Prevention of intrauterine growth retardation by multiple micronutrient supplements during pregnancy in Burkina Faso

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Chapter 6  Prenatal Micronutrient Supplements Cumulatively Increase Foetal Growth

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Introduction

Prenatal multiple micronutrients (UNIMMAP) improve fetal growth only moderately compared to iron and folic acid alone (IFA). Whether this is due to insufficient amounts of UNIMMAP or to IFA being in reality an active control is unknown. We investigated the effect of the overall quantity of micronutrients received during pregnancy on fetal growth. The cumulative micronutrient intake (CMI) was assessed from mid-gestation to delivery in a randomized controlled trial (RCT) in Burkina Faso where daily intake was directly observed. UNIMMAP is a mixture of vitamins and minerals that combines IFA with an additional dose of other micronutrients (2). UNIMMAP tablets contain 15 micronutrients at the level of 1 RDA (1 RDA = Reference Daily Allowance, the level of daily nutrient intake that is estimated to meet the nutritional needs of nearly all healthy people within the main life stages and for most nutritional needs) as specified for women in USA/Canada (3). Recent meta-analyses have suggested that prenatal multiple micronutrients yielded a similar response on birth weight and anthropometry to IFA (1,2). However, the overall effect of UNIMMAP on birth weight is only modest relative to IFA (3–6). However, the overall effect of UNIMMAP on birth weight is only modest relative to IFA (3–6). Nevertheless, a large meta-analysis (7) has shown that IFA tablets could have contributed to this observation. First, IFA, which was used as a control in the vast majority of the UNIMMAP trials, might have exerted its own beneficial effect on fetal growth, as suggested by some previous studies in Nepal (7), Niger (8), and India (9). If IFA were truly an active control, the observable benefit of UNIMMAP would have been expected in women with a higher CMI. Second, higher daily doses of prenatal vitamin/multivitamin supplementation might be necessary to yield a greater effect on fetal growth, as suggested by studies from Tanzania (11), India (12), and India (13), and Guinea-Bissau (14). However, to date, only two trials (15) have assessed the association between micronutrient intake and anthropometry (15). None of these studies considered whether the overall quantity of micronutrients received during pregnancy, as suggested by the above-mentioned studies, higher doses compared to 1 RDA of micronutrient might be necessary to yield a greater effect on fetal growth. The modest reported effect of UNIMMAP relative to IFA could relate to a suboptimal tablet intake, with a stronger effect expected in women with a higher CMI.

Methods

Cumulatively Increase Fetal Growth

In a randomized controlled trial; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.

Analyses were conducted with R (R Core Team, 2013). For maternal and cord blood samples, we used the Swiss-Cohort Study on Women’s Health. The women were from 1991 to 1992, and the study was conducted in Switzerland. The primary objective of the study was to determine the effect of a supplement on birth weight and anthropometry. The study included 1063 single pregnancies who were randomly assigned to receive either IFA or UNIMMAP. The women were followed daily from the start of the study until delivery. The study was conducted in the same Tanzanian population group as the above-mentioned studies. The study was registered at clinicaltrials.gov as NCT00642408. The study was supported by Nutrition Third World and the Belgian Ministry of Development, with no role in study design, data collection, data analysis, or writing of the report. The study was performed in compliance with the Declaration of Helsinki. The Institutional Review Board of the Institute of Tropical Medicine approved the study, and informed consent was obtained from all participants.

Results

The overall quantity of micronutrients received during pregnancy modifies the effect observed on fetal growth. The modest reported effect of UNIMMAP relative to IFA could relate to a suboptimal tablet intake, with a stronger effect expected in women with a higher CMI. The effect on fetal growth is cumulative. The supplementation should therefore begin as early as possible in pregnancy, even if the growth increment per CMI is higher in late than in early pregnancy.
Prenatal Micronutrient Supplements Cumulatively Increase Fetal Growth\textsuperscript{1–3}

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\textbf{Abstract}

Prenatal multiple micronutrients (UNIMMAP) improve fetal growth only moderately compared to iron and folic acid alone (IFA). Whether this is due to insufficient amounts of UNIMMAP or to IFA being in reality an active control is unknown. We assessed the association between cumulative micronutrient intake (CMI) and fetal growth by secondary analysis of a randomized controlled trial in Burkina Faso where tablet intake was directly observed. We applied 2-part residual regression models adjusted for main confounders. Among the 1056 single pregnancies included, the mean CMI (± SD) was 124 ± 54 tablets. The odds of delivering a small-for-gestational-age baby was reduced by 21% (95% CI: 5, 35%; $P = 0.013$) for each additional tertile of CMI. The association between CMI and birth weight was positively modified by gestational age at enrollment ($P$-interaction = 0.001). Each unit of CMI was associated with a 1.6-g (95% CI: 0.3, 3.1; $P = 0.019$) higher birth weight at a mean-centered gestational age at enrollment, with a higher gradient observed later in pregnancy. Maternal BMI at enrollment was also a positive modifying factor ($P$-interaction = 0.02), with no association of CMI with birth weight for low BMI. There was no evidence of an effect modification by group allocation; i.e., we observed the same change in birth weight per unit of CMI with either IFA or UNIMMAP. Yet UNIMMAP increased birth weight by 69 g (95% CI: 58, 81); $P < 0.001$) relative to IFA. We found similar results for thoracic and cephalic circumferences. In conclusion, for both IFA and UNIMMAP, the effect on fetal growth is cumulative. The supplementation should therefore begin as early as possible in pregnancy, even if the growth increment per CMI is higher in late than in early pregnancy. Women with a low BMI should also receive extra energy. J. Nutr. 142: 548–554, 2012.

\textbf{Introduction}

Because pregnant women in poor populations are often deficient in multiple micronutrients (1), UNICEF/WHO/UNU recently recommended the use of UNIMMAP\textsuperscript{8} to replace the usual IFA containing 60 mg iron and 400 μg folic acid (2). UNIMMAP supplements contain 15 micronutrients at the level of 1 RDA as specified for women in USA/Canada (Table 1). Recent meta-analyses reported strong evidence that providing pregnant women with UNIMMAP can improve fetal growth compared to IFA (3–6). However, the overall effect of UNIMMAP on birth weight in the 12 studies that were included was rather modest [+22.4 g (95% CI: 8.3, 36.4); $P = 0.002$] (4). Two mechanisms could have contributed to this observation. First, IFA, which was used as a control in the vast majority of the UNIMMAP trials, might have exerted its own beneficial effect on fetal growth, as suggested by some previous studies in Nepal (7), Niger (8), and the USA (9), but not by others (10). If IFA were truly an active control, the observable benefit of UNIMMAP would have been reduced proportionately. Second, higher daily doses of prenatal micronutrient might be necessary to yield a greater effect on fetal growth, as suggested by studies from Tanzania (11,12), India (13), and Guinea-Bissau (14). However, to date, only two trials were specifically designed to address the differential effect of incremental daily doses and they reported contradictory findings (14,15). In Guinea-Bissau, UNIMMAP was found to increase birth weight only in women randomized to receive a double dose; i.e., 1 RDA yielded no significant effect (14). In the second study, organized in the same Tanzanian population group as the above-mentioned studies, higher doses compared to 1 RDA of multiple micronutrients yielded a similar response on birth anthropometry (15). None of these studies considered whether the overall quantity of micronutrients received during pregnancy (hereafter called CMI) modifies the effect observed on fetal growth. The modest reported effect of UNIMMAP relative to IFA could relate to a suboptimal tablet intake, with a stronger effect expected in women with a higher CMI.

\textsuperscript{1} Supported by Nutrition Third World and the Belgian Ministry of Development, with no role in study design, data collection, data analysis, or writing of the report.

\textsuperscript{2} Author disclosures: D. Roberfroid, L. Huybregts, H. Lanou, J. P. Habicht, M. C. Henry, N. Meda, and P. Kolsteren, no conflicts of interest.

\textsuperscript{3} This trial was registered at clinicaltrials.gov as NCT00642408.

\textsuperscript{4} Abbreviations used: CMI, cumulative micronutrient intake; Hb, hemoglobin; IFA, iron and folic acid; MUAC, mid-upper arm circumference; RCT, randomized controlled trial; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.

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These two hypotheses, an active control and/or an average suboptimal quantity of supplements, can be addressed through the analysis of compliance data (16,17). In case of an active control, a positive association between CMI and fetal growth is also expected in the control group. On the other hand, the existence of a threshold in the number of tablets under which no effect is observed can be indicative of a minimum requirement.

We tested the two hypotheses by secondary analysis of one of the UNIMMAP trials in Burkina Faso in which individual micronutrient intake was accurately recorded (18).

### Methods

Between March 2004 and February 2006, 1426 pregnant women in the catchment area of two health centers in Houndé district, Burkina Faso were randomly assigned to receive either daily IFA or UNIMMAP (18). The randomization scheme was generated by a computer program in permuted blocks of four. UNIMMAP and IFA tablets were identical in appearance (SCAPHARM) and participants and assessors were unaware of the group allocations. Daily supplement intake was directly observed by home visitors recruited by the project. A quality assessment of each home visitor’s work was performed monthly on a random day (19).

Newborn length and weight were measured to the nearest 1 mm using a SECA 207 scale and to the nearest 0.1 g using a SECA 725 scale, respectively. Newborn occipito-frontal head circumference and MUAC were measured to the nearest 1 mm using a SECA girth measuring tape or a SECA 212 tape. All measurements were made in the health centers within the first 24 h after birth. To ensure reliability, all anthropometric variables were measured twice, once by clinic staff and a second time by an anthropometrist hired by the project. The mean of the two measurements was used for analysis. In case of large discrepancy between the two measures, the file was reviewed by a supervisor to check consistency. All weighing scales were calibrated daily. The accuracy and precision of measures were established monthly through a standardization session (20).

The Hb concentration in the mother was measured at her enrollment in the trial and between 30 and 34 wk of gestation by spectrophotometry, using a Hemocue device with a daily calibration check using a Hemocue Control Cuvet. Anemia was defined as Hb concentration < 110 g/L during the first or third trimester and < 105 g/L in the second trimester to account for plasma expansion (21). In case of maternal illness, appropriate treatments were provided according to national guidelines.

The study was approved by the ethics committees of the Center Muraz, Bobo-Dioulasso, Burkina Faso, and the Institute of Tropical Medicine, Antwerp, Belgium.

### Statistical analysis

The analysis included only singleton live-born infants (96.3% of all deliveries). This was due to two separate considerations: first, maternal under-nutrition is not the primary determinant of fetal growth retardation in multiple pregnancies. Second, measuring stillbirths was found unacceptable in this cultural context.

As expected, the correlation between gestational age at enrollment and CMI was important ($r = 0.79; P < 0.0001$), and gestational age at enrollment explained 61.8% of the variance observed in CMI. We addressed this high level of correlation by running a 2-part residual model, i.e., CMI was first regressed on gestational age at enrollment and at delivery, and the residuals of this regression were then inserted as a covariate in the model (22). These residuals reflected deviations from CMI that could not be explained by knowing the individual participation time in the study. The other predictor variables were as follows: type of prenatal micronutrients received (UNIMMAP vs. IFA); type of malaria prevention (sulfadoxine-pyrimethamine vs. chloroquine) and catchment area (health center 1 vs. health center 2) to account for study design; parity (primiparity vs. multiparity), season at randomization (lean vs. fat season), and school education (some vs. none), as preliminary analyses showed that these were independent factors with a small, but significant influence on CMI in our study population; newborn sex, maternal BMI (kg/m²), and Hb concentration (Hb g/L) at enrollment, because previous reports indicated that these predictors could modify the physiological response to prenatal micronutrients (18,23,24); and finally, gestational age at enrollment in the study (weeks), as the effect of prenatal micronutrients might be dependent on when the supplementation is initiated (25,26). Given the high correlation level of this latter variable with CMI, we applied the same 2-part residual procedure as described above; i.e., we used as covariates the residuals of gestational age at enrollment regressed over CMI.

In all the models, CMI was the independent variable of interest, either as a continuous variable or categorized into tertiles. An interaction term was inserted between CMI and gestational age at enrollment (continuous), baseline Hb (continuous), baseline BMI (continuous), type of micronutrient supplements received (IFA vs. UNIMMAP), parity (primiparity vs. multiparity), and newborn sex (male vs. female). Interaction terms were tested together (chunk test). In the presence of an interaction between CMI and a continuous variable, the centering method was applied; i.e., the association between CMI and the outcome was computed at three levels of the interacting covariate (mean − 1 SD, mean, and mean + 1 SD) (27). A stepwise backward procedure was applied with removal of variables when the Likelihood ratio test yielded a $P > 0.10$ for interaction terms (28) or a $P > 0.05$ for all other variables. Multicollinearity was assessed by examining the variance inflation factors (command ESTAT VIP in STATA 11.0).

Birth weight, length, MUAC, and chest and head circumferences were the independent variables tested in the corresponding regression models. Two categorical variables were also considered: small-for-gestational-age was defined as a birth weight < 10th percentile of a reference population with same gestational age (29); large-for-gestational-age was defined as a birth weight > 90th percentile of the study population. Large-for-gestational-age was computed within our cohort population, because the left shift of the whole birth weight distribution rendered any reference population inappropriate for analysis.

The general distribution of CMI against the main outcome variables was visually appraised by a running-line, least-squares smoothing plot (lowess; bandwidth = 0.8) (30). In the presence of important slope variations, a piecewise regression was modeled (31). All analyses were conducted using STATA 11.0 (StataCorp). Values in the text are means ± SD or mean difference (95% CI).

### Results

Birth weight was measured in 1056 singleton live newborns, with no differences in baseline characteristics of the mothers, except a small difference in Hb concentration ($P = 0.02$) (Table 2). The information on maternal micronutrient intake was complete for 98.6% (1041/1056) of the participants. Mean gestational age at enrollment was 16.8 ± 7.3 wk (IQR: 10.3, 22.1 wk). About 40% of participants were recruited during the first trimester of

<table>
<thead>
<tr>
<th>Table 1 Composition of the IFA and UNIMMAP supplements1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient</td>
</tr>
<tr>
<td>Vitamin A, μg</td>
</tr>
<tr>
<td>Vitamin B, mg</td>
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<tr>
<td>Vitamin B, mg</td>
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<td>Vitamin B, mg</td>
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<td>Vitamin B, mg</td>
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<tr>
<td>Vitamin B, μg</td>
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<tr>
<td>Vitamin B, μg</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
</tr>
<tr>
<td>Vitamin D, μg</td>
</tr>
<tr>
<td>Vitamin E, mg</td>
</tr>
<tr>
<td>Zinc, mg</td>
</tr>
<tr>
<td>Iron, mg</td>
</tr>
<tr>
<td>Copper, mg</td>
</tr>
<tr>
<td>Selenium, μg</td>
</tr>
<tr>
<td>Iodine, μg</td>
</tr>
</tbody>
</table>

1 IFA, iron and folic acid; UNIMMAP, UNICEF/WHO/UNU Multiple micronutrient supplement for pregnancy and lactating women.
pregnancy. Pregnant women took 124 ± 54 micronutrient tablets under direct supervision of home visitors, and this number did not differ between study groups (P = 0.58). As expected, early enrollment and high compliance were two strong independent determinants of CMI (Table 3). School education, a lower maternal BMI, and a higher HB concentration were also factors associated with a higher CMI, but this was mainly mediated through an enrollment in the study occurring at a earlier gestational age. There was no difference in maternal age, maternal height, or primiparity percentage among tertiles of CMI or gestational age at enrollment.

Birth weight of all infants was 2898 ± 437 g and of those born at term, 2948 ± 383 g. Birth weight <2500 g occurred in 15% of babies overall and in 11.5% of term babies. In multivariable models, gestational age at enrollment and at delivery, type of micronutrients received, newborn sex, maternal BMI at enrollment, and primiparity were all factors significantly associated with birth weight (Table 4). Women who entered the study in the second and third pregnancy trimester gave birth to babies that were lighter by 84.5 ± 26.2 g (P = 0.001) and 97.9 ± 41.5 g (P = 0.018), respectively, compared to newborns from participants included during the first trimester.

The association between CMI and birth weight was modified by gestational age at enrollment (P-interaction = 0.001) (Table 5). When gestational age was maintained at the mean, the increment in birth weight per tablet was 1.6 g ([95% CI: 0.3, 3.1]); P = 0.019), whereas it was 2.4 g ([95% CI: 0.7, 4.2]; P = 0.006) at (mean + 1 SD) gestational age and only 0.9 g ([95% CI: −0.3, 2.2]; P = 0.15) at (mean − 1 SD) gestational age. In spite of this greater increment in birth weight per tablet, entering the study at a later gestational age tended to be associated with a lower birth weight [−9 g/wk (95% CI: −19, 1); P = 0.07].

Maternal BMI at enrollment (P = 0.02) and primiparity (P = 0.016) were other positive modifying factors. No interaction by newborn sex or maternal HB concentration at enrollment was observed. It is worth mentioning that the type of micronutrient received (IFA vs. UNIMMAP) also did not modify the association between CMI and birth weight (P-interaction = 0.41); the regression curves of CMI relative to birth weight remained quite parallel between the 2 study groups over the entire CMI distribution (Fig. 1).

Overall, the odds of delivering a small-for-gestational-age baby were reduced by 21% ([95% CI: 5, 35]; P = 0.013) for each additional tertile of CMI (Table 6).

The interaction with gestational age at enrollment was also observed for birth length (P = 0.02), thoracic circumference (P = 0.07), and head circumference (P = 0.09); i.e., the association between CMI and anthropometry indicators, except MUAC, was consistently greater in women who had entered the study later during pregnancy (Table 5).

### Table 2: Baseline characteristics and participation pattern by allocation group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>IFA n</th>
<th>UNIMMAP n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>24.2 ± 6.0</td>
<td>23.9 ± 6.1</td>
</tr>
<tr>
<td>Gestational age at enrollment, wk</td>
<td>16.8 ± 7.5</td>
<td>16.9 ± 7.4</td>
</tr>
<tr>
<td>No schooling, %</td>
<td>85.9</td>
<td>82.2</td>
</tr>
<tr>
<td>Primiparity, %</td>
<td>19.2</td>
<td>23.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>161.9 ± 5.9</td>
<td>162.3 ± 5.7</td>
</tr>
<tr>
<td>BMI at enrollment, kg/m²</td>
<td>20.8 ± 2.1</td>
<td>21.0 ± 2.2</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>25.8 ± 2.1</td>
<td>25.6 ± 2.2</td>
</tr>
<tr>
<td>Hb at enrollment, g/dL</td>
<td>11.2 ± 19</td>
<td>109 ± 16</td>
</tr>
<tr>
<td>Anemia at enrollment, %</td>
<td>39.1</td>
<td>46.1</td>
</tr>
<tr>
<td>Participation time, d</td>
<td>152 ± 54</td>
<td>151 ± 54</td>
</tr>
<tr>
<td>CMI, tablet</td>
<td>124 ± 54</td>
<td>129 ± 55</td>
</tr>
<tr>
<td>Compliance, %</td>
<td>82 ± 17</td>
<td>83 ± 19</td>
</tr>
</tbody>
</table>

1 Data are means ± SD or percentages. CMI, cumulative micronutrient intake; Hb, hemoglobin; IFA, iron and folic acid; MUAC, mid-upper arm circumference; UNIMMAP, UNICEFWHO/UNU Multiple micronutrient supplement for pregnancy and lactating women.
2 Only women with a single live newborn whose birth weight was measured are presented.
3 Compliance was calculated as [CMI/(number of participations days)] × 100.

### Table 3: Comparison of participant characteristics stratified per tertile of CMI and per tertile of gestational age at enrollment

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CMI Tertile 1</th>
<th>CMI Tertile 2</th>
<th>CMI Tertile 3</th>
<th>P-trend4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>23.6 ± 5.9</td>
<td>24.3 ± 6.0</td>
<td>24.3 ± 6.3</td>
<td>0.17</td>
</tr>
<tr>
<td>GA enrollment, wk</td>
<td>23.3 ± 6.4</td>
<td>17.1 ± 4.9</td>
<td>10.2 ± 3.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GA delivery, wk</td>
<td>38.5 ± 3.7</td>
<td>39.4 ± 2.4</td>
<td>39.7 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No schooling, %</td>
<td>87.2</td>
<td>86.2</td>
<td>78.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Primiparity, %</td>
<td>25.0</td>
<td>18.9</td>
<td>20.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Height, cm</td>
<td>161.8 ± 5.7</td>
<td>162.0 ± 5.7</td>
<td>162.5 ± 6.0</td>
<td>0.11</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.3 ± 2.1</td>
<td>20.7 ± 2.1</td>
<td>20.8 ± 2.2</td>
<td>0.002</td>
</tr>
<tr>
<td>MUAC, cm</td>
<td>25.5 ± 2.1</td>
<td>25.8 ± 2.1</td>
<td>26.3 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb enrollment, g/dL</td>
<td>105 ± 15</td>
<td>118 ± 18</td>
<td>111 ± 18</td>
<td>&lt;0.001</td>
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<tr>
<td>Participation time, d</td>
<td>98.0 ± 40.5</td>
<td>149.0 ± 30.4</td>
<td>205.5 ± 21.2</td>
<td>&lt;0.001</td>
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<tr>
<td>CMI, tablet</td>
<td>63.2 ± 25.3</td>
<td>123.7 ± 16.5</td>
<td>184.9 ± 20.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compliance, %</td>
<td>67 ± 24</td>
<td>83 ± 12</td>
<td>90 ± 6</td>
<td>&lt;0.001</td>
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<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Gestational age at enrollment2</th>
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<tr>
<td>Maternal age, y</td>
<td>23.5 ± 6.0</td>
<td>24.3 ± 6.0</td>
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<tr>
<td>GA enrollment, wk</td>
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<td>17.1 ± 4.9</td>
</tr>
<tr>
<td>GA delivery, wk</td>
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<td>39.4 ± 2.4</td>
</tr>
<tr>
<td>No schooling, %</td>
<td>78.1</td>
<td>86.6</td>
</tr>
<tr>
<td>Primiparity, %</td>
<td>24.5</td>
<td>19.8</td>
</tr>
<tr>
<td>Height, cm</td>
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<td>BMI, kg/m²</td>
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<tr>
<td>MUAC, cm</td>
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</tr>
</tbody>
</table>

1 Data are means ± SD or percentages. CMI, cumulative micronutrient intake; GA, gestational age; Hb, hemoglobin; MUAC, mid-upper arm circumference.
2 14 missing values.
3 P-trend across tertiles of CMI or gestational age at enrollment for each of the participant characteristic. This was derived from logistic regression for dichotomous variables and from linear regression for continuous ones.
4 Intake was directly observed.
5 Compliance was calculated as [CMI/(number of participations days)] × 100.
TABLE 4 Associations between CMI and birth anthropometry

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Model 1 – Weight, g (n = 1014)</th>
<th>Model 2 – Length, mm (n = 1012)</th>
<th>Model 3 – MUAC, mm (n = 940)</th>
<th>Model 4 – TC, mm (n = 1011)</th>
<th>Model 5 – HC, mm (n = 1014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMI2</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
</tr>
<tr>
<td>CMI3</td>
<td>1 (0, 2) 0.19</td>
<td>0.0 (–0.0, 0.1) 0.82</td>
<td>0.0 (0.0, 0.1) 0.08</td>
<td>0.1 (0.0, 0.2) 0.02</td>
<td>0.0 (0.0, 0.1) 0.08</td>
</tr>
<tr>
<td>UNIMMAP vs. IFA</td>
<td>0.0 (–0.1, 0.1) 0.82</td>
<td>0.0 (0.0, 0.1) 0.08</td>
<td>0.1 (0.0, 0.2) 0.02</td>
<td>0.0 (0.0, 0.1) 0.08</td>
<td>0.0 (–0.1, 0.1) 0.08</td>
</tr>
<tr>
<td>Parity (primi vs. multi)</td>
<td>–0.5 (–1.9, –0.5) &lt;0.001</td>
<td>–0.4 (–0.7, –0.1) 0.22</td>
<td>–0.4 (–0.7, –0.1) 0.22</td>
<td>–0.4 (–0.7, –0.1) 0.22</td>
<td>–0.4 (–0.7, –0.1) 0.22</td>
</tr>
<tr>
<td>GA enrollment, wk</td>
<td>0.0 (–0.0, 0.0) 0.40</td>
<td>0.1 (–0.4, 0.1) 0.22</td>
<td>0.1 (–0.4, 0.1) 0.22</td>
<td>0.1 (–0.4, 0.1) 0.22</td>
<td>0.1 (–0.4, 0.1) 0.22</td>
</tr>
<tr>
<td>GA delivery, wk</td>
<td>1.2 (0.2, 2.2) 0.01</td>
<td>3.3 (1.1, 5.5) 0.003</td>
<td>3.3 (1.1, 5.5) 0.003</td>
<td>3.3 (1.1, 5.5) 0.003</td>
<td>3.3 (1.1, 5.5) 0.003</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>1.0 (–0.1, 0.1) 0.82</td>
<td>1.0 (–0.1, 0.1) 0.82</td>
<td>1.0 (–0.1, 0.1) 0.82</td>
<td>1.0 (–0.1, 0.1) 0.82</td>
<td>1.0 (–0.1, 0.1) 0.82</td>
</tr>
<tr>
<td>Maternal BMI, kg/m²</td>
<td>0.8 (0.2, 1.4) 0.014</td>
<td>0.6 (0.3, 0.8) &lt;0.001</td>
<td>1.2 (0.7, 1.7) 0.001</td>
<td>0.7 (0.3, 1.1) 0.001</td>
<td>0.7 (0.3, 1.1) 0.001</td>
</tr>
</tbody>
</table>

1 Calculated by multiple linear regression models with further adjustment for maternal Hb concentration at enrollment, g/L; type of malaria prevention (sulfadoxine-pyrimethamine vs. chloroquine); school attendance (yes vs. no); season at enrollment (G1 vs. G2); and catchment area (health center 1 vs. health center 2). These models included no interaction terms. CMI, cumulative micronutrient intake; GA, gestational age; Hb, hemoglobin; HC, head circumference; IFA, iron and folic acid; UNIMMAP, mid-upper arm circumference; TC, thoracic circumference; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.

2 Residuals from the recentering method were used, i.e., the association between CMI and the outcome was computed at 3 levels of the interacting covariate (mean – 1 SD, mean, and mean + 1 SD) (27). Mean = 1 SD for GA enrollment was 16.8 ± 7.3 wk and 20.9 ± 2.2 kg/m² for maternal BMI at enrollment.

Discussion

The cumulative supplement intake of prenatal micronutrients was independently associated with a greater fetal growth. This association was modified by gestational age at enrollment, maternal BMI at enrollment, and primiparity. There was no evidence of an interaction with the type of supplements received (UNIMMAP vs. IFA).

A greater CMI resulted in better fetal growth. Although residual confounding cannot be ruled out, this is a plausible finding, considering that each tablet provided 1 RDA of micronutrients in a population with a diet poor in micronutrients (32). Thus, the greatest effect of the supplementation can be expected when it begins early during pregnancy; i.e., when the woman’s needs for micronutrients are addressed during the longest possible period. Indeed, we observed that women who entered the study in the first trimester had heavier newborns than women who were included later on. However, recommending an early prenatal micronutrient supplementation might be wishful thinking in many settings in poor countries, because the first prenatal visit to health services usually occurs quite late. In our study, for example, only 40% of women were included during the first trimester, despite active case finding through monthly home visits. Social communication to encourage earlier antenatal visits and community-based micronutrient supplementation might be helpful strategies. Alternatively, a higher cumulative

TABLE 5 Associations between CMI and birth anthropometry stratified by levels of interacting factors

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Model 1: weight, g (n = 1014)</th>
<th>Model 2: length, mm (n = 1012)</th>
<th>Model 3: MUAC, mm (n = 940)</th>
<th>Model 4: TC, mm (n = 1011)</th>
<th>Model 5: HC, mm (n = 1014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMI2</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
<td>( \beta ) (95% CI) P</td>
</tr>
<tr>
<td>CMI3</td>
<td>0.001 4</td>
<td>0.02*</td>
<td>0.19*</td>
<td>0.07*</td>
<td>0.09*</td>
</tr>
<tr>
<td>Mean GA 1 SD</td>
<td>0.9 (–0.3, 2.2) 0.15</td>
<td>0.01 (–0.0, 0.0) 0.88</td>
<td>—</td>
<td>0.08 (0.0, 0.15) 0.03</td>
<td>0.06 (0.0, 0.10) 0.014</td>
</tr>
<tr>
<td>Mean GA</td>
<td>1.6 (0.3, 3.1) 0.02</td>
<td>0.02 (–0.0, 0.0) 0.83</td>
<td>—</td>
<td>0.10 (0.0, 0.17) 0.01</td>
<td>0.07 (0.0, 0.12) 0.006</td>
</tr>
<tr>
<td>Mean GA + 1 SD</td>
<td>2.4 (0.7, 4.2) 0.006</td>
<td>0.05 (–0.0, 0.0) 0.36</td>
<td>—</td>
<td>0.12 (0.0, 0.21) 0.02</td>
<td>0.08 (0.0, 0.15) 0.009</td>
</tr>
<tr>
<td>CMI2</td>
<td>0.02*</td>
<td>0.32*</td>
<td>0.49*</td>
<td>0.05*</td>
<td>0.02*</td>
</tr>
<tr>
<td>Mean BMI: 1 SD</td>
<td>0.8 (–0.4, 2.1) 0.19</td>
<td>—</td>
<td>—</td>
<td>0.08 (0.0, 0.15) 0.03</td>
<td>0.06 (0.0, 0.10) 0.016</td>
</tr>
<tr>
<td>Mean BMI + 1 SD</td>
<td>1.6 (0.1, 3.1) 0.04</td>
<td>—</td>
<td>—</td>
<td>0.12 (0.0, 0.21) 0.04</td>
<td>0.10 (0.0, 0.15) 0.001</td>
</tr>
<tr>
<td>CMI2</td>
<td>0.02*</td>
<td>0.13*</td>
<td>0.98*</td>
<td>0.29*</td>
<td>0.21*</td>
</tr>
<tr>
<td>Primiparous</td>
<td>2.5 (–0.1, 5.0) 0.05</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Multiparous</td>
<td>0.5 (–1.0, 1.9) 0.52</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1 An interaction term was inserted between CMI and gestational age at enrollment, CMI and newborn sex, CMI and maternal Hb concentration at enrollment, CMI and parity, and CMI and maternal BMI at enrollment in the multiple linear regression models presented in Table 4. Adjustment was made for type of micronutrients received (UNIMMAP vs. IFA); parity (primiparity vs. multiparity), gestational age at enrollment, and at delivery; newborn sex, maternal BMI at enrollment, kg/m²; maternal Hb concentration at enrollment, g/L; type of malaria prevention (sulfadoxine-pyrimethamine vs. chloroquine); school attendance (yes vs. no); season at enrollment (G1 vs. G2); and catchment area (health center 1 vs. health center 2). CMI, cumulative micronutrient intake; GA, gestational age; Hb, hemoglobin; HC, head circumference; IFA, iron and folic acid; UNIMMAP, mid-upper arm circumference; TC, thoracic circumference; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.

2 Residuals from the recentering method were used, i.e., the association between CMI and the outcome was computed at 3 levels of the interacting covariate (mean – 1 SD, mean, and mean + 1 SD) (27). Mean = 1 SD for GA enrollment was 16.8 ± 7.3 wk and 20.9 ± 2.2 kg/m² for maternal BMI at enrollment.

3 Residuals from the recentering method were used, i.e., the association between CMI and the outcome was computed at 3 levels of the interacting covariate (mean – 1 SD, mean, and mean + 1 SD) (27). Mean = 1 SD for GA enrollment was 16.8 ± 7.3 wk and 20.9 ± 2.2 kg/m² for maternal BMI at enrollment.
Independent
variables
Dependent variables
Model 1: small-for-gestational age
Model 2: large-for-gestational age
P
OR (95% CI)
P
CMI
Continuous
0.99 [0.98, 1.00]
0.003
1.00 [0.99, 1.01]
0.85
Per tertile
0.79 [0.65, 0.95]
0.013
1.10 [1.00, 1.20]
0.55
UNIMMAP vs. IFA
0.78 [0.60, 1.02]
0.07
1.76 [1.12, 2.78]
0.014
GA enrollment,
wk
1.13 [1.08, 1.18]<0.001
0.97 [0.88, 1.08]
0.49
GA delivery, wk
—
1.35 [1.17, 1.58]<0.001
Sex (male vs. female)
0.91 [0.70, 1.19]
0.49
1.97 [1.25, 3.11]
0.004
Parity (primi. vs. mul.)
2.12 [1.52, 2.98]<0.001
0.15 [0.05, 0.43]<0.001
Maternal BMI, kg/m²
0.89 [0.83, 0.95]<0.001
1.16 [1.06, 1.28]
0.002

1 Results are from logistic regression models with further adjustment for maternal Hb concentration at enrollment, g/L; type of malaria prevention (sulfadoxine-pyrimethamine vs. chloroquine); school attendance (some vs. none); season at enrollment (lean vs. fat); catchment area (health center 1 vs. health center 2). No interaction terms were inserted in these models. CMI, cumulative micronutrient intake; GA, gestational age; IFA, iron and folic acid; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.
2 Small-for-gestational age was defined as a birth weight less than the 10th percentile of the reference population with same gestational age (29).
3 Large-for-gestational age was defined as a birth weight greater than the 90th percentile of the study population.
4 Residuals from the regression of CMI on gestational age at enrollment and at delivery were used.
5 Residuals from the regression of gestational age at enrollment on CMI were used.

FIGURE 1 Distribution of birth weight over CMI by type of micronutrient supplement. Regression lines and points were fitted from a 2-part residual model, i.e., CMI was first regressed on gestational age at enrollment and at delivery and the residuals of this regression were then inserted as a covariate in the model (22). The model was adjusted for primiparity (yes/no), maternal BMI at enrollment (kg/m²), maternal Hb concentration at enrollment (g/L), type of micronutrient supplement (IFA vs. UNIMMAP), type of malaria prevention (sulfadoxine-pyrimethamine vs. chloroquine), school attendance (some vs. none), season at enrollment (lean vs. fat), newborn sex, and catchment area (health center 1 vs. health center 2). Birth weight was the dependent variable of this model and the resulting fitted values were plotted. The regression lines were obtained by locally weighted scatterplot smoothing (lowess) of the fitted values. Hb, hemoglobin; IFA, iron and folic acid; UNIMMAP, UNICEF/WHO/UNU multiple micronutrient supplement for pregnancy and lactating women.

dose could be achieved by increasing the daily dose of micrornutrients. However, evidence supporting such an alternative approach is scanty; to our knowledge, only two trials addressed that question with an appropriate design. One RCT in Guinea-Bissau reported that the mean birth weight increased by 53 g (95% CI: –19, 125; P = 0.15) with UNIMMAP and by 95 g (95% CI: 24, 166; P = 0.009) when twice the dose of UNIMMAP was provided compared with a placebo (14). Although these results might indicate that a daily dose of more that 1 RDA is more efficient, in reality the birth weight did not significantly differ between the two intervention groups. This might have been due to a low statistical power of the study (n = 360 newborns/group) along with quite a short supplementation duration (mean number of tablets: 81 ± 42). Another trial in Tanzania compared multiple and single RDA of multivitamins and did not detect a difference in birth weight and other pregnancy outcomes between the two groups (15). This lack of difference might simply reflect that multiples of RDA (from 3 to 15 times the RDA for some micronutrients), instead of 1 RDA, exceeded what can be physiologically utilized daily. Measuring urine concentrations of vitamin B metabolites would have generated valuable insights on this question.

Whether prenatal micronutrient supplementation should be started at a specific gestational age for getting an optimal health effect remains uncertain. Nutrients in the first trimester may be more important to development and differentiation of various organs, and later in pregnancy they may be important for overall fetal growth (25). Lee et al. (26) suggested that improvements in iron and folate nutriture were highly dependent on when the supplementation program was initiated. In their study, IFA supplementation initiated during the first pregnancy trimester had more effect on iron nutriture indexes than supplementation initiated at 20 wk of gestation. In reality, their results were more a reflection of iron being stored over a longer period rather than the effect of a differential prenatal timing per se. Although our study was not designed to assess the effect of initiating micronutrient supplementation at various stages of pregnancy, participants were included at very different gestational ages. After controlling for potential confounding factors, we found a stronger association between CMI and birth weight, but also thoracic and cephalic circumferences, for women entering the study at a later gestational age. Although counterintuitive at first sight, this is a plausible observation. Women recruited late in pregnancy had not been supplemented previously; i.e., micronutrient deficiencies were likely to be more pronounced in these individuals than in early participants and the utilization of supplements might have been more efficient. A similar phenomenon has already been described for iron (33). The interaction observed with primiparity (P = 0.016) might relate to the same explanation, because we previously reported that primiparous women in this study population presented a worse nutritional status compared to multiparous ones (18). In spite of this greater gradient of birth weight by unit of CMI, primiparous women...
delivered newborns with a much lower birth weight than multiparous ones, as has also been observed in other settings (34). The observed effect modification on birth weight by parity might also be a chance finding, because it was not observed for the other indices of fetal growth.

Maternal BMI at enrollment was also a factor modifying the association between CMI and birth weight, the amplitude of this association increasing at greater levels of maternal BMI. In women with a low BMI, there was no association between CMI and birth weight. This is a finding consistent with results that we previously published (18) and with the pooled analysis of 12 RCT on prenatal UNIMMAP, where the positive effect of the UNIMMAP on birth weight was greatest in heavier women and around zero among women with a low BMI (4). One plausible explanation is that micronutrients are not optimally utilized in the presence of maternal energy deficiency, because their metabolism and their active transport to the fetus require energy. Another RCT in Burkina Faso found that providing UNIMMAP with additional macronutrients was more effective than UNIMMAP alone in women with a BMI <18 kg/m² (35). Also, this association might be indirect. It has been demonstrated that maternal BMI, placental volume, and birth weight are associated (36). It could be that micronutrient transfer to the fetus is greater when the placenta is better developed, as observed in women with a higher BMI (37).

The association between CMI and fetal growth did not differ in women receiving IFA or UNIMMAP; i.e., a higher birth weight was observed in newborns from mothers who used more micronutrient tablets also in the IFA group. This is an important finding, because IFA has mainly been considered a placebo as micronutrient tablets also in the IFA group. This is an important finding, because it was not observed for the other indices of fetal growth. In conclusion, implementing further studies for assessing the health benefit of higher cumulative UNIMMAP intake by starting the supplementation very early during pregnancy or even before conception is warranted. In settings where low maternal BMI is prevalent, supplements of energy should also be provided. Such studies should include cautious safety monitoring, notably because an association with CMI was also demonstrated for head circumference.

In a recent Cochrane review found no evidence of increased birth weight associated with IFA (10). In that review, however, the mean difference in birth weight between those whose mothers had taken iron supplements and those whose mothers had not was 36.1 g [(95% CI: −4.84, 77.0); P = 0.08], a result that was nonsignificant. Only two of the studies in this meta-analysis of 10 studies were carried out in developing countries (7,8). In Nepal, mothers receiving IFA gave birth to newborns who were on average heavier by 37 kg (95% CI: −16, 90) than those in the control group. Chest circumference and head circumference were also significantly higher (7). In Niger, no increase in birth weight with iron supplements was observed, although the small sample size (n = 197) and late enrollment during pregnancy might have contributed to this negative outcome. Since the Cochrane review was published, a third RCT in rural China also reported no difference in birth weight between women receiving IFA and those receiving only folic acid [24.3 g (95% CI: −10.3, 59.0); P = 0.169] (38). In contrast, another RCT conducted in nonanemic women in the USA reported an important increase in mean birth weight with prenatal iron supplements [205.0 g (95% CI: 48.8, 361.2); P = 0.010] (9). Although the evidence is not definitive, the effect of IFA on fetal growth is physiologically plausible. IFA might affect fetal growth through improving maternal HB concentration and thus oxygen content of maternal blood, which possibly influence the development of the placenta and subsequently human chorionic gonadotropin and human placental lactogen concentrations (23,39). One alternative physiological pathway may be that IFA alleviates the stimulation of the synthesis of corticotrophin-releasing hormone due to iron deficiency, with a subsequent decrease in the production of fetal cortisol (40,41).

In any case, this finding, i.e., the association between CMI and fetal growth with both UNIMMAP and IFA, allows interpreting the results of various meta-analyses on UNIMMAP and birth weight in a new light (3–6). The mean 22.4-g [(95% CI: 8.3, 36.4); P = 0.002] increase in birth weight in the UNIMMAP group relative to the IFA group (4) is in addition to the birth weight increment due to IFA itself. For instance, in our study, UNIMMAP increased birth weight by a mean 69.0 g relative to IFA, but for 100 tablets, UNIMMAP would increase birth weight by a mean 229 g [69.0 + (100 × 1.6)] relative to a hypothetical group receiving no supplements. It is also important to realize that the mean 22.4-g increase in birth weight relates to an average intake of micronutrient tablets observed in the individual studies. This intake was not optimal in every study (42). As suggested by our results, increasing the number of micronutrient tablets provided during pregnancy could result in a greater effect on fetal growth. Increasing the overall micronutrient intake, however, must be weighed against the potential for harm in some context. Each additional tablet of micronutrient, UNIMMAP or IFA, increased the head circumference by 0.07 mm, a finding consistent with the results of another trial in Nepal, where both IFA and multiple micronutrients increased mean head circumference compared to controls (7). These observations bear potentially important health implications as regards delivery complications in mothers receiving high amounts of IFA or UNIMMAP (43), particularly in settings with suboptimal obstetrical care (6,15). Further prenatal supplementation studies should include cautious safety monitoring.

The main limitation of our study was its reliance on adherence data; i.e., women were not randomized to receive different doses of IFA or UNIMMAP. However, our adherence data were based on direct observation of tablet intake and the analyses were adjusted for the effect of potential confounders. Another difficulty was the correlation existing between micronutrient dose and gestational age at enrollment, i.e., women who received the most supplements also entered the trial earlier. This was accounted for by an analysis based on residuals (22).

In conclusion, implementing further studies for assessing the health benefit of higher cumulative UNIMMAP intake by starting the supplementation very early during pregnancy or even before conception is warranted. In settings where low maternal BMI is prevalent, supplements of energy should also be provided. Such studies should include cautious safety monitoring, notably because an association with CMI was also demonstrated for head circumference.

Acknowledgments
D.R. and P.K. designed research; D.R., L.H., M.C.H., H.L., and N.M. oversaw the field work; D.R. and J.P.H. analyzed data; N.M. oversaw the field work; D.R. and J.P.H. analyzed data; D.R. and L.H. wrote the paper; and D.R. and P.K. had primary responsibility for final content. All authors read and approved the final manuscript.

Literature Cited


