Impact of Small-Quantity Lipid-Based Nutrient Supplement on Hemoglobin, Iron Status and Biomarkers of Inflammation in Pregnant Ghanaian Women[1-7]

Seth Adu-Afarwuah*, Anna Lartey*, Harriet Okronipa*, Per Ashorn[†], Mamane Zeilani[‡], Lacey M. Baldiviez[§], Brietta M. Oaks[§], Stephen Vosti[§], Kathryn G. Dewey[§]

*Department of Nutrition and Food Science, University of Ghana, Legon, Accra, Ghana; [†]University of Tampere School of Medicine, Department for International Health, Tampere, Finland; [‡]Nutriset S.A.S., Hameau du Bois Ricard, PB 35, 76770 Malaunay, France; [§]Program in International and Community Nutrition, Department of Nutrition, University of California, Davis, CA, USA

[4]Address correspondence to S Adu-Afarwuah, Department of Nutrition and Food Science, University of Ghana, Legon, Accra, Ghana. Phone: +233 24 914 9385. Email: ct3665@gmail.com. Reprints are not available.

[5]Abbreviations: AGP, Alpha-1 glycoprotein; CRP, C-reactive protein; EFA, Essential Fatty Acid; GA, Gestational age; GW, Gestational weeks; IDA, Iron Deficiency Anemia; IFA, Iron and folic acid; ; LNS, Lipid-based Nutrient Supplement; MMN, Multiple Micronutrients; SQ-LNS, Small-quantity lipid-based nutrient supplement; TfR, Transferrin receptor; ZPP, Zinc Protoporphyrin.

Contributor statement

SA-A, AL, PA, MZ, SV, and KGD designed the research; MZ was responsible for the development and production of the LNS used in the study based on the specifications agreed

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1 **Abstract**

2 We examined hemoglobin (Hb, g/L), iron status (zinc protoporphyrin, ZPP, μmol/mol heme, and

3 transferrin receptor, TfR, mg/L), and inflammation (C-reactive protein, CRP and alpha-1

4 glycoprotein, AGP) in pregnant Ghanaian women who participated in a randomized controlled

5 trial. Women (n=1320) received either 60 mg Fe + 400 μg folic acid (IFA); 18 micronutrients

6 including 20 mg Fe (MMN); or small-quantity lipid-based nutrient supplements (SQ-LNS, 118

7 kcal/d) with the same micronutrient levels as in MMN, plus 4 additional minerals (LNS) daily

8 during pregnancy. Intention-to-treat analysis included 349, 354, and 354 women in the IFA,

9 MMN and LNS groups, respectively, with overall baseline mean Hb and anemia (Hb <100)

10 prevalence of 112 and 13.3%, respectively. At 36 gestational weeks, overall Hb was 117 and

11 anemia prevalence was 5.3%. Compared with the IFA group, the LNS and MMN groups had

12 lower mean Hb (120±11 vs 115±12 and 117±12, respectively; p<0.001), higher mean ZPP

13 (42±30 vs 50±29 and 49 ±30; p=0.010) and TfR (4.0±1.3 vs 4.9±1.8 and 4.6±1.7; p<0.001), and

14 greater prevalence of anemia (2.2% vs 7.9% and 5.8%; p=0.019), elevated ZPP (>60) [9.4% vs

15 18.6% and 19.2%; p=0.003] and elevated TfR (>6.0) [9.0% vs 19.2% and 15.1%; p=0.004]. CRP

16 and AGP concentrations did not differ among groups. We conclude that among pregnant women

17 in a semi-urban setting in Ghana, supplementation with SQ-LNS or MMN containing 20 mg

18 iron resulted in lower Hb and iron status but had no impact on inflammation, when compared

19 with iron (60 mg) plus folic acid (400 μg). The amount of iron in such supplements that is most

20 effective for improving both maternal Hb/iron status and birth outcomes requires further

21 evaluation. This trial was registered at ClinicalTrials.gov as: NCT00970866.

22

25 **Introduction**

26 Poor nutrient intake during pregnancy has been associated with several adverse

27 consequences. It is estimated that up to 50% of the anemia among pregnant women in many

28 developing country settings is due to iron deficiency (van den Broek et al., 1998) usually as a

29 result of low dietary iron intake (World Health Organization, 1992) and poor iron bioavailability

30 due to over-reliance on plant-based diets high in inhibitors of iron absorption such as phytate

31 (Tatala et al., 1998). Consequences of anemia include reduced work capacity and increased risk

32 of mortality for the mother, and premature delivery, low birth weight and poor mental

33 development for the infant (Ren et al., 2007, International Anemia Consultative Group (INACG),

34 2002). While normal pregnancy is found to be associated with an increased inflammatory

35 response (Picklesimer et al., 2008, Sacks et al., 1998), this response may be modified by macro-

36 or micro-nutrients (Roberts et al., 2003). Higher intakes of folic acid (Bertran et al., 2005) and

37 vitamin B_6 (Friso et al., 2001) have been associated with lower concentration of C-reactive

38 protein (CRP), a common biomarker of inflammation. Several other dietary factors including

39 essential fatty acids, EFAs (Rallidis et al., 2003) and antioxidants (Brighenti et al., 2005, Devaraj

40 and Jialal, 2000) are also associated with the reduction of CRP concentration. Elevated CRP

41 concentration in pregnancy is related to the development of pre-eclampsia and preterm delivery

42 (Elovitz, 2006).

43 A major recommendation for increasing nutrient intake among pregnant women in

44 developing countries is the one developed by WHO (WHO), which is the consumption of

45 iron/folic acid (IFA) supplements containing 30-60 mg iron and 400 µg folic acid. In meta-

46 analyses, this strategy, compared with no iron or placebo, reduced the risk of maternal anemia by

47 69-70 % (Imdad and Bhutta, 2012, Pena-Rosas et al., 2012) and iron deficiency by 57% (Pena-

48   Rosas et al., 2012), but appeared to increase the risk of reported side effects (relative risk (RR) =

49   2.36; 95% CI: 0.96 -5.82) particularly at iron doses of 60 mg or higher (Pena-Rosas et al., 2012).

50   The WHO/UNICEF/UNU UNIMMAP (United Nations International Multiple Micronutrient

51   Preparation) formulation containing 15 vitamins and minerals was more recently developed

52   (UNICEF/WHO/UNU) to combat other possible deficiencies, e.g. for vitamins A, C and B_{12},

53   which may also contribute to anemia (World Health Organization, 1992). The dose of iron in the

54   UNIMMAP was set at 30 mg (below 60 mg) for the following reasons: (a) the presence of

55   vitamins A, B_2 and C in the UNIMMAP would enhance the absorption and utilization of iron,

56   and therefore the lower amount of iron should be sufficient, (b) a lower iron dose would be

57   associated with less negative side effects and therefore better adherence, (c) including 60 mg of

58   iron would mean including at least 30 mg of zinc (to avoid possible negative influence of iron on

59   zinc absorption), bringing the total amount of metals to 90 mg, which is likely to increase

60   negative side effects, and (d) UNIMMAP may be used in conjunction with additional iron /folic

61   acid tablets in individual cases of more severe anemia (assuming it is caused by iron deficiency).

62   Meta-analyses suggested that supplementation with UNIMMAP and similar products (mostly

63   containing 11 or more micronutrients including 30 mg iron) had the same effect on maternal

64   hemoglobin and iron status as iron (usually at 60 mg dose) with or without folic acid (Allen and

65   Peerson, 2009, Haider et al., 2011), while also reducing the risk of low birth weight (Fall et al.,

66   2009, Haider and Bhutta, 2012, Ramakrishnan et al., 2012).

67   Our group developed small quantity (20 g/d) lipid-based nutrient supplements (SQ-LNS)

68   for pregnant and lactating women (Arimond et al., 2013) to provide micronutrients together with

69   EFAs, using a minimum food base that supplies a small amount of energy (118 kcal/d) and high

70   quality protein (2.6 g/d). In many populations, total energy intake among pregnant and lactating

71   women may be adequate, but the EFA content of the usual diet may be low (Michaelsen et al.,

72   2011). The micronutrient composition of the SQ-LNS was generally based on the UNIMMAP

73   formulation and a similar product used in Guinea Bissau (Kaestel et al., 2005), but we further

74   reduced the daily iron dose to 20 mg, based on evidence that 20 mg day^{-1} may be an adequate

75   dose to prevent iron deficiency anemia during pregnancy (even for women who are iron deficient

76   at entry to prenatal care) and causes fewer gastrointestinal side effects, compared to higher doses

77   of iron (Zhou et al., 2009). We estimated (Arimond et al., 2013) that in addition to iron coming

78   from the usual diet, the 20 mg of iron from a daily supplement would meet the recommended

79   dietary allowance (RDA) of 27 mg iron during pregnancy (and be close to the 30 mg/d dose in

80   the UNIMMAP formulation) while not greatly exceeding the RDA (9 mg/d) for iron during

81   lactation (IOM, 2001, Arimond et al., 2013).

82   Currently, there is a growing interest in the potential use of Small-Quantity Lipid-based

83   Nutrient Supplements (SQ-LNS) among pregnant women in developing-country settings

84   (Hambidge et al., 2014, Research Engagement on Food Innovation for Nutritional Effectiveness

85   (REFINE), 2013), because of evidence suggesting a positive impact of the product on certain

86   pregnancy outcomes (Adu-Afarwuah et al., 2015). However, little is known about the impact of

87   SQ-LNS on maternal outcomes such as anemia, iron status and inflammation. We previously

88   reported (Adu-Afarwuah et al., 2015), that compared to IFA and a multiple micronutrient

89   (MMN) capsule with most of the same micronutrients as the SQ-LNS, the SQ-LNS promoted

90   fetal growth in vulnerable women, particularly primiparas, whilst the occurrence of serious

91   adverse events did not differ between the 3 groups. In the current analysis, we compare the effect

92   of the 3 supplementation regimens (IFA, MMN and SQ-LNS), on maternal hemoglobin (Hb),

93   iron status, and two biomarkers of inflammation (CRP and alpha-1 glycoprotein, AGP) during

94   pregnancy.

Methods

Study setting, design, participants and blinding

The iLiNS DYAD study in Ghana was conducted in several adjoining semi-urban communities in the Yilo Krobo and the Lower Manya Krobo Districts about 70 km north of Accra, Ghana. Details of the study setting, participants, design, randomization and masking schemes, and other key procedures have been reported elsewhere (Adu-Afarwuah et al., 2015). In brief, the study was designed as a partially double-blind, parallel, individually randomized, controlled trial with three equal-size groups. Pregnant women attending usual ante-natal clinics in four main health facilities in the area between December 2009 and December 2011 completed a screening questionnaire if they were ≥18 years old, ≤20 weeks gestation (as determined by the antenatal clinics mostly by fundal height), and had an antenatal card complete with history and examination. Informed consent for the screening was obtained by trained study workers at the antenatal clinics. Following screening, women were excluded if the antenatal card indicated HIV infection, asthma, epilepsy, tuberculosis or any malignancy. Additional exclusion criteria were known milk or peanut allergy, not residing in the area, intention to move within the next two years, unwillingness to receive field workers or take study supplement, participation in another trial, or gestational age (GA) >20 weeks before completion of the enrolment process.

Women who passed the screening were visited in their homes, where details of the study were provided, and those willing to participate were recruited, after signing or thumb-printing informed consent. Recruited women remaining eligible underwent a baseline laboratory assessment after consent, and were immediately randomized to receive one of three treatments daily: (a) 60 mg iron plus 400 µg folic acid (hereafter, IFA supplement or group); (b) multiple micronutrient capsule containing 18 vitamins and minerals (including 20 mg iron) (hereafter,

118 MMN supplement or group); and (c) SQ-LNS with similar micronutrients as the MMN

119 supplement, plus other minerals and macronutrients (hereafter, LNS supplement or group).

120 Group allocations were developed by the Study Statistician at UC Davis using a computer-

121 generated (SAS version 9.3) randomization scheme (in blocks of nine), and were placed in

122 sealed, opaque envelopes. At each enrolment, a Study Nurse offered nine envelopes at a time,

123 and the woman picked one to reveal the allocation. Allocation information was kept securely by

124 the Field Supervisor and the Study Statistician only.

125   The compositions of the 3 supplements were reported previously (Adu-Afarwuah et al.,

126 2015), as well as the considerations underlying the concentrations of the nutrients in the MMN

127 and SQ-LNS (Arimond et al., 2013). Apart from iron which was kept at 20 mg/day in the MMN

128 and SQ-LNS, the vitamin and mineral contents were either 1x or 2x the RDA for pregnancy, or

129 in a few cases, the maximum amount that could be included in the supplement given technical

130 and organoleptic constraints. The IFA and MMN supplements were provided as capsules in

131 blister packs, and were intended to be consumed with water after a meal, one capsule per day

132 throughout pregnancy. The LNS supplement was in 20-g sachets, and was intended to be mixed

133 with any prepared food, one sachet per day throughout pregnancy. To maintain blinding, two

134 individuals independent of the study placed color-coded stickers behind the blister packs (three

135 different colors for IFA and three for MMN supplements) so that the capsules were known to the

136 study team and participants only by the colors of the stickers. Laboratory staff and data analysts

137 had no knowledge of group assignment until all preliminary analyses had been completed and

138 the allocation codes were broken. The study was registered on ClinicalTrials.gov (Identifier:

139 NCT00970866) and was approved by ethics committees of the University of California, Davis,

140   the Ghana Health Service, and the University of Ghana Noguchi Memorial Institute for Medical

141   Research.

142

143   **Procedures**

144   We collected socio-demographic information at baseline, and determined GA mostly by

145   ultrasound biometry (Aloka SSD 500, Tokyo, Japan). During follow-up, field workers visited

146   women in their homes every two weeks, whereupon they delivered a fresh supply of supplement

147   and monitored supplement intakes. At each of laboratory assessments at baseline and at 36 GW,

148   women's weight (Seca 874) and height (Seca 217) were measured, and peripheral malaria

149   parasitemia (Clearview Malarial Combo, Vision Biotech, South Africa), hemoglobin, Hb

150   (HemoCue AG, Wetzikon, Switzerland) and zinc protoporphyrin, ZPP (hematofluorometer, Aviv

151   Biomedical Co. NJ, USA), were determined using venous blood (Adu-Afarwuah et al., 2015).

152   We used the original Aviv cover-slides and 3-level control material for the ZPP measurements,

153   after red blood cells were washed three times with normal saline. Plasma samples obtained after

154   blood was centrifuged at 1,252 x g for 15 min were stored in Ghana at -20 $^{\circ}$C, before being air-

155   freighted on dry ice to UC Davis, where soluble transferrin receptor (TfR, mg/L), C-reactive

156   protein (CRP, mg/L), and alpha-1 glycoprotein (AGP, g/L) concentrations were determined

157   using a Cobas Integra 400 plus Automatic Analyzer (Roche Diagnostic Corp., Indianapolis, IN).

158   At 36 GW, the continuous outcomes measures were Hb (g/L), ZPP (µmol/mol heme),

159   and plasma TfR (mg/L), CRP (mg/L), and AGP (g/L) concentrations, whilst the binary outcome

160   measures were the percentages of women with low Hb, high Hb, and elevated ZPP, TfR, CRP

161   and AGP.

162

163   **Sample size and data analysis**

164   For the Ghana iLiNS-DYAD Study, an effect size (Cohen's d: difference between group

165   means divided by the pooled standard deviation) of 0.3 (considered a small-to-moderate effect

166   size) (Cohen) was the basis for sample size calculation. Thus, our sample size was based on

167   detecting an effect size of 0.3 between any two groups for any continuous variable at 36 GW,

168   with a two-sided 5% test and 80% power. As described previously (Adu-Afarwuah et al., 2015),

169   we enrolled 1320 pregnant women into the study, but after excluding 177 who received both IFA

170   and MMN supplements during pregnancy because of a temporary mislabeling of supplements, as

171   well as 86 in the LNS group who were pregnant during the same time period, 1,057 women were

172   included in the current analysis. Based on a sample size of 827 women (~275 per group) for

173   whom data were available at 36 GW, we had 94% power to detect an effect size of 0.3 between

174   any two groups for hemoglobin, ZPP, or TfR. This would allow a difference of 3.4 g/L in Hb, 8.9

175   μmol/mol heme in ZPP, and 0.5 mg/L in TfR (given SD of 11.0, 30.0, and 2.0, respectively) to

176   be detected between any 2 groups.

177   We posted the statistical analysis plan (www.ilins.org) before analysis. Statistical

178   analysis, by intention-to-treat, was performed using SAS for Windows Release 9.3 (Cary, NC,

179   USA). Background socio-demographic characteristics were summarized as mean ± SD for

180   continuous variables, or number of participants and percentages for categorical variables. As

181   done previously (Adu-Afarwuah et al., 2015), we used 2 indices, namely assets index and

182   housing index as proxy indictors for socioeconomic status, and calculated household food

183   insecurity access (HFIA) score (Coates et al., 2007) as a measure of degree of household food

184   insecurity. Higher values of the assets and housing indices represented higher socioeconomic

185   status, and higher values of the food insecurity index represented higher food insecurity.

186   We calculated adherence to treatment as percentage of days from enrolment to the home

187   visit closest to the laboratory assessment at 36 GW, when women reported consuming the

188   supplement. We used Hb <100 g/L as our primary definition for low Hb (representing anemia).

189   This was based on previous WHO (WHO, 2007, WHO/UNICEF/UNU, 2001) and International

190   Nutritional Anemia Consultative Group, INACG (Nestel and INACG Steering Committee, 2002)

191   documents that suggest lowering the standard 110 g/L cut-off by 10 g/L for pregnant women of

192   African extraction to achieve adequate sensitivity and specificity for screening purposes

193   (WHO/UNICEF/UNU, 2001). In addition, we defined low Hb using the standard cut-off of Hb

194   <110 g/L, based on a recent WHO recommendation (WHO, 2011) to maintain that cut-off (110

195   g/L) without any adjustment, because of scarce evidence to support the adjustment. A meta-

196   analysis (Haider et al., 2013) revealed that Hb cut-offs ranging from <100 g/L to 115 g/L have

197   been used in studies to define anemia in pregnant women. We defined high Hb as >130 g/L

198   (Pena-Rosas et al., 2012), elevated ZPP (proxy for iron deficiency) as >60 μmol/mol heme

199   (Walsh et al., 2011) and elevated TfR (proxy for tissue iron deficiency) as > 6.0 mg/L (Pfeiffer et

200   al., 2007, Vandevijvere et al., 2013). Because there is no generally accepted cut-off value for

201   TfR, we derived the 6.0 mg/L cut-off based on the evidence that TfR values obtained using the

202   Automatic Analyzer assay (as used in this study) were on average 30% lower than values

203   obtained with the ELISA assay (Pfeiffer et al., 2007). Therefore, we reduced by 30% the 8.5

204   mg/L cut-off value used when TfR was determined using ELISA (Vandevijvere et al., 2013) to

205   obtain the cut-off of approximately 6.0 mg/L for our analysis. Because we used 2 cut-offs to

206   define anemia, we also defined iron deficiency anemia (IDA) in two ways: first as Hb <100 g/L

207   and at least one marker of iron deficiency (ZPP > 60 (μmol/mol heme) or TfR >6.0 mg/L

208   (Pfeiffer et al., 2007, Vandevijvere et al., 2013)), and second, as Hb <110 g/L and at least one

209 marker of iron deficiency. We defined elevated inflammatory markers using the cut-off values of

210 >5.0 mg/L for CRP and >1.0 g/L for AGP (Thurnham and McCabe), and categorized women

211 with inflammation as either elevated CRP only (indicative of incubation phase of infection),

212 elevated CRP and AGP (indicative of early convalescence) or elevated AGP only (indicative of

213 late convalescence) (Thurnham and McCabe).

214   At 36 GW, we calculated overall mean (±SD) values and percentages for Hb and markers

215 of iron status and inflammation. We compared groups by using general linear models

216 (continuous outcomes) and logistic regression models (binary), with Tukey-Kramer adjustment

217 for multiple comparisons. Along with the group comparisons, we calculated pairwise mean

218 differences (continuous outcomes, ANOVA) and relative risks (binary outcomes, Logistic

219 regression) with their 95% CI and p-values. Relative risks were calculated using Poisson

220 regression (Spiegelman and Hertzmark, 2005). In addition, we analyzed changes in the

221 prevalence of anemia, high Hb, and elevated ZPP, TfR, CRP and AGP from enrolment using

222 mixed model logistic regression (SAS PROC GLIMMIX). Where the mixed model logistic

223 regression failed to converge because of sparse data, we used generalized estimating equations

224 model (SAS PROC GENMOD). We analyzed each outcome twice, first without any covariate

225 adjustments, and then with adjustment for covariates significantly associated ($p < 0.10$) with the

226 outcome in a bivariate analysis. Because ZPP, TfR, AGP and CRP are not normally distributed,

227 we calculated the group means (± SD or SE), group percentages, and pair-wise mean differences

228 and relative risks with their 95% CI based on untransformed data, but generated the p-values for

229 group or pair-wise comparisons using logarithmically transformed data.

230   To investigate the possible effect of group differences in adherence to treatment, we

231 performed a per-protocol analysis, which was restricted to women with adherence ≥ 70%. We

232   evaluated potential interaction of treatment group with pre-specified baseline variables for

233   maternal characteristics, anemia and iron status. These variables were: age, years of schooling,

234   BMI, gestational age at enrolment, household assets index, housing index, food insecurity access

235   score, season at enrolment (dry or wet), primiparous, anemia, and elevated ZPP, TfR, and AGP

236   or CRP. Where an interaction was significant (alpha <0.10), we performed subgroup analysis by

237   including an interaction term between treatment and the effect modifier in the ANCOVA or

238   logistic regression model. For continuous effect modifiers, we used data from all participants to

239   create a linear regression model to predict the values of the outcome at the 10th and 90th

240   percentile of the effect modifier distribution. Each effect modifier was considered separately in

241   the models to avoid collinearity.

242   In a sensitivity analysis aimed at correcting for the effect of inflammation (CRP and

243   AGP) on the Hb and iron status outcomes, we repeated the above analyses using values of Hb

244   and iron status markers corrected for inflammation (WHO, 2007). These corrected values were

245   calculated by grouping women into 3 inflammation categories, estimating the correction factor

246   (CF) for each inflammation category, and multiplying the Hb and iron status values of each

247   woman by the inflammation category-specific CF (Grant et al., 2012). The 3 inflammation

248   categories were: reference (normal CRP and AGP), incubation (raised CRP and normal AGP),

249   and early (raised CRP and AGP) or late (normal CRP and raised AGP) convalescence [these two

250   phases of convalescence were combined because of small sample sizes and little indication of

251   differences]. For ZPP at 36 GW, women were grouped into 2 inflammation categories (normal

252   versus any inflammation), since the 3-category grouping did not yield consistent results.

253

254 **Results**

255   We collected data from December 2009 to August 2012. The study flow diagram, as well

256 as the main reasons women were not eligible, or were eligible but not enrolled, were reported

257 elsewhere (Adu-Afarwuah et al., 2015). Among eligible women, those enrolled (n=1320) and

258 those not enrolled (n=606) did not differ in most background characteristics (results not shown).

259 The background characteristics of the 1057 women included in the current analysis are presented

260 in **Table 1**. These characteristics were well-balanced across the three groups. On average,

261 women were about 26 years of age, had about 7 years of formal education, and BMI of about 25

262 kg/m^3. Nearly all of the women said they were married or living with a partner, slightly more

263 than a third were primiparous, and nearly 10% tested positive for malaria. The average GA at

264 enrolment was 16 weeks. At baseline, we obtained Hb values for all 1057 women, ZPP values

265 for 1055 women, and TfR, CRP and AGP values for 1032 women.

266   At 36 GW, dropout (4.4%) was low, and did not differ among groups (p = 0.65). Women

267 who dropped out – mainly because of miscarriage (2.8%) and movement from the study site

268 (1.2%) –did not differ in the baseline characteristics from those who were present at 36 GW,

269 except for GA (weeks) at enrolment, which was significantly lower (p = 0.001) for the former

270 (14.5) than for the latter (16.3). Mean (±SD) adherence (% of days from enrolment to the home

271 visit closest to the laboratory assessment at 36 GW when supplement was reportedly consumed)

272 was lower (p=0.001) in the LNS group (68 ± 24) compared to the IFA (74 ± 21) and the MMN

273 (72 ±23) groups. Women usually reported mixing the LNS supplement with porridge, but

274 sometimes mixed it with other foods including soups and stews, or consumed it alone. We

275 obtained Hb values for 827 women, and ZPP, TfR, CRP and AGP values for 822 women; the

276 number (%) of women without Hb values did not differ between groups (p = 0.10). The women

277   with Hb values did not differ from those without Hb values in most of the baseline

278   characteristics, except that the latter had lower mean housing index (-0.17 ± 1.09 vs. 0.04 ± 0.99;

279   p = 0.011), BMI (24 ± 4.0 vs. 25 ± 4.7 kg/m^2; p = 0.022) and GA at enrolment (16 ± 3.1 vs. 16 ±

280   3.3 weeks; p = 0.042).

281   In the intention-to-treat analysis, the overall mean (±SD) values at baseline and 36 GW

282   were 112± 12 and 117 ± 12, respectively, for Hb (g/L), 45 ± 32 and 47 ± 30 for ZPP (μmol/mol

283   heme) and 4.1 ± 2.5 and 4.5 ± 1.7 for TfR (mg/L). From baseline to 36 GW, the prevalence of

284   anemia decreased significantly from 13% to 5.3% (p < 0.001), whilst the reverse was true for the

285   prevalence of high Hb (4.9% vs 12%, p < 0.001) and elevated TfR (9.2% to 15%; p = 0.001),

286   with a moderate change in the prevalence of elevated ZPP (13% vs. 16%; p = 0.06). The mean

287   (±SD) CRP (mg/L) and AGP (g/L) at baseline (6.9 ± 11 and 0.6 ± 0.2, respectively) were slightly

288   greater than at 36 GW (5.7 ± 17 and 0.5 ± 0.2, respectively), which was also reflected in the

289   percentages of women with elevated CRP and AGP at baseline vs. 36 GW (38.2% vs 24.1%; p <

290   0.001 and 6.3% vs 2.8%; p < 0.001, respectively).

291

292   **Main group comparisons at 36 gestational weeks**

293   **Table 2** shows the unadjusted mean (± SD) Hb (g/L), ZPP (μmol/mol heme), TfR (mg/L),

294   CRP (mg/L) and AGP (g/L) concentrations, by intervention group, at baseline and 36 GW in the

295   intention-to-treat analysis. Baseline values did not differ significantly among groups. At 36 GW,

296   mean Hb was significantly (p < 0.001) greater in the IFA group (120 ±11) than in the LNS

297   (115±12) or MMN (117±12) group; ZPP was significantly (p <0.001) lower in the IFA group (43

298   ± 30) than in the LNS (50± 29) or MMN (49 ± 30) group; and TfR was significantly (p < 0.001)

299   lower in the IFA group (4.0 ± 1.3) than in the LNS (4.9±1.8) or MMN (4.6±1.7) group. Further

300   (**Table 3**), the percentage of women with anemia defined either as Hb <100 g/L or Hb <110 g/L

301   was significantly lower in the IFA group compared with the LNS and MMN groups, and when

302   using the latter definition, this percentage was also significantly greater in the LNS compared

303   with the MMN group. Compared with the IFA group, the LNS and MMN groups had greater

304   percentages of women with elevated ZPP (9.4% vs 19% and 19%, respectively; p = 0.003) and

305   elevated TfR (9.0% vs 19% and 15 %, respectively; p = 0.004). Differences among the 3 groups

306   in the prevalence of IDA were marginally significant (p = 0.07) when the Hb cut-off of 100 g/L

307   was used in the definition of IDA, but were significant (p <0.001) when the Hb cut-off of 110

308   g/L was used. In the latter situation, the risk of IDA was significantly greater in the LNS group

309   compared with the IFA group (p < 0.001), and marginally greater in the MMN compared to the

310   IFA group, and in the LNS compared with the MMN group. The prevalence of high Hb did not

311   differ among groups (p = 0.15).

312   From baseline to 36 GW, the decrease in the prevalence of anemia (based on our primary

313   definition of Hb <100 g/L) and increase in the prevalence of high Hb were significant for all

314   groups; the change in prevalence over time differed between groups for anemia (*P*-interaction =

315   0.099) but not for high Hb (*P*-interaction = 0.95). For elevated ZPP, the increase in prevalence

316   from baseline was marginally significant in the LNS group (p = 0.06) and non-significant in the

317   other two groups, and the change in prevalence over time did not differ between groups (*P*-

318   interaction = 0.14). For elevated TfR, the increase in prevalence was significant for the LNS (p =

319   0.003) and MMN (p = 0.009) groups only, and the change in prevalence over time did not differ

320   between groups (*P*-interaction = 0.17). Apart from the prevalence of anemia defined using the

321   Hb cut-off of 110 g/L, the LNS and MMN groups did not differ significantly in any of the

322   continuous (Table 2) or binary (Table 3) Hb and iron status outcomes.

323   There were no significant differences among groups in 36 GW mean (±SD) concentrations

324   of CRP (p = 0.98) and AGP (p = 0.35) (Table 2), or the percentages of women in the incubation

325   phase of infection (elevated CRP only; p = 0.26), early convalescence (both CRP and AGP

326   elevated; p = 0.67) or late convalescence (elevated AGP only; p = 1.00). From baseline to 36

327   GW, the decrease in the percentage of women in the incubation phase of infection was

328   significant for all groups. For percentage of women in early convalescence, only the decrease in

329   the IFA group (p = 0.010) was significant, and for percentage of women in late convalescence

330   (where the mixed model logistic regression did not converge because of sparse data, and hence

331   generalized estimating equations model was used), only the decrease in the MMN group (p =

332   0.024) was significant.

333   Adjustments by covariates significantly associated with Hb and the iron status (ZPP and

334   TfR) and inflammatory (CRP and AGP) outcomes (including the baseline value for each

335   outcome) did not alter the unadjusted results (data not shown). In addition, correcting the Hb and

336   iron status values for inflammation in the sensitivity analysis (results not shown) did not change

337   the above findings.

338   The per-protocol analysis (results not shown) revealed that among women with adherence

339   to treatment ≥ 70% (samples sizes at 36 gestational weeks: 213 in IFA, 222 in MMN and 166 in

340   the LNS group, for Hb), the above findings remained unchanged, except for the fact that the

341   prevalence of anemia (defined as Hb < 100 g/L or < 110 g/L) did not differ between the LNS and

342   MMN groups, and the prevalence of IDA (if definition included Hb < 110 g/L) was greater only

343   in the LNS compared with the IFA group.

344

345   **Effect modification**

346   Interactions of treatment group with BMI, season of enrolment and elevated CRP

347 concentration at baseline were not significant for any of the outcomes. As shown in **Table 4**, the

348 effect of intervention on ZPP concentration at 36 GW was modified by baseline anemia (Hb <

349 100 g/L; *P*-interaction = 0.099), elevated ZPP (*P*-interaction = 0.013) and TfR (*P*-interaction =

350 0.090), GA at enrolment (*P*-interaction = 0.041), and household assets score (*P*-interaction =

351 0.061). Specifically, the difference in mean (±SD) ZPP concentration at 36 GW among

352 intervention groups (MMN and LNS groups compared with the IFA group) was greater among

353 women with anemia at baseline, elevated baseline ZPP, greater GA at enrolment, or lower

354 household assets scores . Similarly, the difference in risk of elevated ZPP among intervention

355 groups was greater in women with greater GA at enrolment (*P*-interaction = 0.057), and elevated

356 TfR at baseline (*P*-interaction = 0.049)

357   The difference in mean TfR concentration at 36 GW among intervention groups was

358 greater among women who were anemic at baseline (*P*-interaction = 0.079), did not have

359 elevated AGP at baseline (*P*-interaction = 0.051), or had greater GA at enrolment (*P*-interaction

360 = 0.016), whereas the difference in risk of elevated TfR among intervention groups was greater

361 in women with less food insecurity.

362   For elevated CRP and AGP at 36 GW, there were significant interactions between

363 intervention group and elevated ZPP at baseline (*P*-interaction = 0.008) and household assets

364 score (*P*-interaction = 0.091), but the stratified analyses (Table 4) did not show consistent results.

365

366 **Discussion**

367   In the iLiNS-DYAD-Ghana study, pregnant women who were provided with standard

368 iron (60 mg) and folic acid supplements from ≤20 GW had significantly greater mean Hb, lower

369 mean ZPP and TfR and lower prevalence of anemia and iron deficiency (elevated ZPP and TfR)

370 at 36 GW than pregnant women who were provided with either the MMN or LNS supplements,

371 both of which contained 20 mg iron. Overall, however, the prevalence of anemia (Hb < 100 g/L)

372 at 36 GW was relatively low (2.2-7.9%), and iron deficiency was evident in <20% of women in

373 all groups. At 36 GW, the three groups did not differ in the percentage of women with high Hb

374 (12.3% overall) or inflammation (CRP and AGP). These findings remained unchanged when

375 analyses were restricted to women who were more adherent to treatment.

376   A few weaknesses of our study were described previously (Adu-Afarwuah et al., 2015),

377 namely: (a) a fully double-blind study design was not possible because of the physical

378 differences between the SQ-LNS in the form of sachets, and the other two supplements (MMN

379 and IFA) in the form of capsules, and (b) adherence to treatment was assessed by self-report and

380 not direct observation. However, none of the individuals involved in sample collection,

381 laboratory measurements or data analysis had any knowledge of group assignment. The good

382 collaboration with the antenatal clinics (which gave women the confidence to cooperate),

383 relatively low rate of attrition, intense follow-up of participants, and detailed attention we paid to

384 ensuring data quality were notable strengths of the study.

385   Several possible explanations may be relevant for the observed lower Hb and higher ZPP

386 and TfR values in the MMN and LNS groups compared to the IFA group. First, the 20 mg iron

387 dose used in the MMN and LNS supplements may have been too low for this Ghanaian

388 population of pregnant women. In most (but not all) similar studies, the iron dose of the iron +

389   folic acid supplement was 60 mg/day and that of the multiple micronutrient supplement was 30

390   mg/day (Allen and Peerson, 2009, Roberfroid et al., 2011, Mei et al., 2014), although in

391   Indonesia (Suprapto et al., 2002), Mexico (Ramakrishnan et al., 2004), Nepal (Christian et al.,

392   2003) and Tanzania (Makola et al., 2003), both the iron + folic acid and multiple micronutrient

393   supplement groups received iron doses of at least 50 mg/ day. In these studies (even for those

394   that used multiple micronutrients containing 30 mg Fe), pregnant women consuming the multiple

395   micronutrient supplements generally did not differ in Hb or iron status indicators compared to

396   those consuming iron + folic acid (Allen and Peerson, 2009, Mei et al., 2014, Roberfroid et al.,

397   2011). A study of Australian women (Zhou et al., 2009) suggested that 20 mg iron per day may

398   be an adequate dose to prevent iron deficiency anemia during pregnancy compared with higher

399   doses of iron. However, the diet of the women in our sample, as is typical of Ghana, is mainly

400   plant-based and high in phytate (Gibson, 1994), which reduces iron absorption (Baech et al.,

401   2003), so dietary iron needs during pregnancy may be higher in Ghana than in Australia.

402   Another possibility is that the relatively high dose of zinc (30 mg) in the MMN and LNS

403   supplements may have interfered with iron absorption, as suggested by the results of a

404   supplementation trial in Nepal (Christian et al., 2003). The lack of a good biomarker of zinc

405   status at the individual level makes it difficult to explore this potential mechanism for the

406   differences in iron status between the IFA and other two groups.

407   It is noteworthy that there were no significant differences in mean Hb or iron status

408   between the LNS and MMN groups, despite the fact that SQ-LNS is a food-based supplement

409   rather than a capsule and some differences in composition could have affected these outcomes.

410   For instance, it is possible that the calcium or phytate in SQ-LNS could have limited the

411   absorption of iron in the LNS group. The lack of differences in Hb and iron status between these

412   two groups suggests that the iron or other micronutrient content (which was identical in these

413   two supplements, except for the macro-minerals) was the most critical factor.

414   In Ghana, anemia (Ghana Statistical Service (GSS) et al., 2009) and infections including

415   malaria (Yatich et al., 2009) are common among pregnant women even in relatively high income

416   communities, and evidence (Mockenhaupt et al., 2000) suggests that malaria is a major risk

417   factor for anemia. Thus, it is noteworthy that the prevalence of both anemia and elevated CRP

418   declined significantly in all three groups between baseline and 36 weeks gestation.

419   It is important to consider the implications of the observed lower mean Hb of the SQ-LNS

420   and MMN groups compared to the IFA group at 36 weeks gestation. Low Hb concentration or

421   anemia in the first or second trimester of pregnancy is linked with poor pregnancy outcomes

422   including low birth weight (Murphy et al., 1986), but no such association has been established

423   for low Hb concentration or anemia in the third trimester (Allen, 2000). Further, Hb

424   concentrations substantially above 110–119 g/L during pregnancy may be independent of iron

425   status and have been linked with poorer health outcomes for the mother and fetus (Yip, 2000,

426   Zhou et al., 1998). Therefore, the higher mean Hb concentrations observed for women in the IFA

427   group compared to those in the LNS and MMN groups in the third trimester (36 GW) may not

428   necessarily be beneficial with respect to birth outcomes. In fact, we previously demonstrated that

429   in this same study (Adu-Afarwuah et al., 2015) the prenatal consumption of LNS (compared to

430   IFA) was associated with greater birth weight, weight-for-age z-score and BMI-for-age z-score,

431   and that, in first-time mothers, prenatal LNS supplementation also increased birth length and

432   head circumference and reduced the proportion of infants with low birth weight, low birth length,

433   and small-for-gestational age. In this cohort, there was no relationship between Hb at 36 wk

434   gestation and infant birth size, and there was actually a significant *negative* relationship between

435   maternal iron status at 36 wk and birth size (Oaks et al., 2015). Thus, the difference between the

436   LNS and IFA groups in mean maternal Hb, ZPP, and TfR concentrations needs to be weighed

437   against the difference (in the opposite direction) in birth outcomes. In two sets of analyses (Garn

438   et al., 1981, Steer et al., 1995) each involving a large number of pregnant women, the lowest risk

439   of adverse birth outcomes including low birth weight was seen in women with Hb ~95-105 g/L

440   (Steer et al., 1995) or Hb ~100 -110 g/L (Garn et al., 1981). Appropriate cut-offs for ZPP and

441   TfR in pregnancy are not well documented, particularly with respect to functional outcomes.

442   Therefore, it is difficult to judge whether our results should be interpreted as "improvements" in

443   maternal iron status in the IFA group compared to the MMN or LNS group.

444   We conclude that among pregnant women in a semi-urban setting in Ghana,

445   supplementation with SQ-LNS or MMN containing 20 mg iron resulted in lower Hb and iron

446   status but had no impact on inflammation, when compared with iron (60 mg) plus folic acid (400

447   µg) treatment. The amount of iron in such supplements that is most effective for improving both

448   maternal Hb/iron status and birth outcomes requires further evaluation.

449

450   **Key messages**

451   1.   In this semi-urban Ghanaian population, the prevalence of anemia (Hb <100 g/L) among

452   pregnant women who received IFA, MMN or SQ-LNS was reduced from 13% at <20

453   gestational weeks (GW) to 5% at 36 GW.

454   2.   Provision of IFA (with 60 mg Fe) was associated with a greater mean concentration of

455   Hb and lower prevalence of anemia at 36 GW than provision of MMN or SQ-LNS (both

456   with 20 mg Fe).

457   3.   The prevalence of high Hb (>130 g/L) or elevated inflammatory biomarkers (CRP and

458   AGP) at 36 GW was not affected by the type of prenatal supplement provided.

459   4.   More research is needed to determine the concentration of iron in MMN and SQ-LNS

460   supplements that is most effective for improving both maternal Hb/iron status and birth

461   outcomes.

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663   *Journal of Clinical Nutrition* **63,** 183-190

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TABLE 1

Background characteristics of pregnant Ghanaian women whose hemoglobin, and iron status and inflammatory

markers were analyzed at 36 gestational weeks, by intervention group[1]

Background characteristics	IFA (N = 349)	MMN (N = 354)	LNS (N = 354)
Age, y	27 ± 5.3 [349]	27 ± 5.7 [354]	27 ± 5.4 [354]
Formal education, y	7.6 ± 3.5 [349]	7.5 ± 3.6 [354]	7.7 ± 3.7 [354]
Body Mass Index, kg/m^2	25 ± 4.3 [342]	25 ± 5.0 [348]	25 ± 4.4 [349]
Low BMI, n/N (%)	11/342 (3.2)	8/348 (2.3)	6/349 (1.7)
Gestational age at enrolment, weeks	16.3 ± 3.3 [346]	16.2 ± 3.2 [353]	16.2 ± 3.3 [349]
Assets index[2]	0.09 ± 0.98 [342]	0.1 ± 0.9 [349]	-.01 ± 0.91 [348]
Housing index[2]	0.04 ± 0.99 [342]	-0.03 ± 1.03 [349]	0.00 ± 1.00 [348]
HFIA Score[3]	2.9 ± 4.6 [345]	2.5 ± 3.9 [346]	2.5 ± 3.9 [348]
Married or co-habiting, n/N (%)	320/349 (91.7)	332/354 (93.8)	328/354 (92.7)
Primiparous women, n/N (%)	131/349 (37.5)	110/354 (31.1)	128/354 (36.2)
Tested positive for malaria[4], n/N (%)	31/349 (8.9)	30/354 (8.5)	40/354 (11.3)

[1] IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group. LNS= Small Quantity Lipid-based Nutrient

Supplement group. HFIA is Household Food Insecurity Access Score. N=total number of participants in the group

in question; n = number of participants positive on the variable in question; % = percent of participants positive on

the variable in question. Values are Mean ± SD [N] or n/N (%).

[2] Proxy indicators for household socioeconomic status; higher values represent higher socioeconomic status.

[3] HFIA (Household food insecurity access) is a proxy indicator for household food insecurity (Coates et al., 2007);

higher values represent higher food insecurity

[4] Rapid Diagnostic Test (Clearview Malarial Combo, Vision Biotech, South Africa), which detected *P. falciparum*

and non-*P. falciparum* histidine-rich protein-2

TABLE 2

Unadjusted hemoglobin, and iron status and inflammatory markers of pregnant Ghanaian women at baseline and 36 gestational weeks, by intervention group, and pair-wise comparison of groups[1]

Variable	IFA[2] [N=349]	MMN[2] [N=354]	LNS[2] [N=354]	P[3]	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Mean difference (95 % CI)	p	Mean difference (95 % CI)	p	Mean difference (95 % CI)	p
Hb, g/L										
Baseline	112 ± 13 [349]	111 ± 12 [354]	112 ± 12 [354]		-1 (-4, 1)		0 (-2, 2)		1 (-1, 3)	
36 GW	120 ± 11 [270]	117 ± 12 [291]	115 ± 12 [266]	<0.001	-3 (-6, -1)	0.002	-5 (-7, 3)	<0.001	-2 (-4, 1)	0.18
ZPP, μmol/mol heme										
Baseline	43 ± 28 [347]	46 ± 36 [354]	45 ± 33 [354]		3 (-3, 8)		2 (-4, 8)		-1 (-6, 5)	
36 GW	42 ± 30 [267]	49 ± 30 [291]	50 ± 29 [264]	<0.001	6 (0, 12)	<0.001	7 (1, 13)	<0.001	1 (-5, 7)	0.91
TfR, mg/L										
Baseline	4.0 ± 1.9 [338]	4.0 ± 1.7 [348]	4.3 ± 3.5 [346]		0.0 (-0.4, 0.5)		0.3 (-0.1, 0.8)		0.3 (-0.1, 0.8)	
36 GW	4.0 ± 1.3 [266]	4.6 ± 1.7 [291]	4.9 ± 1.8 [265]	<0.001	0.5 (0.2, 0.9)	<0.001	0.8 (0.5, 1.2)	<0.001	0.3 (-0.0, 0.6)	0.07
CRP, mg/L										
Baseline	7.9 ± 14 [338]	5.8 ± 8.3 [348]	6.9 ± 11 [346]		-2.1 (-4.1, -0.1)		-1.1 (-3.1, 1.0)		1.0 (-1.0, 3.0)	
36 GW	5.6 ± 18 [266]	5.7 ± 14 [291]	5.9 ± 18 [265]	0.85	0.1 (-3.3, 3.5)	0.95	0.3 (-3.2, 3.8)	0.97	0.2 (-3.2, 3.6)	0.84
AGP, g/L										
Baseline	0.7 ± 0.2 [338]	0.6 ± 0.2 [348]	0.6 ± 0.2 [346]		0.0 (-0.1, 0.0)		0.0 (-0.1, 0.0)		0.0 (0.0, 0.0)	

36 GW	0.5 ± 0.2 [266]	0.5 ± 0.2 [291]	0.5 ± 0.2 [265]	0.30	0.0 (0.0, 0.1)	0.65	0.0 (0.0, 0.0)	0.80	0.0 (-0.1, 0.0)	0.27

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS= Small Quantity Lipid-based Nutrient Supplement group. AGP, CRP, GW, Hb, TfR, and ZPP are alpha-1 acid glycoprotein, C-reactive protein, gestational weeks, hemoglobin, transferrin receptor, and zinc protoporphyrin, respectively. Analyses are based on ANOVA (SAS, PROC GLM). Group means (±SD) and pair-wise mean difference (95% CI) were calculated using untransformed data. Except for Hb, all p-values for group or pair-wise comparisons were generated from log-transformed data; untransformed data were used for comparisons of mean Hb values. N=total number of participants in the group in question.

[2] Values are Mean ± SD [N].

[3]P-values, with Tukey-Kramer adjustments, compare all three groups.

TABLE 3

Number (percentage) of pregnant Ghanaian women with abnormal hemoglobin, and iron status and inflammatory markers at baseline and 36 weeks of gestation,

by intervention group and pairwise relative risks (RR) between groups[1]

	IFA[2] [N=349]	MMN[2] [N=354]	LNS[2] [N=354]	P[3]	Comparison of IFA and MMN[4]		Comparison of IFA and LNS[4]		Comparison of MMN and LNS[4]	
					RR (95 % CI)	p	RR (95 % CI)	p	RR (95 % CI)	p
Low Hb (< 100 g/L)[5]										
Baseline	39/349 (11.2)	51/354 (14.4)	47/354 (13.3)		1.29 (0.81, 2.05)		1.19 (0.74, 1.91)		0.92 (0.59, 1.43)	
36 GW	6/270 (2.2)	17/291 (5.8)	21/266 (7.9)	0.019	2.63 (0.88, 7.86)	0.09	3.55 (1.22, 10.3)	0.013	1.35 (0.65, 2.83)	0.60
Low Hb (< 110 g/L)[6]										
Baseline	139/349 (39.8)	157/354 (44.4)	134/354 (37.9)		1.11 (0.90, 1.37)		0.95 (0.76, 1.19)		0.85 (0.69, 1.06)	
36 GW	38/270 (14.1)	69/291 (23.7)	88/266 (33.1)	<0.001	1.68 (1.10, 2.59)	0.011	2.35 1.56, 3.53)	<0.001	1.40 (1.01, 1.92)	0.038
High Hb[7]										
Baseline	21/349 (6.0)	15/354 (4.2)	16/354 (4.5)		0.70 (0.33, 1.52)		0.75 (0.35, 1.60)		1.07 (0.47, 2.43)	
36 GW	42/270 (15.6)	32/291 (11.0)	28/266 (10.5)	0.15	0.71 (0.42, 1.18)	0.25	0.68 (0.40, 1.16)	0.20	0.96 (0.54, 1.70)	0.98
Elevated ZPP[8]										
Baseline	40/347 (11.5)	54/354 (15.3)	46/354 (13.0)		1.32 (0.84, 2.09)		1.13 (0.70, 1.81)		0.85 (0.55, 1.32)	
36 GW	25/267 (9.4)	56/291 (19.2)	49/264 (18.6)	0.003	2.06 (1.21, 3.48)	0.003	1.98 (1.16, 3.40)	0.007	0.96 (0.64, 1.46)	0.98
Elevated TfR[9]										
Baseline	29/338 (8.6)	29/348 (8.3)	37/346 (10.7)		0.97 (0.54, 1.75)		1.25 (0.72, 2.17)		1.28 (0.74, 2.23)	
36 GW	24/266 (9.0)	44/291 (15.1)	51/265 (19.2)	0.004	1.68 (0.96, 2.94)	0.08	2.13 (1.24, 3.67)	0.003	1.27 (0.82, 1.97)	0.40
IDA[10]										
Baseline	20/338 (5.9)	22/348 (6.3)	22/346 (6.4)		1.07 (0.53, 2.15)		1.07 (0.53, 2.17)		1.01 (0.51, 1.99)	
36 GW	2/264 (0.8)	11/291 (3.8)	11/263 (4.2)	0.07	4.99 (0.83, 29.9)	0.09	5.52 (0.92, 33.1)	0.06	1.11 (0.42, 2.95)	0.97
IDA[11]										
Baseline	36/338 (10.7)	37/348 (10.6)	37/346 (10.7)		1.00 (0.59, 1.68)		1.00 (0.60, 1.69)		1.01 (0.60, 1.68)	
36 GW	12/264 (4.5)	27/291 (9.3)	41/263 (15.6)	<0.001	2.04 (0.93, 4.49)	0.08	3.43 (1.63, 7.20)	<0.001	1.68 (0.97, 2.90)	0.07
Elevated CRP only[12]										

	IFA	MMN	LNS	p	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
Baseline	113/338 (33.4)	108/348 (31.0)	121/346 (35.0)		0.93 (0.72, 1.20)		1.05 (0.82, 1.34)		1.13 (0.87, 1.45)	
36 GW	49/266 (18.4)	70/291 (24.1)	59/265 (22.3)	0.26	1.31 (0.89, 1.93)	0.24	1.21 (0.81, 1.81)	0.52	0.93 (0.64, 1.33)	0.87
Elevated CRP+AGP[13]										
Baseline	26/338 (7.7)	15/348 (4.3)	11/346 (3.2)		0.56 (0.27, 1.17)		0.41 (0.18, 0.94)		0.74 (0.30, 1.84)	
36 GW	7/266 (2.6)	9/291 (3.1)	5/265 (1.9)	0.67	1.18 (0.37, 3.77)	0.94	0.72 (0.18, 2.79)	0.83	0.61 (0.17, 2.22)	0.64
Elevated AGP only[14]										
Baseline	3/338 (0.9)	5/348 (1.4)	5/346 (1.4)		1.62 (0.30, 8.88)		1.63 (0.30, 8.93)		1.01 (0.23, 4.38)	
36 GW	1/266 (0.4)	0/291 (0.0)	1/265 (0.4)	1.00						

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS=Small Quantity Lipid-based Nutrient Supplement group. AGP, CRP, GW, Hb, IDA, TfR, and ZPP are alpha-1 acid glycoprotein, C-reactive protein, gestational weeks, hemoglobin, iron deficiency anemia, transferrin receptor, and zinc protoporphyrin, respectively. N=total number of participants in the group in question.

[2]Values are number of participants positive on the variable in question/N (% of participants positive on the variable in question).

[3]P-vaues compare all three groups, with Tukey-Kramer adjustment, using logistic regression (SAS PROC LOGISTIC).

[4]Relative Risks, RR(95% CI) and their p-values are based on Poisson regression (Spiegelman and Hertzmark, 2005).

[5] Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001.

[6] WHO, 2011.

[7]Hb >130 g/L (Pena-Rosas et al., 2012).

[8]ZPP > 60.0 (μmol/mol heme).

[9]TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013).

[10]Hb <100 g/L (Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001) and at least one marker of iron deficiency (ZPP > 60.0 (μmol/mol heme) or TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013)).

[11]Hb <110 g/L (WHO, 2011) and at least one marker of iron deficiency (ZPP > 60.0 (µmol/mol heme) or TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et

al., 2013)).

[12]CRP >5.0 mg/L and AGP not >1.0 g/L (Thurnham and McCabe).

[13] CRP >5.0 mg/L and AGP >1.0 g/L (Thurnham and McCabe).

[14] CRP not > 5.0 mg/L and AGP >1.0 g/L (Thurnham and McCabe).

TABLE 4

Effect of intervention on iron status and inflammatory outcomes of pregnant Ghanaian women, stratified by baseline characteristics[1]

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
ZPP, µmol/mol heme[5]											
Baseline anemia				0.099			.		.		.
No[†]	44 ± 2 [233]	47 ± 2 [245]	48 ± 2 [226]		0.09	3 (-2, 9)	0.34	5 (-0, 11)	0.08	2 (-4, 8)	0.71
Yes	36 ± 6 [26]	54 ± 5 [41]	53 ± 5 [33]		0.013	18 (3, 34)	0.017	18 (1, 34)	0.032	-1 (-16, 14)	0.99
Elevated baseline ZPP				0.013			.		.		.
No	42 ± 2 [230]	46 ± 2 [242]	46 ± 2 [226]		0.19	3 (-2, 9)	0.34	4 (-2, 10)	0.20	1 (-5, 7)	0.94
Yes	47 ± 5 [29]	64 ± 5 [44]	72 ± 5 [33]		0.001	18 (3, 32)	0.015	25 (9, 41)	0.001	8 (-7, 22)	0.43
Elevated baseline TfR				0.090			.		.		.
No	43 ± 2 [239]	47 ± 2 [261]	49 ± 2 [233]		0.06	4 (-2, 9)	0.28	6 (0, 12)	0.048	2 (-4, 8)	0.65
Yes	41 ± 6.5 [20]	63 ± 6 [25]	59 ± 6 [26]		0.017	22 (3, 41)	0.017	18 (-1, 36)	0.07	-4 (-22, 14)	0.84
GA at enrolment				0.041			.		.		.
At 10th percentile[‡]	46 ± 3	51 ± 3	44 ± 3		0.28	5 (-5, 15)	0.44	-1 (-11, 9)	0.97	-6 (-16, 4)	0.29
At 90th percentile	40 ± 3	48 ± 3	54 ± 3		0.002	7 (-1, 16)	0.12	13 (5, 22)	0.001	6 (-3, 15)	0.25
Assets score				0.061			.		.		.
At 10th percentile	40 ± 3	46 ± 3	54 ± 3		0.003	5 (-4, 15)	0.38	14 (4, 24)	0.002	8 (-1, 18)	0.11
At 90th percentile	46 ± 3	50 ± 3	46 ± 3		0.37	5 (-4, 13)	0.44	-0 (-9, 9)	1.00	-5 (-14, 4)	0.46
Elevated ZPP[6]											
GA at enrolment				0.057			.		.		.
At 10th percentile[§]	11.1 (5.7, 20.5)	23.2 (15.3, 33.6)	11.6 (6.4, 20.0)		0.06	3.0 (0.9, 4.6)	0.12	1.0 (0.4, 2.5)	1.00	0.5 (0.2, 1.1)	0.11
At 90th percentile	5.8 (2.9, 11.4)	14.9 (9.6, 22.3)	20.7 (14.1, 29.2)		0.004	2.4 (1.0, 5.7)	0.049	3.4 (1.5, 7.7)	0.003	1.4 (0.8, 2.5)	0.46

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
Elevated baseline TfR				0.049		.		.		.	
No[¶]	17/234 (7.4)	44/256 (17.4)	32/229 (14.0)		0.008	2.2 (1.2, 4.0)	0.006	1.8 (1.0, 3.4)	0.08	0.8 (0.5, 1.3)	0.61
Yes	1/20 (3.0)	3/25 (10.0)	8/25 (31.3)		0.014	1.3 (0.4, 4.6)	0.41	2.7 (0.8, 9.4)	0.016	2.1 (0.7, 6.1)	0.14
TfR, mg/L[7]											
Baseline anemia				0.079		.		.		.	
No	4.1 ± 0.1 [232]	4.5 ± 0.1 [245]	4.8 ± 0.1 [226]		<0.001	0.4 (0.1, 0.8)	0.004	0.7 (0.4, 1.0)	<0.001	0.3 (-0.0, 0.6)	0.11
Yes	3.5 ± 0.3 [26]	4.8 ± 0.3 [41]	5.0 ± 0.3 [33]		<0.001	1.3 (0.4, 2.1)	0.002	1.5 (0.6, 2.4)	<0.001	0.2 (-0.6, 1.1)	0.76
Elevated baseline AGP				0.051		.		.		.	
No	4.1 ± 0.1 [238]	4.6 ± 0.1 [272]	4.9 ± 0.1 [250]		<0.001	0.6 (0.3, 0.9)	<0.001	0.8 (0.5, 1.2)	<0.001	0.3 (-0.02, 0.6)	0.07
Yes	4.0 ± 0.4 [20]	4.0 ± 0.4 [14]	3.3 ± 0.5 [9]		0.503	-0.0 (-1.2, 1.2)	1.00	-0.7 (-2.1, 0.8)	0.51	-0.66 (-2.2, 0.8)	0.56
GA at enrolment				0.016		.		.		.	
At 10th percentile	4.4 ± 0.2	5.0 ± 0.2	4.8 ± 0.2		0.023	0.9 (0.1, 1.1)	0.019	0.4 (-0.1, 0.9)	0.16	-0.2 (-0.7, 0.3)	0.64
At 90th percentile	3.8 ± 0.1	4.3 ± 0.1	4.9 ± 0.1		<0.001	0.5 (0.0, 0.9)	0.041	1.1 (0.7, 1.6)	<0.001	0.7 (0.2, 1.1)	0.002
Elevated TfR[8]											
HFIA score				0.047		.		.		.	
At 10th percentile	5.3 (2.9, 9.7)	15.7 (10.8, 22.2)	15.3 (10.5, 21.7)		0.004	2.3 (1.1, 4.7)	0.006	2.5 (1.2, 5.0)	0.007	1.1 (0.6, 1.8)	1.00
At 90th percentile	9.7 (5.1, 17.5)	4.9 (1.7, 13.5)	18.7 (10.3, 31.5)		0.06	0.7 (0.2, 2.0)	0.50	1.9 (0.8, 4.6)	0.27	2.8 (0.9, 8.7)	0.06
Elevated CRP[9]											
Elevated baseline ZPP				0.008		.		.		.	
No	41/225 (18.3)	72/238 (30.1)	47/221 (21.4)		0.013	1.6 (1.1, 2.4)	0.014	1.2 (0.8, 1.8)	0.71	0.7 (0.5, 1.1)	0.11
Yes	9/29 (30.1)	6/43 (13.5)	11/33 (32.0)		0.09	0.5 (0.2, 1.1)	0.17	1.1 (0.5, 2.1)	0.99	2.1 (0.9, 4.9)	0.10

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
Elevated AGP[10]											
Assets score				0.091			.		.		.
At 10th percentile	1.0 (0.2, 4.8)	1.0 (0.2, 4.5)	2.8 (0.9, 8.4)		0.39	1.0 (0.1, 9.8)	1.00	2.8 (0.6, 14.1)	0.51	2.8 (0.3, 28.0)	0.48
At 90th percentile	4.3 (1.5, 11.3)	4.4 (1.7, 10.8)	0.7 (0.1, 3.9)		0.14	1.0 (0.2, 4.5)	1.00	0.16 (0.0, 1.3)	0.16	0.2 (0.0, 1.4)	0.14

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS=Small Quantity Lipid-based Nutrient Supplement group. GA, Hb, HFIA, TfR, and ZPP are gestation age, hemoglobin, household food insecurity access, transferrin receptor, and Zinc protoporphyrin, respectively. Baseline anemia is Hb <100 g/L (Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001). Analyses are based on ANCOVA (SAS PROC MIXED, with SLICE option) for continuous outcomes or logistic regression (SAS PROC GLIMMIX, with SLICE option) for binary outcomes. Linear regression modeling was used to predict the outcome at the 10th and 90th percentile of continuous baseline effect modifiers.

[2]Values are mean $\pm$ SE [total number of participants], or mean $\pm$ SE, or percent of participants positive on the variable in question (95% CI) or number of participants positive on the variable in question/total number of participants (percent of participants positive on the variable in question). Values are adjusted for variables significantly associated with the outcome variable in bivariate analysis.

[3]P-values are for interaction with the outcome in question

[4]P-values compare all three groups in each stratum.

[5]ZPP values are adjusted for age, parity, season at enrolment, and baseline Hb, ZPP, and TfR concentrations.

[6]Elevated ZPP (ZPP >60 μmol/mol heme) adjusted for age, education, and baseline Hb, ZPP and TfR concentrations.

[7]TfR values are adjusted for age, season at enrolment, and baseline Hb, ZPP, TfR and AGP concentrations.

[8]Elevated TfR (TfR > 6.0 mg/L) adjusted for age, gestational age enrolment, season at enrolment, and baseline Hb, ZPP, and TfR concentrations.

[9]Elevated CRP (CRP > 5.0 mg/L) adjusted for BMI, household food insecurity access score, and baseline ZPP, AGP and CRP concentrations.

[10]Elevated AGP (AGP > 1.0 g/L) adjusted for age, parity, and season as enrolment.

[†]Values are group mean ± SE [number of participants], and difference in means (95% CI) and p-values. All such values.

[‡]Values are group mean ± SE, and difference in means (95% CI) and p-values. All such values.

[§]Values are group % (95% CI), and relative risk (95% CI) and p-values. All such values.

[¶]Values are number of participant (%), and relative risk (95% CI) and p-values. All such values.