**Supplementary File 1: Further study details**

**Study area and participants**

**Procedures**

**Pregnant cohort study assessments**

**Data collection and monitoring**

**Table S1: P values for Table 3 correlation coefficients in pregnant and non-pregnant women**

**Table S2: P values for a comparison of the relationships between biomarkers in pregnant and non-pregnant women in Table 3 main paper**

The study received ethical approval in Burkina Faso (Comité d’Ethique pour la Recherche en Santé), England (Liverpool School of Tropical Medicine), and Belgium (University Hospital, Antwerp). Full details of the study protocol and primary trial analyses have been published. [1, 2]

***a. Study area and participants:*** The Health and Demographic Surveillance System (HDSS) area was established in 2009. By December 2011, the HDSS recorded approximately 61 000 inhabitants, predominantly of Mossi origin. Health care was provided by twelve peripheral health centres and one referral hospital.

Individual participant information about the trial was provided by a study investigator who read this to women who were illiterate before signed consent (part 1) was obtained. Unmarried minor girls were accompanied by a legal representative who agreed to their participation. A second signed consent procedure (part 2) was instigated for participants who became pregnant during the trial, who agreed to attend for a study antenatal visit and to continue weekly follow-up visits.

***b. Procedures:*** At trial enrolment demographic data and a general history of past and present illnesses and obstetrical history including last menstrual period, age at menarche, sexual activity, use of contraceptive methods and current complaints were recorded. A study clinician completed a physical and clinical examination. Women were not recruited if they had any significant illness at the time of screening that required hospitalization, including clinical signs of severe anemia (conjunctival or mucosal pallor, tachycardia, respiratory distress), or a history or presence of major clinical disease likely to influence pregnancy outcome (sickle cell disease, diabetes mellitus, severe renal or heart disease, open tuberculosis, epilepsy, known HIV/AIDS infection). Following recruitment participants were individually randomized to one of two trial arms and received weekly one of identical red coloured vegetable cellulose (hypromellose) capsules (disintegration time less than 30 minutes, mean 9.5 minutes), containing either ferrous gluconate (60mg) and folic acid (2.8mg), or folic acid alone (2.8mg)(G and G Food Supplies Ltd, West Sussex, UK), which were provided in tamper evident opaque containers of 20 capsules. A block allocation sequence was used with randomly determined block lengths. Supplement codes, unknown to investigators and maintained independently by the sponsor, were revealed only after data base lock and completion of data analysis. Women received a card with a unique study number corresponding to the randomisation list. The dose of supplements was based on recommendations made by WHO.

***c. Pregnant cohort study assessments:*** Women who became pregnant within the 18 month follow up period and consented to enter the pregnancy cohort were referred to Nanoro hospital for a scheduled antenatal visit (standardized at about 13-16 weeks gestation according to the last menstrual period). This was termed ANC1 and was performed by one of the study nurses/doctors. The weekly supplement was withdrawn when antenatal care commenced and haematinics provided according to national policy as daily iron (60mg) and folic acid (400µg) tablets, but weekly follow-up continued. Women in the first trimester (≤13 weeks gestation), if malaria positive, were treated with oral quinine. Severely anaemic pregnant (Hb <7g/dL) women were referred to Nanoro hospital.

***d. Data collection and monitoring:*** The study was implemented according to the approved protocol and study specific study operating procedures (SOPs). Questionnaire data was entered directly into an electronic Case Report Form (CRF) on MACRO (InferMed, UK), Good Clinical Practice (GCP) compliant software for clinical trials. Weekly follow-up visits were recorded on electronic questionnaires using Personal Digital Assistant (PDA) handheld devices with in-built consistency checks. PDA data was uploaded weekly onto Macro. An external independent trial monitor from the Institute of Tropical Medicine in Antwerp assessed SOP adherence and reported to the Sponsor on GCP compliance and trial conduct on three occasions. An internal monitor not involved in this trial, verified on a continuous basis that the rights and well-being of human subjects were protected and that the trial was conducted in compliance with the approved protocol on a six monthly basis. A Data Safety Monitoring Board met four times during the course of the trial. Adverse outcomes were reported in reference [2].

**References**

[1] Gies S, Diallo S, Roberts SA, Kazienga A, Powney M, Brabin L, Ouedraogo S, Swinkels DW, Geurts-Moespot AJ, Claeys Y, D'Alessandro U et al. Effects of weekly iron and folic acid supplements on malaria risk in nulliparous women in Burkina Faso: A periconceptional double-blind randomized non-inferiority trial. J Infect Dis. 2018. doi: 10.1093/infdis/jiy257.

[2] Brabin L, Roberts SA, Gies S, et al. Effects of long-term weekly iron and folic acid supplementation on lower genital tract infection—a double blind, randomised controlled trial in Burkina Faso. BMC Med 2017; 15:206.

An Abstract summarising this analysis was presented at 10th The European Congress of Tropical Medicine and International Health, Antwerp, Belgium, 10-16 October, 2017.

 **Table S1: P values for Table 3 correlation coefficients in pregnant and non-pregnant women.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Ferritin\* | Adjusted ferritin | sTfR\* | ZnPP\* | Hepcidin\* | sTfR/log10Fer\* | Hb | RDW | MCHC | MCV | AdjustedBIS | CRP |
| **Pregnant** |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Ferritin\* | - |  |  |  |  |  |  |  |  |  |  |  |
| sTfR\* | 0.362 | 0.040 | - |  |  |  |  |  |  |  |  |  |
| ZnPP\* | 0.761 | 0.249 | <0.001 | - |  |  |  |  |  |  |  |  |
| Hepcidin\* | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |  |  |
| sTfR/log10Fer\* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |  |
| Hb | <0.001 | 0.022 | <0.001 | <0.001 | 0.026 | <0.001 | - |  |  |  |  |  |
| RDW | 0.797 | 0.294 | <0.001 | <0.001 | 0.001 | <0.001 | <0.001 | - |  |  |  |  |
| MCHC | 0.955 | 0.339 | <0.001 | 0.029 | 0.071 | <0.001 | <0.001 | <0.001 | - |  |  |  |
| MCV | <0.001 | <0.001 | 0.014 | 0.636 | 0.773 | 0.956 | 0.006 | 0.171 | <0.001 | - |  |  |
| Adjusted BIS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.461 | <0.001 | 0.012 | 0.011 | - |  |
| CRP\* | <0.001 | 0.090 | <0.001 | <0.001 | 0.029 | 0.932 | <0.001 | 0.006 | <0.001 | 0.016 | 0.96 | - |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Non-pregnant** |  |  |  |  |  |  |  |  |  |  |  |  |
| Ferritin\* | - |  |  |  |  |  |  |  |  |  |  |  |
| sTfR\* | <0.001 | <0.001 | - |  |  |  |  |  |  |  |  |  |
| ZnPP\* | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |  |  |  |
| Hepcidin\* | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |  |  |
| sTfR/log10Fer\* | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |  |
| Hb | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |  |
| RDW | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |  |
| MCHC | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |  |  |
| MCV | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.013 | - |  |  |
| Adjusted BIS | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - |  |
| CRP\* | <0.001 | 0.003 | 0.002 | <0.001 | <0.001 | 0.982 | 0.043 | 0.208 | 0.060 | 0.113 | <0.001 | - |

 sTfR:serum transferrin receptor; sTfR/log10Fer: sTfR/log10ferritin 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.

**Table S2: P values for a comparison of the relationships between biomarkers in pregnant and non-pregnant women in Table 3**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Ferritin\* | Adjusted ferritin | sTfR\* | ZnPP\* | Hepcidin\* | sTfR/log10Fer\* | Hb | RDW | MCHC | MCV | Adjusted BIS | CRP\* |
|  |  |  |  |  |  |  |  |  |  |  |  |  |
| Ferritin\* | - | - | <0.001 | <0.001 | 0.961 | 0.657 | <0.001 | <0.001 | <0.001 | 0.501 | 0.005 | <0.001 |
| Adjusted ferritin | - | - | <0.001 | <0.001 | 0.954 | 0.625 | <0.001 | <0.001 | <0.001 | 0.126 | 0.009 | 0.002 |
| sTfR\* | <0.001 | <0.001 | - | 0.906 | 0.283 | 0.011 | 0.027 | 0.281 | 0.004 | <0.001 | 0.009 | 0.108 |
| ZnPP\* | <0.001 | <0.001 | <0.001 | - | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.072 |
| Hepcidin\* | 0.257 | 0.809 | 0.636 | 0.137 | - | 0.078 | <0.001 | 0.046 | <0.001 | <0.001 | 0.051 | 0.983 |
| sTfR/log10Fer\* | <0.001 | <0.001 | <0.001 | 0.007 | 0.074 | - | <0.001 | 0.001 | <0.001 | <0.001 | <0.001 | 0.936 |
| Hb | <0.001 | <0.001 | 0.518 | <0.001 | <0.001 | <0.001 | - | 0.639 | <0.001 | <0.001 | <0.001 | <0.001 |
| RDW | <0.001 | <0.001 | 0.060 | 0.764 | 0.006 | 0.011 | 0.004 | - | 0.667 | <0.001 | <0.001 | 0.101 |
| MCHC | <0.001 | <0.001 | 0.672 | 0.002 | 0.009 | 0.119 | <0.001 | <0.001 | - | <0.001 | 0.001 | 0.006 |
| MCV | 0.939 | 0.62 | <0.001 | <0.001 | 0.003 | <0.001 | <0.001 | <0.001 | <0.001 | - | 0.01 | 0.002 |
| Adjusted BIS | <0.001 | <0.001 | <0.001 | <0.001 | 0.588 | 0.798 | <0.001 | <0.001 | <0.001 | <0.001 | - | 0.053 |
| CRP\* | 0.005 | 0.002 | 0.089 | <0.001 | 0.881 | 0.930 | <0.001 | 0.063 | 0.083 | 0.003 | 0.068 | - |

Interaction test comparing slopes of regression lines between pregnant and non-pregnant groups. Note this table is not symmetric and the slopes compared are by row

against column, with upper triangle X against Y and lower triangle Y against X

Asterisk: log 10 transformed