

# Randomized controlled trial of 2 prenatal iron supplements: is there a dose-response relation with maternal hemoglobin?<sup>1–3</sup>

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## ABSTRACT

**Background:** The most appropriate dose of iron to prevent maternal anemia is still unclear.

**Objective:** We assessed the dose-response relation between maternal hemoglobin and 2 prenatal iron supplements.

**Design:** An intention-to-treat, double-blind, randomized controlled trial compared 30 mg Fe + folic acid and 13 other micronutrients (UNIMMAP; UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women) with 60 mg Fe + folic acid (IFA) only in rural Burkina Faso. Home visitors directly observed tablet intake. Mixed-effects models were used for the data analysis.

**Results:** At inclusion, 43.2% of the 1268 participants were anemic. On average, the hemoglobin concentration decreased over gestation by 0.019 g/dL (95% CI: 0.012, 0.025 g/dL) per week in the IFA and UNIMMAP groups. An increment in hemoglobin concentration per micronutrient tablet [ $\beta$  ( $\pm$ SE) = 0.006  $\pm$  0.001 g/dL;  $P < 0.001$ ] was observed only in women who were anemic at inclusion, whereas a decrease was observed in the other mothers ( $-0.003 \pm 0.001$  g/dL;  $P = 0.002$ ,  $P$  for interaction  $< 0.0001$ ); the finding was similar in both the IFA and UNIMMAP groups. Women with baseline anemia achieved the same hemoglobin concentration (mean  $\pm$  SD: 11.1  $\pm$  0.64 g/dL) as their counterparts who received  $\pm 180$  tablets of either UNIMMAP or IFA. Despite this, micronutrient intake did not significantly prevent anemia (51.0% in the third trimester). It was, however, a risk factor for hemoconcentration (odds ratio per tertile of tablet intake: 2.10; 95% CI: 1.12, 3.94), independently of supplement type or initial hemoglobin concentration.

**Conclusions:** UNIMMAP triggered the same hemoglobin dose response with half the amount of iron as provided by IFA treatment. The benefit of iron supplements in nonanemic women is unclear. Despite micronutrient supplementation, anemia remained highly prevalent during gestation, partly because of physiologic hemodilution. This trial was registered at [clinicaltrials.gov](http://clinicaltrials.gov) as NCT00642408. *Am J Clin Nutr* 2011;93:1012–8.

## INTRODUCTION

Iron deficiency anemia occurs frequently during pregnancy because the diet cannot provide the extra iron required by the increased maternal red blood cell mass, the placenta, and the growing fetus (1). Pregnant women in developing countries are even more at risk because of a diet poor in absorbable iron and iron losses due to frequent parasitic infections (2). The worldwide prevalence of maternal anemia is estimated at 41.8%, but amounts

to 57.1% and 48.2% in sub-Saharan Africa and Southeast Asia, respectively (3). This is an important public health concern because maternal anemia has been consistently linked with adverse pregnancy outcomes, such as preterm birth and low birth weight (4, 5), and is potentially linked to maternal mortality and morbidity (6, 7). Universal prenatal supplementation with 60 mg Fe (with or without folic acid) provided either daily or weekly is effective at preventing maternal anemia and iron deficiency at term (2, 8). However, there is considerable debate over the doses of iron likely to yield the best risk-benefit balance for both mothers and their offspring (2, 9–11). For instance, Ekström et al (12, 13) have suggested that the current international recommendation for iron supplementation in pregnancy could be decreased by 78–83%. Other researchers have also proposed using lower daily doses of iron (10, 14, 15). In addition, providing too much iron may result in hemoconcentration and oxidative stress—2 conditions associated with adverse pregnancy outcomes (16, 17). It is thus crucial to define and to use the lowest dose of supplementary iron that fulfills the prophylactic criteria (10).

Apart from iron, pregnant women in poor populations are often also deficient in other nutrients (18). This is why UNICEF/WHO/UNU recently recommended the use of multiple micronutrient supplements for pregnant and lactating women (UNIMMAP) instead of iron+folic acid (IFA) only (19). Of note, UNIMMAP contains half the amount of iron of IFA, ie, 30 mg (**Table 1**). A 2009 meta-analysis reported no significant difference in maternal anemia and in hemoglobin and ferritin concentrations between gravidae provided in UNIMMAP or IFA (18). However, that meta-analysis focused on end outcomes only, so the dynamic of the physiologic response remains unknown. For in-

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<sup>2</sup> Supported by Nutrition Third World and the Belgian Ministry of Development.

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Received October 13, 2010. Accepted for publication February 14, 2011.

First published online March 2, 2011; doi: 10.3945/ajcn.110.006239.

**TABLE 1**

Composition of the UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women (UNIMMAP) and the iron + folic acid (IFA) supplement

Nutrient	IFA	UNIMMAP	Unit
Vitamin A	—	800	μg
Vitamin D	—	200	IU
Vitamin E	—	10	mg
Thiamine	—	1.4	mg
Riboflavin	—	1.4	mg
Niacin	—	18	mg
Folic acid	400	400	μg
Vitamin B-6	—	1.9	mg
Vitamin B-12	—	2.6	μg
Vitamin C	—	70	mg
Zinc	—	15	mg
Iron <sup>1</sup>	60	30	mg
Copper	—	2	mg
Selenium	—	65	μg
Iodine	—	150	μg

<sup>1</sup> In the form of ferrous fumarate.

stance, the effect on maternal hemoglobin might have been reached more rapidly with IFA than with UNIMMAP given the higher iron dose. Also, in both supplementation groups the optimal effect on maternal hemoglobin could have been reached with a limited number of tablets. These 2 hypotheses are particularly relevant in contexts with low resources and/or inefficient health care service delivery.

We therefore aimed to assess the existence and the pattern of a dose-response relation between prenatal iron supplements and maternal hemoglobin concentration for 2 types of micronutrient supplements: UNIMMAP and IFA.

## SUBJECTS AND METHODS

The methods of the randomized controlled trial were described elsewhere (20). Briefly, between March 2004 and February 2006, 1426 pregnant women in the catchment area of 2 health centers in the Houndé district (Burkina Faso) were randomly assigned to receive daily either IFA or UNIMMAP from the date of inclusion up to 3 mo after delivery. Participants were also randomly assigned to receive either 300 mg chloroquine weekly or intermittent preventive treatment with sulfadoxine (1500 mg) and pyrimethamine (75 mg) once in the second and third trimesters of pregnancy for malaria prevention. Twenty-five locally trained home visitors visited every compound monthly to detect pregnancy early, and possible cases were referred to the health center for pregnancy testing. Once pregnancy was confirmed, the field coordinator (MD) explained individually, in a private room, the purpose of the study and procedures in 1 of the 3 local languages (Bwamu, Moré, or Dioula) as appropriate. That participation into the study could be refused or withdrawn at any moment without affecting the quality of health care received was duly emphasized. Every study participant signed an informed consent form. The randomization scheme was generated by a computer program in permuted blocks of 4. UNIMMAP and IFA tablets were identical in appearance (Scanpharm, Copenhagen, Denmark). Daily supplement intake was directly observed by the project home visitors. Home visitors updated their visit reporting sheets daily,

and 2 sociologists performed a quality assessment of each home visitor's work on a monthly random day (21). The number of micronutrient tablets ingested was therefore measured with precision.

Maternal height, weight, and midupper arm circumference (MUAC) were measured at study inclusion and at each antenatal consultation. The hemoglobin concentration in the mother was measured at inclusion in the trial and between 30 and 34 wk of gestation by spectrophotometry with a HemoCue device (Dronfield, United Kingdom), and a daily calibration check was done by using a HemoCue Control Cuvette (22). In case of maternal illness, appropriate treatments were provided according to national guidelines. Anemia was defined as a hemoglobin concentration <11.0 g/dL during the first or third trimester and <10.5 g/dL in the second trimester to account for plasma expansion (8). Hemoconcentration was defined as a hemoglobin concentration >13.0 g/dL at any time (2). Severely anemic women (hemoglobin <7.0 g/dL, without dyspnea) received ferrous sulfate (200 mg) + folic acid (0.25 mg) twice daily for 3 mo, regardless of allocation group. All participants also received 400 mg albendazole during the second and third trimesters for deworming. If malaria occurred despite chemoprophylaxis, quinine (300 mg, 3 times/d) was given for 5 d. The study was approved by the ethics committees of the Center Muraz, Bobo-Dioulasso, Burkina Faso, and the Institute of Tropical Medicine, Antwerp, Belgium.

Adherence data were used to assess the dose-response relation (23). Because women entered the study at various gestational ages, a large array of total supplements intake was available for analysis. Hemoglobin concentration was regressed over the number of supplements up to the time of hemoglobin measurement. Because of the repeated measurements at the individual level, we used multilevel models (24). *Measurement occasion* and *participant* (unit of analysis) were defined as the first and second model levels, respectively. Adjustment was made for the following fixed effects: gestational age at the time of measurement, maternal MUAC as measured at each antenatal consultation, type of micronutrient supplements received (UNIMMAP or IFA), type of malaria prevention (chloroquine or sulfadoxine-pyrimethamine), and parity (primiparity or multiparity). All models were further adjusted for the effect of potential confounders: educational achievement of both the mother and her partner, mother's age, profession of the husband, religion, ethnicity, catchment area, home delivery, and season at inclusion (lean or fat season). Model 2 and model 1 contained all of the above covariates, with and without interaction terms, respectively. Model 3 tested further the significance of introducing a random individual-specific slope in model 2, ie, the assumption that the individual specific regression lines were parallel was relaxed (24). The matrix of covariance was unstructured. The comparative fitness of the models was assessed by a restricted maximum likelihood (REML) ratio test.

As a second step, we assessed by logistic regression the association of micronutrient intake (per tertile of tablet number) with the presence of maternal anemia or hemoconcentration at the second measurement and the probability of recovering from baseline anemia. The same adjustment used in the mixed-effects models was used for the confounders. The analysis was by intention-to-treat. No imputation for missing values was performed.

Lowess smoothed plots (band width = 0.8) were used to visualize dose-response relations. Effect modification of supplements on maternal hemoglobin by covariates was tested by a chunk test (25). When this test was significant, specific interactions were looked at. In case of a significant interaction with a continuous covariate, we assessed the regression coefficient of micronutrient supplement intake by the method of recentering, ie, by holding the value of the interacting covariate constant either at the mean, a lower value (mean - 1 SD), or a higher value (mean + 1 SD) (26).

To adjust as much as possible for potential confounding factors, we used saturated models, ie, no procedure for the removal of model parameters was applied. Statistical significance was determined with a 2-sided test at the 5% level. Stata 11.0 was used for all analyses (StataCorp, College Station, TX).

## RESULTS

The hemoglobin concentration was measured initially in 1268 participants, at a mean ( $\pm$ SD) gestational age of  $17.3 \pm 7.6$  wk. The baseline characteristics were not significantly different between study groups (Table 2). The hemoglobin concentration was measured a second and a third time in 858 (67.7%) and 48 (3.8%) mothers at a median gestational age of 34.0 and 35.6 wk, respectively. Women with a missing second hemoglobin measurement were recruited significantly later (mean  $\pm$  SE) in pregnancy ( $7.2 \pm 0.5$  wk;  $P < 0.00001$ ) and delivered earlier ( $-2.5 \pm 0.3$  wk;  $P < 0.00001$ ) than the other women, which resulted in a significantly shorter participation in the study. Missing hemoglobin measurements were also more frequent in the second catchment area (37.5% compared with 19.4%) and were not related to the baseline hemoglobin concentration or randomization group.

The mean ( $\pm$ SD) hemoglobin concentration in pregnant women at inclusion was  $11.0 \pm 1.7$  g/dL, and 43.2% (548/1268) of women were anemic at the time. The prevalence of anemia was 26.9% (102/379) in participants included during the first

trimester (Table 2); 0.79% (10/1268) and 1.05% (9/858) of the women were severely anemic at the first and second measurements, respectively. During pregnancy, 20.3% (257/1268) of the participants received quinine treatment for malaria. The proportion of women treated was evenly distributed in the IFA and UNIMMAP groups and having received a malaria treatment was not significantly associated with maternal hemoglobin concentration.

On average, the hemoglobin concentration decreased over the gestation period by 0.019 g/dL (95% CI: 0.012, 0.025) per week (Figure 1). The rate of anemia was 45.7% (247/600) and 52.0% (531/1021) in the second and third trimesters, respectively. The variation in hemoglobin concentration over gestational time was not related to the type of micronutrients used. Including a random slope in the model significantly improved its fit (REML ratio test,  $P < 0.0001$ ): the higher the hemoglobin concentration at inclusion, the steeper the decreasing slope over the gestation period (data not shown).

The number of micronutrient tablets predicted maternal hemoglobin concentration ( $\beta$ -coefficient  $\pm$  SE), although the overall effect size was modest ( $\beta = 0.003 \pm 0.001$  g/dL;  $P = 0.004$ ) (Table 3, model 1). The dose-response relation was negatively modified by hemoglobin concentration at baseline ( $P$  for interaction  $< 0.001$ ), ie, the effect of micronutrient intake was more important when the hemoglobin concentration at baseline was lower (Table 3, model 2). We therefore computed the slope for maternal hemoglobin on tablet count while holding the value of baseline hemoglobin constant. At the higher baseline hemoglobin value (mean + 1 SD), there was a decrease in hemoglobin concentration over the number of supplements, whereas the slope was flat at the mean and increased significantly ( $\beta = 0.009 \pm 0.001$  g/dL;  $P < 0.0001$ ) at a lower baseline hemoglobin concentration. This effect modification is also apparent in Figure 2, where the dose-response relation stratified by anemic status at inclusion is shown. The maternal hemoglobin

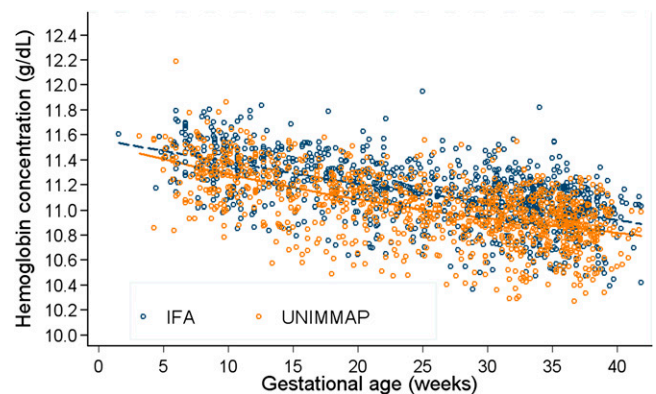
**TABLE 2**  
Baseline characteristics of participants, by allocation group<sup>1</sup>

Characteristics	IFA (n = 632)	UNIMMAP (n = 636)
Maternal age (y)	24.4 $\pm$ 6.2 <sup>2</sup>	24.3 $\pm$ 6.3
Gestational age (wk)	17.1 $\pm$ 7.5	17.3 $\pm$ 7.7
Illiteracy (%)	82.9	79.1
Primiparity (%)	18.8	21.7
BMI (kg/m <sup>2</sup> )	20.8 $\pm$ 2.0	21.0 $\pm$ 2.2
<18.5 kg/m <sup>2</sup> (%)	12.1	9.4
Midupper arm circumference (cm)	25.8 $\pm$ 2.1	25.9 $\pm$ 2.2
Hemoglobin at inclusion (g/dL)	11.1 $\pm$ 1.8	10.9 $\pm$ 1.6
Anemia		
At inclusion (%)	41.3	44.8
In first trimester [n/total n (%)]	49/185 (26.5)	53/194 (27.3)
In second trimester [n/total n (%)]	122/291 (41.9)	145/296 (49.0)
In third trimester [n/total n (%)]	91/156 (58.3)	88/146 (60.3)
Micronutrient intake (no. of tablets) <sup>3</sup>	117 $\pm$ 56	118 $\pm$ 58

<sup>1</sup> Only data from women with at least one hemoglobin measurement are presented. UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women; IFA, iron + folic acid supplement.

<sup>2</sup> Mean  $\pm$  SD (all such values).

<sup>3</sup> Tablet intake was directly observed.



**FIGURE 1.** Maternal hemoglobin concentrations during gestation in the iron+folic acid (IFA) and UNIMMAP (UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women) groups.  $\beta = 0.019$  g/dL (95% CI: 0.012, 0.025 g/dL) per week. Regression lines and points were fitted from a mixed-effects model that regressed hemoglobin concentrations over gestational age at the time of hemoglobin measurement. The data were adjusted for primiparity, maternal midupper arm circumference, type of micronutrient supplement, type of malaria prevention, maternal age, place of delivery, religion, educational achievement of the woman and her husband, ethnicity, season at random assignment, and health center. The model also included a random slope factor. The regression lines were obtained by locally weighted scatterplot smoothing (lowess) of the fitted values.

**TABLE 3**Multivariate mixed-effects models of factors associated with maternal hemoglobin (Hb) concentrations during gestation ( $n = 2027$ )<sup>1</sup>

Covariates	Maternal Hb concentration (g/dL)		
	Model 1: random intercept	Model 2: model 1 + interaction term	Model 3: model 2 + random slope
Micronutrient intake (tablets)			
Overall	0.003 ± 0.001 <sup>2,*</sup>	—	—
Baseline Hb = mean + 1 SD	—	-0.011 ± 0.001**	-0.011 ± 0.001**
Baseline Hb = mean	—	-0.001 ± 0.001	0.000 ± 0.001
Baseline Hb = mean - 1 SD	—	0.009 ± 0.001**	0.011 ± 0.001**
Gestational age (wk) <sup>3</sup>	-0.030 ± 0.005**	0.006 ± 0.003	-0.003 ± 0.002
Primiparity (yes or no)	-0.287 ± 0.115	-0.043 ± 0.070	-0.007 ± 0.046
Maternal MUAC (mm)	0.051 ± 0.019*	0.001 ± 0.011	-0.007 ± 0.008
Micronutrient supplements (UNIMMAP or IFA)	-0.137 ± 0.077	0.005 ± 0.047	-0.005 ± 0.031
Malaria prevention (SP or chloroquine)	0.040 ± 0.78	0.017 ± 0.047	-0.004 ± 0.031
Catchment area (health center 2 or center 1)	-0.443 ± 0.111**	-0.163 ± 0.067*	-0.082 ± 0.47
Interaction term (baseline Hb × micronutrient intake) <sup>4</sup>	—	-0.006 ± 0.002**	-0.006 ± 0.000**
Log REML	—	-2941.04 <sup>5,***</sup>	-2519.91 <sup>6,***</sup>

<sup>1</sup> MUAC, midupper arm circumference; REML, restricted maximum likelihood; IFA, iron+folic acid; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women; SP, sulfadoxine-pyrimethamine. Because of the interaction between micronutrient intake and Hb concentration at baseline, we used the recentering method to present the increment in Hb concentration per micronutrient tablet at 3 different concentrations of hemoglobin at baseline. Models were adjusted for gestational age at the time of Hb measurement, primiparity, maternal MUAC, type of micronutrient supplement, type of malaria prevention, maternal age, place of delivery, religion, educational achievement of the woman and husband, ethnicity, and season at random assignment. \* $P < 0.05$ , \*\* $P < 0.001$ , \*\*\* $P < 0.0001$ .

<sup>2</sup>  $\beta$  Coefficient ± SE (all such values).

<sup>3</sup> Gestational age at the time of Hb measurement.

<sup>4</sup> Interaction term between Hb at baseline and micronutrient intake.

<sup>5</sup> The  $P$  value was derived from the REML ratio test, which compared model 2 with model 1.

<sup>6</sup> The  $P$  value was derived from the REML ratio test, which compared model 3 with model 2.

increment per tablet was  $0.006 \pm 0.001$  g/dL ( $P < 0.001$ ) in women with baseline anemia, whereas a decrease was observed in other mothers ( $-0.003 \pm 0.001$  g/dL;  $P = 0.002$ ) ( $P$  for interaction  $< 0.0001$ ). The hemoglobin concentration of women with baseline anemia reached the hemoglobin concentration of their nonanemic counterparts ( $11.1 \pm 0.64$  g/dL) at  $\approx 180$  tablets, independently of the type of micronutrient received (Figure 2). The type of micronutrient or malaria prevention had no significant effect on hemoglobin concentration and did not modify the dose-response relation.

Model 3 replicated model 2 with allowance for random variation of the individual regression slopes (Table 3, model 3). This significantly improved the model fit (REML ratio test,  $P < 0.00001$ ): the higher the hemoglobin concentration at baseline, the flatter the individual-specific incremental slope of hemoglobin by micronutrient intake.

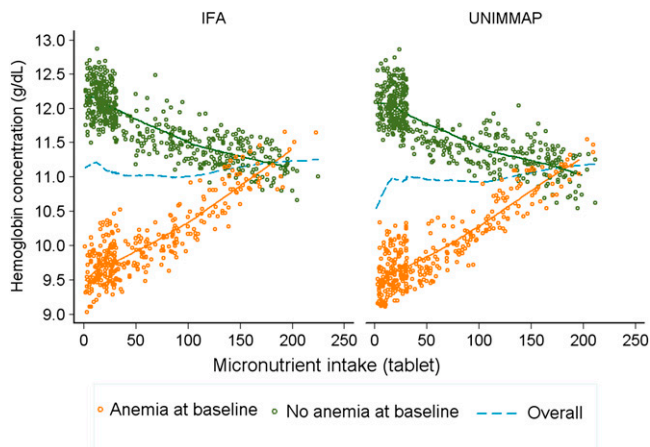
Despite the positive effect of micronutrient intake on hemoglobin concentration, the proportion of anemic women was still 51.0% (462/906) at a mean ( $\pm$ SD) gestational age of  $34.3 \pm 6.5$  wk. Baseline anemia was an important risk factor for anemia during pregnancy (Table 4). However, even in women without anemia at baseline, 42.1% (214/508) became anemic despite receiving the micronutrient supplements. As shown in Table 4, micronutrient intake did not significantly reduce the risk of anemia at the second hemoglobin measurement, although this protective effect was nearly statistically significant (odds ratio: 0.62; 95% CI: 0.38, 1.02) in women who were anemic at baseline ( $P$  for interaction = 0.002). Micronutrient intake was also not significant determinant of recovering from initial anemia, but this aspect was assessed in smaller numbers ( $n = 327$ ),

and the results were only marginally insignificant. In contrast, micronutrient intake increased the risk of hemoconcentration. The proportion of women who became hemoconcentrated between the first and second measurements was 10.0% (22/220) in the highest tertile of micronutrient intake compared with 4.8% and 8.6% in the first and second tertiles, respectively (odds ratio per tertile: 2.10; 95% CI: 1.12, 3.94). The risk of hemoconcentration by micronutrient supplementation was not modified by hemoglobin concentration at baseline nor by type of micronutrient received.

## DISCUSSION

An identical dose-response relation between tablet intake and maternal hemoglobin was observed in both the IFA and UNIMMAP groups. An increment in hemoglobin concentration per tablet was observed only in women who were anemic at baseline. Women with anemia at baseline achieved the same hemoglobin concentration as their counterparts with 180 tablets, independently of the supplement type. However, the level of micronutrient intake was not significantly related to recovery from baseline anemia, but was a risk factor for hemoconcentration, independently of supplement type or initial hemoglobin concentration.

Our study showed that 30 mg Fe associated with other enhancing micronutrients can produce the same effect on maternal hemoglobin as 60 mg Fe, consistently with previous studies (18, 27). Not only was the total effect equivalent between groups but so was the hemoglobin increment per supplement tablet. The presence in UNIMMAP of vitamin A, riboflavin, and vitamins



**FIGURE 2.** Maternal hemoglobin concentrations in relation to micronutrient intakes by anemia status at inclusion in the iron+folic acid (IFA) and UNIMMAP (UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women) groups. Regression lines and points were fitted from a multilevel regression model of hemoglobin concentrations over micronutrient intakes (tablets) up to the time of hemoglobin measurement. The data were adjusted for gestational age at the time of hemoglobin measurement, hemoglobin concentration at baseline, primiparity, maternal midupper arm circumference, type of micronutrient supplement, type of malaria prevention, maternal age, place of delivery, religion, educational achievement of the woman and her husband, ethnicity, season at random assignment, and health center. The model also included an interaction term between micronutrient intake and anemia at baseline and a random slope effect. The regression lines were obtained by locally weighted scatterplot smoothing (lowess) of the fitted values.

B-6 and B-12 (which also affect hemoglobin synthesis) and of vitamin C (which increases iron bioavailability) may explain such an equivalence (18). The previously reported positive effect of

UNIMMAP compared with IFA on fetal growth (28, 29) was thus unlikely to be due to changes in maternal hemoglobin concentrations.

We found no evidence that 60 mg Fe given daily during pregnancy, the current international recommendation, was an excessive dose in this population, although 30 mg Fe/d in association with multiple other micronutrients could yield the same result. First, there was no indication of a saturation point in the dose-response relation within the range of tablet intakes observed in this study. This finding differs from the results reported by Ekström et al (12, 13) in Bangladesh and Tanzania, where a plateau at  $\approx 2400$  mg (40 tablets) and no further hemoglobin increment with additional tablets were reported. However, these study populations were small (90 and 27 anemic women, respectively), the supplementation duration was short (12 wk), and compliance was suboptimal, which resulted in a low median number of tablets (23 and 52 in the weekly and daily group, respectively, in the Bangladesh study). Thus, very little data were available from which to assess the effect of  $>40$  supplements, and the plateau was possibly an artifact due to lowess plotting on small numbers. Other researchers have also argued that 20 mg Fe/d (14), 27 mg Fe/d (15), or 40 mg Fe/d (10) would be sufficient to prevent iron deficiency anemia during pregnancy. However, these studies were carried out in Western countries, where iron deficiency is much less prevalent. It is worth mentioning that when a direct comparison of various iron doses was carried out in some of these studies, a daily dose of  $\approx 20$  mg Fe was significantly less effective than were higher doses (40–80 mg Fe) at preventing anemia (10, 30) or at treating it (16). Second, the incidence of hemoconcentration did not differ significantly between supplement types (11.5% and 8.6% for IFA and UNIMMAP, respectively), regardless of the hemoglobin

**TABLE 4**

Multivariate logistic regression of factors associated with anemia at the second measurement, with recovery from anemia at baseline, and with hemoconcentrations between the first and second hemoglobin (Hb) measurements<sup>1</sup>

	OR (95% CI)		
	Anemia at second measurement (n = 807)	Recovery from baseline anemia (n = 327)	Hemoconcentration <sup>2</sup> (n = 719)
Micronutrient intake (per tertile) <sup>3,4</sup>			
Overall	—	—	2.10* (1.12, 3.94)
In women with baseline anemia	0.62 (0.38, 1.02)	1.56 (0.95, 2.56)	—
In women without baseline anemia	1.12 (0.73, 1.70)	—	—
Primiparity (yes or no)	1.07 (0.68, 1.67)	1.37 (0.68, 2.78)	0.29 (0.08, 1.02)
Maternal MUAC (per tertile)	0.89 (0.74, 1.07)	0.89 (0.46, 1.38)	0.78 (0.54, 1.14)
Micronutrient supplement (UNIMMAP or IFA)	0.84 (0.63, 1.13)	0.93 (0.57, 1.52)	0.66 (0.36, 1.18)
Malaria prevention (SP or chloroquine)	0.89 (0.67, 1.19)	0.81 (0.50, 1.32)	1.16 (0.65, 2.09)
Catchment area (health center 2 or center 1)	1.71* (1.14, 2.57)	0.58 (0.28, 1.19)	0.53 (0.23, 1.22)
Anemia at inclusion (yes or no)	2.38*** (1.74, 3.23)	—	0.43* (0.22, 0.84)
Hb concentration at baseline (g/dL)	—	1.38* (1.05, 1.80)	—
Interaction term (baseline anemia $\times$ micronutrient intake)	0.35* (0.18, 0.67)	—	1.36 (0.61, 3.07)

<sup>1</sup> OR, odds ratio; MUAC, midupper arm circumference; IFA, iron+folic acid; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnant and lactating women; SP, sulfadoxine-pyrimethamine. The results were obtained by multivariate logistic regression. The *P* values were computed by comparing each subcategory of the factor with the subcategory of reference by the Wald test. All models were adjusted for gestational age at the time of Hb measurement, Hb concentration at baseline, primiparity, maternal MUAC, type of micronutrient supplement, type of malaria prevention, maternal age, place of delivery, religion, educational achievement of the woman and her husband, ethnicity, and season at random assignment. \**P* < 0.05, \*\*\**P* < 0.0001.

<sup>2</sup> This model included women who did not have hemoconcentration at inclusion.

<sup>3</sup> The interaction between micronutrient intake (tertiles) and anemia at inclusion was highly significant (*P* < 0.01). Therefore, the results for micronutrient intake were stratified by anemia status at inclusion.

<sup>4</sup> Micronutrient intake up to the second Hb measurement.

concentration at baseline. To our knowledge, this was the first time that the dose response of hemoglobin was examined in a large group of pregnant women and using accurate micronutrient intakes. Sloan et al (31) addressed the dose-response issue through a systematic review of supplementation studies (compared with no supplementation). Their indirect comparison, which included 23 studies—15 of which were conducted in developing countries—also suggested the existence of a positive dose-response relation. Women who received  $\leq 60$ , 61–90, and 91–120 mg Fe/d had  $0.41 \pm 0.027$ ,  $0.86 \pm 0.018$  and  $1.87 \pm 0.027$  g/dL higher hemoglobin concentrations, respectively (31). Although the results were not adjusted for hemoglobin concentration at baseline, the findings are consistent with the current results.

One important finding was that baseline maternal hemoglobin concentration modified dramatically the effect of micronutrient supplements (both IFA and UNIMMAP) on hemoglobin concentrations further during gestation. In women without baseline anemia, the hemoglobin concentration decreased throughout pregnancy, even at doses of 60 mg Fe/d, which was also observed in another study (27). This finding likely reflects the physiologic expansion in plasma volume that occurs during pregnancy and could partially explain why, overall, the hemoglobin increment per tablet was modest. The decrease in hemoglobin could also have been caused by malaria and helminthes. However, all participants received effective preventive treatment against these 2 conditions, and it is highly implausible that women without anemia at baseline would have been more affected by these diseases than would women with anemia.

Approximately 180 tablets (10.8 g Fe with IFA and 5.4 g Fe with UNIMMAP) were necessary for anemic women at baseline to achieve the hemoglobin concentrations of women without anemia at baseline. Whereas this differential response between women with and without anemia underpins the adaptability of pregnancy physiology, it also raises the question of the relevance of general relative to selective supplementation, ie, supplementation targeted toward anemic women (9, 11, 32). However, besides the programmatic difficulties of selective supplementation in the primary health care services of many developing countries, firm evidence supporting such an approach is still lacking. First, iron might affect health through mechanisms different from an increase in maternal hemoglobin (33). For instance, even a low dose of iron (30 mg/d) can increase the birth weight of children of nonanemic and iron-replete pregnant women (34, 35). Second, we found no evidence in this study that the risk of hemoconcentration from micronutrient supplementation was greater in women without anemia than in those with anemia.

Our study had some limitations. First, the main outcome was hemoglobin concentration. Although the hemoglobin concentration is commonly used to screen for iron deficiency, low hemoglobin values are not specific to iron deficiency. Because we did not measure ferritin concentrations, we could not assess specifically the potential differential effect of IFA on iron stores (36). Also, the interpretation of hemoglobin concentration at a given time is blurred by the physiologic plasma volume expansion that occurs during pregnancy, as also shown in our study. However, even if the mechanisms of prenatal anemia are multifactorial, the measurement of hemoglobin concentration is appropriate for assessing dose-response patterns. A second limitation of this study was its reliance on compliance data, ie,

women were not randomly assigned to receive different doses of iron. Although women in the UNIMMAP group received half the amount of iron received by the IFA group, they also received many other micronutrients. However, because of the accuracy of our compliance data, thanks to the direct observation of intake and because all our analyses were adjusted for the potential confounding effect of many factors, the internal validity of our results was high. Third, although 1426 women participated in the supplementation trial, the hemoglobin concentration was measured at least once in only 1268 (89%) women, mainly because of logistical difficulties in the first weeks of the trial. This problem reduced slightly the statistical power of our hemoglobin analysis, but was very unlikely to affect its validity. Last, we did not determine whether IFA and UNIMMAP differed in their incidence of side effects. However the existence of such side effects is still debated (37).

In conclusion, both prenatal IFA and UNIMMAP supplementation had a positive dose-dependent effect on maternal hemoglobin concentration, but only in women with anemia at baseline and had no significant effect at preventing anemia further during pregnancy. The finding that hemoglobin concentrations decreased even in women without anemia at baseline, despite micronutrient supplementation, was particularly striking and most probably underpinned the physiologic aspect of such a decrease. Cohort studies with repeated measurements of individual hemoglobin concentrations throughout pregnancy in non-iron-deficient populations would help to differentiate between normal and pathologic curves of gestational hemoglobin.

The MISAME (Micronutriments et Santé de la Mère et de l'Enfant) Study Group thanks the families of Karaba and Koho, who participated in the study; the health staff of the Houndé district; and especially the district directors (Pascal Korgo and Noufou Sankara) and staff of the Center Muraz for logistical and administrative support. The Field Investigator Team consisted of J-P Ki (sociologist), V Koudougbo (sociologist), L Toe (MD), E Da (OB-GYN), G Lougue (pharmacist), B Negalo (nurse), B Hien (laboratory), and O Guebe (nurse). The Logistic Team consisted of S Ouattara (Coordination), B Bicaba, C Kouakou Yameogo, N Diallo, Michel Sanou, and A Hien (pharmacist). The Scientific Committee consisted of Jane Kusin (Professor at the Amsterdam University, Netherlands), Francis Delpeuch (Head of Tropical Nutrition Unit in IRD, Montpellier, France), John Van Camp (Food Safety and Food Quality, Faculty of Bioscience Engineering, Ghent University, Belgium), Pierre Bourdoux (Head of Pediatric Laboratory, Université Libre de Bruxelles, Belgium), Serge Diabougba (Head of Center Muraz, Burkina Faso), Sylvestre Tapsoba (National Director for Nutrition, Ministry of Public Health, Burkina Faso), Mete Boncougou (Regional Director for Health, Ministry of Public Health, Haut-Bassin, Burkina Faso), and Philippe Nikiema (Biochemist, University of Ouagadougou, Burkina Faso).

The authors' responsibilities were as follows—PK and DR: designed the study; DR: implemented and followed up the study, conducted the analysis, interpreted the data, and drafted the manuscript; M-CH: made substantial contributions to the execution and supervision of the study; HL: coordinated the field investigations; NM: contributed to the execution and supervision of the study; and LH: made substantial contributions to the supervision of the field investigations and data management. All authors substantially contributed to the manuscript and reviewed and approved the final version. The funding agencies had no role in the study design, data collection, data analysis, or writing of the manuscript. None of the authors declared a conflict of interest.

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