

Prenatal multiple micronutrient supplementation impact on biochemical indicators during pregnancy and postpartum

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Abstract

Objective. The objective of this analysis was to test the impact of daily supplementation with multiple micronutrients (MM) during pregnancy on Zn, vitamin A and folate status compared to iron only (Fe). **Material and Methods.** The study was carried out during 1997-2000 in a semi-urban community in Morelos state, Mexico. Women were randomly assigned to MM (n= 249) or Fe (n= 258) and received supplements daily (6 d/wk) under supervision by the field team from recruitment (approximately 9 weeks pregnancy) until delivery. Blood samples were collected on a sub-sample of women at baseline, 32 weeks pregnancy and one month postpartum (IPP) and assessed for serum zinc, retinol and whole blood folate (baseline and IPP only). A breast milk sample was extracted at IPP and assessed for retinol content. **Result.** At baseline there was no significant difference between supplementation groups in mean Zn, retinol or folate concentrations or the prevalence of deficiencies (Zn 12.2%, vitamin A 2.8%, folate 5.3%). Mean change in Zn and retinol concentrations from baseline to 32 weeks pregnancy did not differ between groups or between baseline and IPP for Zn, retinol or folate. At IPP, there was a tendency (p= 0.09) towards a lower prevalence of folate deficiency/depletion in the MM group (10.0%) than the Fe group (18.5%).

Resumen

Objetivo. Evaluar el efecto de la suplementación diaria con múltiples micronutrientos (MM) durante el embarazo en el estado de zinc, vitamina A y folato comparado con la suplementación sólo con hierro (Fe). **Material y métodos.** El estudio se realizó en una comunidad semiurbana en el estado de Morelos, México, entre 1997 y 2000. Las mujeres fueron asignadas aleatoriamente a recibir un suplemento de MM (n= 249) o Fe (n= 258) diariamente (6 días/semana), cuyo consumo fue supervisado por personal de campo, desde la evaluación basal (aproximadamente 9 semanas de gestación) hasta el parto. En una submuestra de mujeres participantes, se tomaron muestras de sangre venosa, a las 9 y 32 semanas de embarazo y al mes posparto (IPP). Se midieron las concentraciones séricas de zinc y retinol y la concentración de folato en sangre total, esta última sólo en la evaluación basal y al IPP. Además se colectó una muestra de leche materna al IPP, en donde se midió la concentración de retinol. **Resultados.** En la evaluación basal no hubo diferencias significativas entre grupos en las concentraciones promedio de zinc y retinol, ni en la concentración de folato o en la prevalencia de deficiencias (Zn 12.2%, vitamina A 2.8%, folato 5.3%). El cambio promedio en zinc y retinol de la evaluación basal a la semana 32 de embarazo, no fue diferente entre grupos, tampoco

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Conclusions. MM supplementation during pregnancy did not improve zinc or vitamin A status compared to Fe only. There is some indication that folate status may have improved with MM supplementation despite low prevalence of deficiency. While lack of response in serum retinol may be explained by generally adequate status, the lack of impact on zinc status requires further exploration.

Key words: zinc; retinol; folic acid; randomized controlled trial; dietary supplements; Mexico

entre la evaluación basal y al IPP en zinc, retinol o folato. Al IPP hubo una tendencia ($p=0.09$) a menor prevalencia de deficiencia/depleción de folato en el grupo de MM (10.0%) que en el grupo Fe (18.5%). **Conclusiones.** La suplementación con MM durante el embarazo no mejoró el estado de zinc y vitamina A comparada con la suplementación sólo con Fe. Sin embargo, el estado de folato puede haber mejorado con la suplementación con MM, a pesar de la baja prevalencia de deficiencia de folato. La falta de efecto en vitamina A podría ser explicada por el adecuado estado de la misma en las mujeres estudiadas y la falta de efecto en el estado de zinc requiere futura exploración.

Palabras clave: zinc; retinol; ácido fólico; ensayo controlado aleatorio; suplementos dietéticos; México

During pregnancy, metabolic demands are increased as a result of physiological and hormonal changes in the mother and growth of the fetus.¹ During this period, micronutrient deficiencies may have serious consequences for pregnancy outcomes. Zinc deficiency in animal models causes intrauterine growth retardation (IUGR) and congenital malformations.² In humans, zinc deficiency during pregnancy is associated with low birth weight, IUGR and preterm delivery.^{3,4} Vitamin A deficiency is associated with adverse maternal, fetal and neonatal outcomes and may be a cause of anemia.⁵ The relationship between maternal hemoglobin concentration and perinatal mortality is well documented^{6,7} and periconceptual folate status is important in the development of neural tube defects.^{8,9}

Micronutrient deficiency during pregnancy continues to be an important public health problem throughout the world. In Mexican women 12 to 49 years of age, iron and zinc deficiency are high (40% and 30%, respectively).¹⁰ In a large nationally representative nutrition survey, folate deficiency was detected in 5% and vitamin A depletion in 4 % of women of reproductive age.¹¹

Although several multiple micronutrient supplementation trials have been conducted, the findings are mixed for birth outcomes. A recent Cochrane review of supplementation trials concluded that multiple micronutrient supplementation improves fetal growth compared to placebo but not compared to iron or iron-folate supplementation.¹² In Nepal, multiple micronutrient supplementation during pregnancy was not associated with markers of inflammation¹³ and may be associated with higher early infant mortality.¹⁴ Evidence for the impact of multiple micronutrient supplementation on a number of outcomes is still considered inadequate and further research is required before a switch from iron-

folate supplementation is implemented.¹² Few supplementation trials have reported biochemical markers of micronutrient status. Functional outcomes are often difficult to measure due to measurement imprecision or the need for large sample sizes and the documentation of impact on intermediary variables such as micronutrient status is vital. In Nepal,¹⁵ multiple micronutrient supplementation resulted in improved folate status during pregnancy and reduced the prevalence of riboflavin, vitamin B₆, vitamin B₁₂, folate, and vitamin D deficiencies.

We previously reported that a randomized controlled multiple micronutrient supplementation trial conducted in Mexico had no effect on birth weight, length or gestational age compared to iron only supplementation.¹⁶ In the same trial, hemoglobin concentration was lower in the multiple micronutrient group during the third trimester, with no lasting difference between the groups at one month postpartum.¹⁷ We report here the impact of daily supplementation with multiple micronutrients during pregnancy on zinc, vitamin A and folate status during pregnancy and 1 month postpartum, zinc and vitamin A in cord blood, and breast milk retinol concentration at one month postpartum in women who participated in a randomized controlled trial in Mexico, using supplementation with iron only as comparison.

Material and Methods

A detailed description of the study population, the intervention, data collection and primary results have been published previously.¹⁶⁻¹⁸ We will review the methods briefly here, with emphasis on those that are relevant to the assessment of micronutrient status. This study was approved by the Human Subjects Committee at Emory

University, Atlanta, GA, USA, and the Research, Ethics and Biosecurity Commissions at National Institute of Public Health (INSP), Cuernavaca, Mexico. Women were invited to participate after receiving complete details of the study objectives procedures, risks and benefits and if interested, the consent declaration was read to the participants and if agreed, they were asked to sign or provide a finger print.

Intervention

All women participants were randomly assigned to receive a multiple micronutrient (MM) or iron only (Fe) tablet once daily from recruitment until delivery. Both tablets contained 60 mg of iron in the form of ferrous sulfate and were identical in color and size. The MM tablet also contained 100 to 150% of the recommended dietary allowance¹⁹ for zinc, iron, vitamins A, C, B₆, folate and several other micronutrients. The exact formula of the tablets has been published previously.¹⁶ Four color coded supplementation groups were created (two per type of supplement) to reduce the possibility of unblinding of the trial and tablets were distributed in color-coded bottles with the participants name and ID number written on the bottle. The supplement was distributed daily to each woman in her home by trained field workers who observed and noted consumption. The study field staff, investigators and the participating women remained blind to the treatment throughout the field stage and until after analyses of primary outcomes were completed. The analyses reported here were conducted after breaking the supplement code.

The study was carried out during 1997-2000 in semi-urban community in Morelos state, Mexico. A total of 873 women were assigned to treatment and 645 (73.9%) completed the trial and resulted in a singleton live births. The reasons for loss to follow-up have been explained elsewhere.^{16,17} The original design was to obtain a venous blood sample from a 30% random sub-sample of all pregnancies, but samples were available for many more women and serum zinc was assessed on 565 (64.7% of women recruited) (MM, n= 249; Fe, n= 258). Due to budgetary limitations, we assessed serum zinc for the follow-up visits (32 weeks pregnancy and one month postpartum) on a random sub-sample of those for whom baseline zinc had been assessed. For the same reason, serum retinol was assessed on a random sub-sample of women who had sample available at baseline and at least one of the two follow-up visits. The methodology for assessment of whole blood folate was implemented at INSP after data collection was underway. We therefore obtained samples on the last women recruited into the trial (approximately 100) and

included only baseline and one month postpartum due to budgetary constraints. Cord blood and breast milk samples were collected from the first approximately 100 women enrolled in the study. Cord blood was assessed for zinc and retinol and breast milk for retinol.

Data collection and sample analysis

Women with confirmed or suspected pregnancy were identified through a monthly community census, provided with details of the study and offered an immunological urine test for pregnancy (Gestaltex, Applied Inc. Biotech, San Diego, USA). Duration of pregnancy was then estimated based on last menstrual period, except in the case of an uncertain date in which the woman was referred to receive an ultrasound examination. This method has been used successfully by our group in similar populations.²⁰ Women with confirmed pregnancies of less than 13 completed weeks were provided with details of the study and invited to participate. Additional exclusion criteria for the present study included multiple pregnancies, use of micronutrient supplements and refusal to participate.^{16,17}

Socio-economic, demographic and reproductive history information was collected by questionnaire at recruitment and height and weight were measured. Information on the status and progress of pregnancy was collected at three additional visits (26, 32 and 37 weeks of pregnancy) and included weight, dietary intake and blood pressure. Details of the blood sampling and processing methods have been published previously.¹⁷ Briefly, a 7 ml venous blood sample was taken at the field clinic by trained phlebotomists at baseline, 32 weeks pregnancy and one month postpartum. Trace element free collection tubes (Vacutainer, Becton Dickinson, Franklin Lakes, New Jersey, USA) and sample processing materials were used. Whole blood samples for folate determinations were collected on labeled filter paper (filter paper No. 903, S&S, Inc., New York, NY, USA) and stored in sealed plastic bags with desiccant until analysis. The remaining sample was centrifuged at 2000 rpm at room temperature in the field clinic. Serum was transferred to trace element-free microtubes and frozen immediately.

To collect cord blood samples and weight and measure the newborns as soon as possible after birth, a trained and standardized field-worker from the community was on-call 24 hr a day. Women were asked to notify the field worker when they went into labor and to advice where they planned to deliver. Arrangements were made with local midwives, clinics and hospitals to permit the study field-worker into the delivery room (with the mothers consent). Once the cord had been clamped, it

was allowed to drain and the sample represented a mix of cord venous and arterial blood. Sample processing and analysis were as described for maternal serum.

A breast milk sample was obtained at one month postpartum by electric pump (Engell Elite, Ameda Egnell, Switzerland). Milk was extracted from one breast until empty, with three samples during one day (approximately 10:00, 14:00 and 16:00 h); a minimum of two h after the infant was fed. When the final sample was obtained, the three samples were pooled and aliquots of 15 ml obtained. The serum and human milk samples were stored temporarily in the field site at -20°C then transferred within a week to the INSP laboratory and stored at -70°C until analysis.

Serum and human milk retinol concentration and folate whole blood concentration were analyzed at the INSP Nutrition Laboratory and serum zinc concentration was analyzed at the Nutrition and Physiology Laboratories of the National Institute of Medical Sciences and Nutrition, *Salvador Zubirán* (Mexico, City). The quantitative measurement of serum and human milk retinol was determined by high-performance liquid chromatography (HPLC) in a Waters instruments (Waters Co., Milford, MA, USA).²¹ The milk sample was saponified with potassium hydroxide at 60°C during 45 min before the retinol extraction. Folate in whole blood was assessed using dried blood spots on filter paper using a microbiological assay.^{22,23} Serum zinc in maternal and cord blood was assessed using an atomic absorption spectrophotometer (AAS) (Perkin Elmer; model 2380, Norwalk, Connecticut, USA) with a wavelength of 213.9 nm. A zinc standard (Sigma N.C. 2750, ST, Louis Missouri, USA) was used for each run. Before analysis, serum was defrosted and a dilution 1:5 with deionized water was made. The corresponding dilutions were made for the standard curve. The average of three readings from the AAS for each sample was obtained.

Variable creation and statistical analysis

Adherence to supplementation was assessed using the tablet consumption data recorded daily by the field worker and reported as the percentage of tablets offered that were consumed. Overweight was defined as BMI >25²⁴ at recruitment. An index of economic status was calculated using principal components analysis.¹⁶ This index provides a comparative classification of each household within the actual sample, but does not permit comparison with the broader society. All participants were from low income households (based on housing characteristics and household possessions).

Zinc deficiency was defined as serum zinc <8.6 $\mu\text{mol/L}$ at baseline, <7.6 $\mu\text{mol/L}$ at 32 wk pregnancy.²⁵

An appropriate cut-off point for one month postpartum has not been well defined,²⁵ and we present, with caution, the prevalence of deficiency using the cut-off for non-pregnancy women in fasting blood samples (<10.0 $\mu\text{mol/L}$). As has been reported previously for Mexico,¹⁰ vitamin A deficiency (serum retinol <0.35 $\mu\text{mol/L}$) is very low in adult women.¹⁰ We defined vitamin A depletion/deficiency as serum vitamin A concentration <0.70 $\mu\text{mol/L}$.^{26,27} The prevalence of folate deficiency (whole blood folate <129.2 nmol/L) is also low in Mexico¹¹ and we present the prevalence of depletion/deficiency together as the concentration of whole blood folate <147.29 nmol/L.²⁸

Demographic, socioeconomic, health and nutrition characteristics of the women were compared at baseline between the sub-sample included in this analysis and all other women assigned to treatment, regardless of whether they completed the study. In this way, we attempt to follow as close as possible an intention to treat analysis. The impact of supplementation on micronutrient status is assessed by comparing the change in serum zinc, retinol and whole blood folate between baseline and the 2 follow-up periods (32 weeks pregnancy and one month postpartum). Unadjusted comparisons of change in micronutrient status and percent deficiency were compared between supplementation groups using χ^2 and Student's *t* or Kruskal Wallis test. Multiple linear or logistic regression was used to assess the impact of supplementation on micronutrient status at 32 weeks pregnancy and one month postpartum without adjustment, and adjusting for baseline BMI, statistically significantly different in the full sample and micronutrient sub-sample between supplement groups at baseline.¹⁶ Since folate concentrations were non-normally distributed, all analyses were conducted with log transformed values. Cord blood zinc and breast milk retinol were compared between supplementation groups, with and without adjustment for potential confounders as described above. Data were analyzed with Stata 9.2 package (College Station, Texas, USA, Stata Corp., 2006).

Results

Of the 873 women assigned to treatment,¹⁶ 507 (58.1%) met the criteria of blood sample available to calculate at least one difference (baseline to 32 weeks and/or baseline to one month postpartum) or cord blood or breast milk for assessment of at least one nutrient and were included in this analysis. Samples were available for one of the above mentioned analysis only from 89 women and 142, 92 and 103 women contributed samples for 3, 4 or 5-8 analysis, respectively. Duration of pregnancy,

remunerated work, hemoglobin concentration and compliance with supplementation were significantly ($p<0.05$) different at recruitment in women included in this sub-sample than those excluded (Table I). Similar to the overall sample¹⁶ mean weight and BMI were significantly different ($p<0.05$) between supplement groups at baseline among those included in the analyses (Table II), but there were no other significant differences between them. Compliance with supplementation was high (consumed on >80% of days that it was offered) and did not differ between groups in the entire study sample¹⁶ or among those included or excluded from this analysis or between supplementation groups in the sub-sample. In addition, comparisons were made of some characteristics of the different subsamples for biochemical analysis (among those included in the sub-sample of zinc, vitamin A and folate), finding that within the zinc and folate sub-sample, the socio-economic status was marginally lower ($0.05 < p < 0.1$) than that of women included in this analysis. Likewise, hemoglobin concentration was marginally lower in the zinc sub-sample, compared to the sample included in this analysis.

There was no difference in the change in serum zinc or retinol concentrations from baseline to 32 weeks

Table I
COMPARISON OF SELECTED CHARACTERISTICS
AT RECRUITMENT FOR WOMEN INCLUDED
AND NOT INCLUDED IN THE BIOCHEMICAL ANALYSES

	Included in at least one biochemical analysis (n= 507)	Not included in biochemical analysis (n= 366)
Maternal age, yrs	23.2 ± 5.4*	23.9 ± 5.5
Duration of pregnancy, wk	9.0 ± 2.3	8.5 ± 3.0 [‡]
Weight, kg	53.2 ± 9.5	53.8 ± 10.3
Height, cm	148.6 ± 4.8	148.5 ± 4.9
Body mass index	24.7 ± 4.4	23.7 ± 3.6 [‡]
Socio-economic status [§]	0.06 ± 1.05	0.02 ± 1.05
Indigenous status [#] , %	31.1	27.9
Literate, %	94.1	91.9
Married or in stable relationship, %	95.5	95.1
Remunerated work, %	7.9	15.0 [‡]
Hemoglobin concentration, g/L	123.8 ± 13.9	127.2 ± 14.8 [‡]
Compliance with supplementation ^{&} , %	88 (84, 90)	82 (63, 90) [‡]

* Mean ± SD, or percent

[‡] Different from those included in any analysis ($p<0.05$)

[§] Based on household characteristic and possessions using principal components analysis (16)

[#] Based on response to question whether she can speak and/or understand Nahuatl or any other indigenous language

[&] Number of days supplement was consumed as a total of the number of days that it was offered. Presented as median and interquartile range

Table II
COMPARISON OF SELECTED CHARACTERISTICS
AT RECRUITMENT FOR WOMEN INCLUDED
IN THE BIOCHEMICAL ANALYSES BY TREATMENT GROUP

	Iron only (n= 258)	Multiple micronu- trients (n= 249)
Maternal age, yrs	23.4 ± 5.5%*	23.0 ± 5.2
Duration of pregnancy, wk	9.0 ± 2.2	8.9 ± 2.4
Weight, kg	54.3 ± 10.1	52.1 ± 8.7 [‡]
Height, cm	148.7 ± 4.6	148.6 ± 5.1
Body mass index	24.7 ± 4.4	23.7 ± 3.6 [‡]
Socio-economic status [§]	0.09 ± 1.05	0.02 ± 1.05
Indigenous status [#] , %	28.5	33.9
Literate, %	93.7	94.6
Married or in stable relationship, %	96.9	93.9
Remunerated work, %	6.9	8.8
Hemoglobin concentration, g/L	124.4 ± 12.9	123.2 ± 14.9
Anemia, %	11.8	14.1
Serum zinc, µmol/L	11.7 ± 3.5	11.8 ± 2.9
Serum retinol, µmol/L	1.13 ± 0.25	1.13 ± 0.27
Whole blood folate, nmol/L	320.8 (225.5, 429.2)	329.9 (210.7, 455.0)

* Mean ± SD, or percent. Data for whole blood folate presented as median and interquartile range

[‡] Different from iron only group ($p<0.05$)

[§] Based on household characteristic and possessions using principal components analysis (16)

[#] Based on response to question whether she can speak and/or understand Nahuatl or any other indigenous language

pregnancy or in zinc, retinol or folate concentrations from baseline to one month postpartum among women who received MM or Fe only supplements during pregnancy (Table III). Nor did zinc concentration in cord blood or breast milk retinol concentration at one month postpartum differ between groups. Adjustment for BMI at baseline, which differed between supplementation groups, did not modify any of the results (data not shown).

The prevalence of zinc deficiency and vitamin A depletion increased in both groups from recruitment to 32 weeks pregnancy with no difference between groups (Figure 1). Using the available cut-off point, the prevalence of zinc deficiency at 1 mo postpartum was 78.2% in the Fe group and 85.5% in the MM group ($p=0.11$). Because there is question whether this cut-off point is appropriate, we have not included these values in Figure 1. By one month postpartum, the prevalence of vitamin A depletion was very low (<1%). The prevalence of folate depletion/deficiency was low at recruitment (approximately 5%) but was high at 1 month postpartum

Table III
CHANGE IN SERUM ZINC, RETINOL, AND WHOLE BLOOD FOLATE CONCENTRATIONS, CORD BLOOD ZINC AND RETINOL AND BREAST MILK RETINOL CONCENTRATIONS BY TREATMENT GROUP

	<i>n</i>	<i>Iron only</i> <i>Mean ± SD*</i>	<i>n</i>	<i>Multiple micronutrients</i> <i>Mean ± SD</i>	<i>p-value</i>
Serum zinc (maternal blood), µmol/L					
Change from baseline to 32 wks pregnancy	158	-3.2 ± 4.5	132	-3.0 ± 2.7	0.78
Change from baseline to 1 mo postpartum	138	-2.4 ± 3.4	137	-2.7 ± 3.2	0.44
Serum zinc (umbilical cord blood), µmol/L	78	14.4 ± 2.4	62	14.0 ± 2.6	0.31
Serum retinol (maternal blood), µmol/L					
Change from baseline to 32 wks pregnancy	101	-0.13 ± 0.27	95	-0.15 ± 0.30	0.72
Change from baseline to 1 mo postpartum	133	0.43 ± 0.34	127	0.41 ± 0.37	0.64
Serum retinol (umbilical cord blood), µmol/L	79	0.65 ± 0.20	63	0.66 ± 0.22	0.69
Breast milk retinol (1 mo postpartum), µmol/L	122	0.57 (0.35, 0.87)	115	0.63 (0.38, 0.84)	0.92
Whole blood folate (maternal blood), nmol/L					
Change from baseline to 1 mo postpartum	69	-56.9 ± 153.2	63	-38.0 ± 205.5	0.55

* Data for breast milk retinol presented as median (interquartile range)

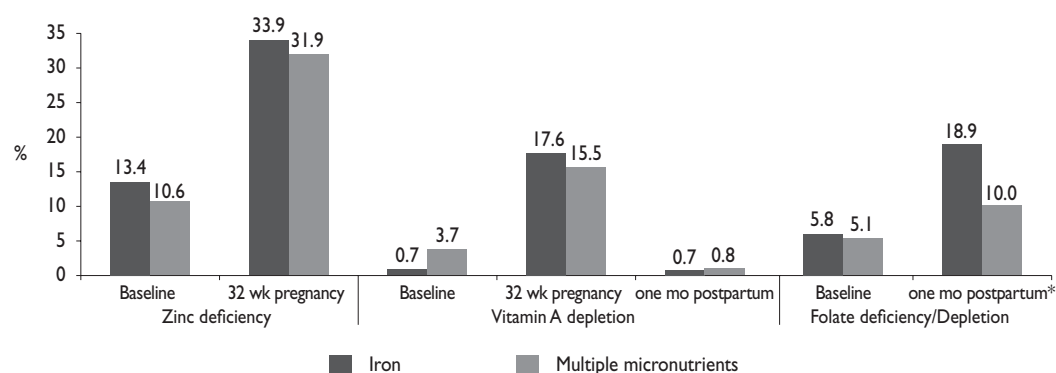


FIGURE 1. PREVALENCE OF ZINC DEFICIENCY AND VITAMIN A AND FOLATE DEPLETION DURING PREGNANCY AND ONE MONTH POSTPARTUM BY TREATMENT GROUP.^{A,B} SOLID BARS REPRESENT IRON ONLY,^A AND OPEN BARS, MULTIPLE MICRONUTRIENTS.^B ZINC DEFICIENCY WAS DEFINED AS SERUM ZINC <8.6 µmol/L AT BASELINE AND <7.6 µmol/L AT 32 WK OF PREGNANCY, VITAMIN A DEPLETION AS SERUM RETINOL <0.70 µmol/L AND FOLATE DEFICIENCY/DEPLETION AS WHOLE BLOOD FOLATE <147.29 nmol/L. *0.05<p<0.10 BETWEEN GROUPS.

(18.5% in iron group and 10.0% in MM group) (Figure 1). In unadjusted group comparisons there was a non-statistically significant trend ($p=0.09$) towards a lower prevalence of folate depletion/depletion in the MM group than the Fe group.

Discussion

Supplementation with multiple micronutrients during pregnancy did not improve zinc or vitamin A status during pregnancy or in the early postpartum period

compared to supplementation with iron only. There is some suggestion that multiple micronutrient supplementation may result in lower risk of folate deficiency/depletion at one month postpartum. The results of this randomized controlled trial provide evidence for the lack of effect of multiple micronutrient supplementation on biochemical indicators of zinc, vitamin A and folate status among pregnant women where the prevalence of deficiency in early pregnancy is low for folate and vitamin A, and moderate for zinc.

We included in this study only the subsample of women from whom we had blood sample of micronutrient biochemical analysis, which is not the same as loss to follow-up, given that these are complete follow-up for each micronutrient studied. We found that there were differences in some characteristics among the sub-sample included in this analysis and those excluded, such as compliance, duration of pregnancy and hemoglobin concentration. The last one, being significantly lower ($p < 0.05$) among women included in this study, resulting, probably in a group of women with a greater potential to respond to supplementation. However, we did not find improvement in iron status in this subsample.

Unfortunately, we do not have evidence whether the MM supplements may have had additional benefits for the women. For example, in a study from Nepal, a positive impact of iron+folic acid+zinc but not multiple micronutrient supplements on the prevalence ratio of sub-clinical infections was reported.¹⁵

To date, few studies have reported the impact of multiple micronutrient supplementation during pregnancy on biochemical indicators. In Nepal, women consuming multiple micronutrient supplements had better vitamin E and A status compared to controls at 32 weeks pregnancy.²⁹ Results are not presented in this study for serum values of other micronutrients. A latter study in the same country reported that supplementation with multiple micronutrients improved folate, vitamin B₁₂, B₆ and riboflavin status between baseline and the third trimester compared to controls receiving vitamin C only.¹⁵ Contrary to our findings, serum zinc improved in the group receiving multiple micronutrients, but not in those receiving folic acid, iron and zinc without other micronutrients. In general, the women who participated in the studies in Nepal had worse zinc, folate and vitamin A status than the women in Mexico, which may explain the difference in findings.

It is unlikely that the lack of impact observed here is related to compliance with supplementation. Compliance was high (over 80% in both groups) and began at an average of nine weeks pregnancy, leaving ample time for impact. In the case of vitamin A there may have been little potential to respond given the low prevalence of

depletion and lack of overt deficiency in the population. A similar prevalence of depletion was reported a large national nutrition survey which was conducted during the same time period (1999)¹¹ and suggests that vitamin A deficiency is no longer a public health problem in adult women in Mexico, even during pregnancy.

Given the relatively high zinc content of the MM tablet (15 mg) and high compliance, the lack of impact on serum zinc and the increase in the prevalence of deficiency is surprising. Post-hoc power calculations suggest that we had power (>80%) to detect a difference in the mean change in zinc concentration of 1.1 $\mu\text{mol/L}$. One study in Peruvian pregnant women³⁰ found that supplementation with 15 mg of zinc plus 60 mg of iron and 250 μg of folate during pregnancy improved maternal zinc status by 0.4-1.7 $\mu\text{mol/L}$ between 28 y 30 wk pregnancy compared to supplementation with iron/folate only. This population had a lower mean serum zinc concentration ($10.6 \pm 2.1 \mu\text{mol/L}$) than the women in our study ($11.9 \pm 2.8 \mu\text{mol/L}$) at baseline. Serum zinc concentration is normally low during pregnancy and is highly affected by hemodilution among other factors related to pregnancy.²⁵ It is possible that these factors outweighed the impact of supplementation in this sample of women with marginal deficiency. The extremely high prevalence of deficiency at one month postpartum (almost 80%) seems improbable in this population and the appropriateness of using the cut-off point for non-pregnant women at this time should be evaluated. Even if the actual prevalence is overestimated, we have no reason to believe that the use of this cut-off point would differentially affect the supplement groups and thus we conclude that supplementation with MM did not have an impact on the prevalence of zinc deficiency.

Another possibility for the lack of effect of supplementation on serum zinc concentration is the interaction between nutrients in the supplement, particularly zinc and iron. Recent studies have reported reduced zinc absorption from food³¹ and from supplements³² in the presence of supplemental iron. In both studies, iron content of the supplement was considerably higher than zinc content (100 mg of supplemental iron in the first with no zinc and 60 mg of iron and 15 mg of zinc in the second). When the iron:zinc ratio is very high, for example 25:1,³³ zinc absorption is diminished. Others³⁴ have reported that 25 mg of zinc sulphate consumed with 25 mg of iron (1:1 ratio) in a watery solution results in reduced plasma zinc concentration compared to supplementation with zinc without iron. This effect was magnified when the dose of iron is of 50 or 75 mg, that is, when the ratio of iron to zinc is 2:1 or 3:1. In the supplement distributed in our study, the ratio of iron

to zinc was 4:1 and it is possible that this resulted in an inhibition of zinc absorption.

Multiple micronutrient supplementation may have prevented a worsening of folate status during pregnancy, compared to iron only supplementation. Dietary intake of folate during pregnancy did not differ between supplementation groups at baseline, 32 weeks or one month postpartum,³⁵ further supporting the possibility that the difference in folate status between groups, albeit small, can be attributable to the supplement. The folate content of the supplement used here was lower than that reported by others in similar studies.¹³ It is possibly that higher doses of folate are needed to significantly improve status in this type of population with only marginal deficiency. Clear benefits of improved folate status before and during pregnancy have been documented, including prevention of neural tube defects, fetal death, among other outcomes,³⁶ and possibly, reduced risk of low birth weight, preterm delivery and intrauterine growth retardation.^{37,38} It is important to note that at the time that this study was conducted, standard practice during pregnancy in Mexico included supplementation with iron only, not iron plus folate—a policy which has since been modified.

Micronutrient malnutrition during pregnancy, particularly zinc and iron deficiency, continue to be important public health problems among the poor in Mexico. Although the prevalence of folate deficiency is low and severe deficiency is rare, it is still of concern due to the important implications for pregnancy outcomes. We report here and previously¹⁷ no clear benefit of multiple micronutrient supplementation over iron only supplementation for zinc, iron, vitamin A or folate status during pregnancy or early postpartum. Furthermore, even in this sample with high compliance, consumption of multiple micronutrients did not eradicate micronutrient deficiency. In populations like Mexico and many other middle income and developing countries where micronutrient deficiencies exist in non-pregnant women of reproductive age, strategies to improve status before women get pregnant, such as food fortification are clearly needed. The impact of multiple micronutrient supplements with lower doses of iron and an iron:zinc ratio closer to one on iron and zinc status during pregnancy should be explored. The potential of other interventions such as home fortification should also be explored. Moreover the programs for supplementation with micronutrients would be desirable are directed specifically to population, where magnitude and severity of nutritional deficiencies are a major public health problem.

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