07]	1
GM sTfR μg/ml, (n)	6.2 [6.0:6.4] (520)	6.8 [6.6:7.1] (372)	0.09 [0.04:0.14]	<0.001
sTfR >8.3, μg/ml, n/N (%)	98/520 (18.8)	105/372 (28.2)	1.50 (1.18:1.91)	0.001
GM sTfR/log ferritin ratio,(n)	3.8 [3.6:4.0] (520)	4.0 [3.9:4.2] (372)	0.06 [−0.01:0.13]	0.10
sTfR/log ferritin >5.6, n/N (%)	100/520 (19.2)	82/372 (22.0)	1.15 [0.88:1.49]	0.31
Mean adjusted BIS, mg/kg (n)[b]	4.7 [4.3:5.0] (520)	4.4 [4.1:4.8] (372)	−0.24 [−0.74:0.26]	0.346
Low adjusted body iron < 0 mg/kg, n/N (%)[b]	60/520 (11.5)	44/372 (11.8)	1.03 [0.71:1.48]	0.916
GM CRP, mg/l, (n)	0.4 [0.4:0.5] (520)	0.8 [0.7:0.9] (373)	0.68 [0.48:0.89]	<0.001
CRP <10, mg/l, n/N (%)	512/520 (98.5)	356/373 (95.4)	0.97 (0.95:0.99)	0.012
CRP <5, mg/l, n/N (%)	497/520 (95.6)	334/373 (89.5)	0.94 (0.90:0.97)	<0.001
GM ZnPP, μmol/molHeme, (n)	99.0 [96:102.] (522)	103 [99.5.:107] (375)	0.04 [−0.01: 0.09]	0.096
ZnPP >70 n/N, (%)	451/522 (86.4)	339/375 (90.4)	1.05 [1.00:1.10]	0.076
ZnPP >85 n/N, (%)	306/522 (58.6)	263/375 (70.1)	1.20 [1.08:1.32]	<0.001
GM hepcidin, nmol/l, (n)	2.7 [2.4:3.0] (518)	3.0 [2.7:3.4] (374)	0.14 [−0.02: 0.29]	0.077
Hepcidin <0.7, n/N (%)	85/518 (16.4)	46/374 (12.3)	0.75 [0.54:1.05]	0.10
Mean Hb, g/dl, (n)	12.0 [11.9:12.1] (522)	11.9 [11.8:12.0] (374)	−0.11 [−0.26: 0.05]	0.167
Hb < 12 g/dl, (%)	206/522 (39.5)	183/374 (48.9)	1.24 [1.07:1.44]	0.005
Mean RDW, % (n)	13.8 [13.7:14.0] (517)	14.0 [13.9:14.2] (370)	0.19 [−0.05: 0.42]	0.129
RDW <14.5, n/N (%)	404/517 (78.1)	266/370 (71.9)	0.92 [0.85:0.99]	0.039
Mean MCHC, g/dl (n)	34.4 [34.3:34.5] (520)	34.4 [34.2:34.5] (374)	−0.02 [−0.22: 0.18]	0.833
MCHC <32, n/N (%)	27/520 (5.2)	13/374 (3.5)	0.67 [0.35:1.28]	0.253
Mean MCV, fl (n)	80.3 [79.7:80.9] (520)	79.8 [79.1:80.5] (374)	−0.53 [−1.47: 0.40]	0.261
MCV <75, n/N (%)	97/520 (18.7)	84/374 (22.5)	1.20 [0.93:1.56]	0.177

GM: geometric mean; BIS: body iron store; Square parenthesis: 95% confidence interval.
[a] Risk-ratio associated with malaria for dichotomous outcomes or difference in (log-transformed) for continuous outcomes.
[b] Adjusted with internal regression correction using common slope for non-pregnant and pregnant women.

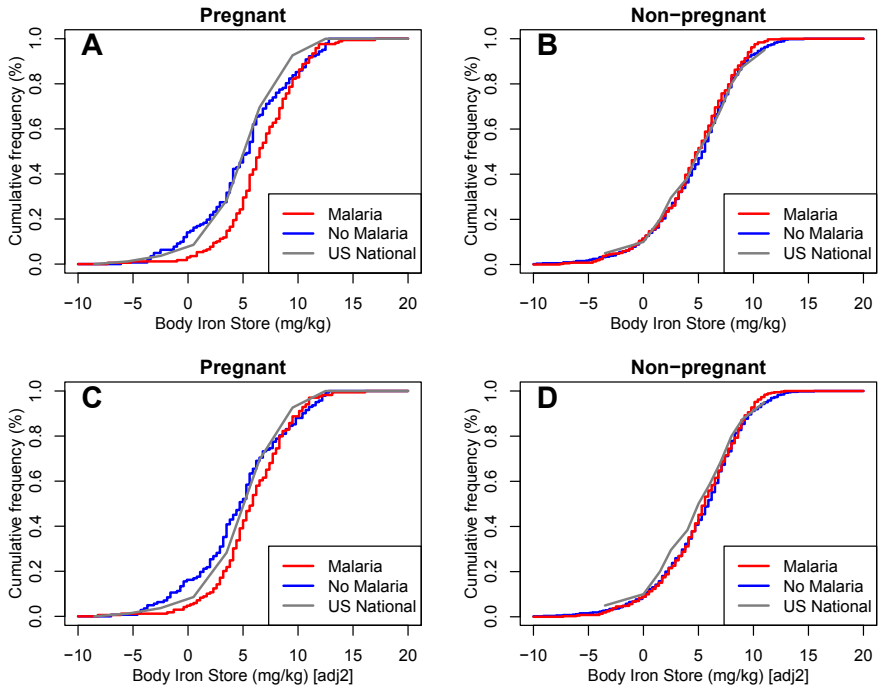

Fig. 3. Cumulative body iron distributions by CRP cut-off or presence of malaria. Cumulative frequency of body iron distribution in women in presence or absence of *P.falciparum* parasitaemia. Body iron distributions are shown using adjusted ferritin estimations. Distributions are compared to those of US women in the first trimester (n = 189) (12–49 years) in the National Health and Nutrition Examination Survey (NHANES) in the US population from 1999 to 2006 Ref. [33], and for non-pregnant women (n = 409) (20–45 years) in the NHANES III study in the US population from 1988 to 1994 as reported by Cook et al. Ref. [21]. Malaria sub-group indicates blood slide negative and positive cases. Panels A and B use a combined (pregnant and non-pregnant) regression slope correction estimate for log ferritin against log CRP. Panels C and D [adj2] use a specific correction, based on separate regression slope correction estimates for primigravidae, or nulliparae, for log ferritin against log CRP.

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In conclusion this study is to the best of our knowledge the first to examine the association of an extensive series of iron-related biomarkers in nulliparae and primigravidae in early pregnancy from the same setting, and with high malaria exposure. Low iron deficiency but high anaemia prevalence characterised both groups of women. Iron deficiency was less prevalent in early pregnancy and mean BIS was higher than in non-pregnant women which may predispose to increased malaria infection in early pregnancy. The biological associations between iron status in young women and *P.falciparum* malaria in early gestation are probably not different from other populations with equivalent malaria exposure in pregnancy. In this, or similar populations with anaemia prevalence ≥40%, WHO guidelines would recommend daily iron supplementation, although iron deficiency was uncommon and anaemia mostly attributable to infection and inflammation. The association between early gestational malaria in the first pregnancy with higher BIS may also indicate that periconceptional iron supplementation could be detrimental.

Conflict of interest

Dorine Swinkels is Medical Director of the "Hepcidinanalysis.com" initiative, which aims to serve the scientific, medical and pharmaceutical communities with high-quality hepcidin measurements (www.hepcidinanalysis.com). The remaining authors disclose they do not have any conflict of interest.

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Availability of data and materials

Until placed in a public repository, data relating to the current study can be requested from the corresponding author and will be made available following an end user data agreement and sponsor approval.

CRediT authorship contribution statement

Salou Diallo: Investigation, Methodology, Project administration, Writing - review & editing. **Stephen A. Roberts:** Data curation, Formal analysis, Validation, Writing - original draft. **Sabine Gies:** Conceptualization, Methodology, Project administration, Supervision, Validation, Writing - review & editing. **Toussaint Rouamba:** Supervision. **Dorine W. Swinkels:** Methodology, Writing - review & editing. **Anneke J. Geurts-Moespot:** Methodology. **Sayouba Ouedraogo:** Project administration, Validation. **Georges Anicet Ouedraogo:** Supervision. **Halidou Tinto:** Project administration, Resources, Supervision. **Bernard J. Brabin:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

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