Effects of Maternal Micronutrient Supplementation on Newborn Size and Infant Health and Survival

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Strategies to lower the rates of low birth weight and perinatal and neonatal mortality in the Third World have had limited success. In south Asia alone, 10–15% of infants are stillborn, die within 7 days of birth, or are born alive but fail to survive beyond the 1st month of life. Micronutrient deficiencies in the first months of life may influence this high risk of mortality in the perinatal and neonatal period. Direct supplementation may be one of the strategies that may impact on early infant health and survival; however, half of all infant deaths occur in the 1st month of life, two thirds of which tend to be in the 1st week. This suggests that intrauterine factors, including materno-fetal transfer of essential nutrients, could influence these outcomes.

Inadequate maternal intakes or stores of single micronutrients such as folic acid, iron, zinc and others have been associated with adverse pregnancy outcomes such as preterm birth, stillbirth, intrauterine growth retardation and perinatal and neonatal morbidity and mortality. Yet causal evidence is weak or lacking. Maternal supplementation can influence 2 groups of infant outcomes [1]. The teratogenic effect of deficiency during the periconceptional period requires a different approach for intervention programs, especially in the context of developing countries where pregnancies are unplanned and reported late in gestation. The example for such an infant outcome would be neural tube defects associated with periconceptional folate deficiency. The 2nd group of infant outcomes is those that are influenced by maternal nutrient deficiencies in later pregnancy such as low birth weight, preterm delivery and infant mortality. This chapter deals with the latter group of outcomes that may be amenable to prenatal micronutrient interventions.

Unlike in the US and other Western countries, the practice of taking a daily prenatal multivitamin/mineral supplement is uncommon in the developing
world. Prenatal iron-folate supplementation, despite international recommen-
dations [2], has had poor success in many Third World countries, primarily due
to inadequate supplies and poorly managed logistics of distribution [3]. Because
maternal micronutrient deficiencies coexist and are widespread in developing
countries, there has been a recent global movement to advance the use of a
daily prenatal multivitamin-mineral supplement that goes beyond iron-folate
alone. However, empirical evidence for a beneficial effect of such a supplement
on either maternal or infant outcomes is yet to be known. Numerous random-
ized clinical trials of multiple micronutrient supplementation in the developed
world have failed to show any beneficial impact on birth weight or perinatal
mortality [4], although the populations in which these trials were conducted
had a low probability of being deficient. In a randomized clinical trial among
HIV-1-infected pregnant women in Tanzania, multivitamins (given in multiples
of the recommended dietary allowance, RDA) resulted in significant reductions
in low birth weight, severe preterm (<34 weeks) birth, and fetal deaths [5]. The
implications of these findings for non-HIV populations are not clear. It is critical
that the health and nutritional effects of a prenatal micronutrient supplement
are well established before initiating interventions on a global scale in the
developing world. This chapter summarizes the existing knowledge on the
efficacy of single micronutrients in improving infant outcomes and presents
the results of one of the first trials from Nepal that tested the effects of a
multiple micronutrient supplement on infant outcomes. Data on maternal
micronutrient supplementation on infant health outcomes are scant. This
chapter focuses on the study from Nepal to provide initial insights into the
potential effects, both beneficial and harmful, of maternal micronutrient sup-
plementation on fetal and infant outcomes. Because half of the world's low birth
weight babies are born in south Asia, the results from this study have broad-
based implications.

Impact of Maternal Supplementation with Single
Micronutrients on Infant Outcomes

Vitamin A

Few studies have examined the impact of antenatal vitamin A supple-
mentation on infant and maternal health due largely to the concerns regarding
its teratogenic effects at large doses (>10,000 IU) [6]. Yet in many regions of
the developing world, maternal vitamin A deficiency is common and women
develop night blindness during pregnancy which is associated with an
increased risk of both maternal [7] and infant mortality [8]. Recent surveys
show that maternal night blindness may range from 5 to 18% in many
countries of south and southeast Asia [9, 10]. A recent trial in Nepal showed
that weekly vitamin A or β-carotene supplementation among women of
reproductive age reduced maternal mortality by 40% [11]. Yet, the same study
showed that neither supplement had any impact on infant mortality [12] (table 1) or early neonatal weight (unpublished, West et al.). Two studies among HIV-1-infected pregnant women have found no impact of vitamin A supplementation on the incidence of low birth weight [5, 13]. One of these studies [13] showed a significant reduction in preterm delivery (<37 week of gestation). One study in Malawi showed that vitamin A supplementation reduced the occurrence of low birth weight from 21 to 14% (p < 0.03) among HIV-1 women [14]. Thus, the evidence regarding a beneficial impact of vitamin A supplementation among pregnant women on newborn weight or infant health is conflicting and derived largely from studies among HIV-1-infected women. More work is needed in this area among non-HIV populations. The impact on maternal mortality is currently being tested in other regions of the world.

**Vitamin D**

A recent Cochrane review found 2 trials involving a small number of women (n = 232) on vitamin D supplementation during pregnancy [15]. One trial showed a lower number of low birth weight infants among supplemented mothers, while the other showed that the supplemented group had lower birth weight. The authors conclude that ‘there is not enough evidence to evaluate the effects of vitamin D supplementation during pregnancy’.

**Folic Acid**

Based on the Cochrane review, there is little evidence to support any effect of folic acid supplementation on maternal and infant outcomes, except for a nonsignificant reduction in the incidence of low birth weight (relative risk, RR, = 0.73, 95% confidence limit, CL, 0.47, 1.13) [16]. Studies examining the impact of periconceptional folate and/or multivitamin supplementation on neural tube defects have found a small reduction in stillbirth rate of 22%, although the confidence interval around this reduction includes 1 [17].

**Vitamin B₆**

Only 1 trial of 371 women was found that examined the impact of pyridoxine supplementation compared to a control group of pregnant women [18]. This trial did not examine any pregnancy-related outcomes but found that supplementation was associated with a significant reduction in dental decay.

**Iron**

A comprehensive review on the impact of iron and/or folate supplementation concluded that ‘the currently available evidence from studies with designs appropriate to establish a causal relationship is insufficient to support or reject this practice (of supplemental iron during pregnancy) for the purposes of raising birth weight or lowering the rate of preterm birth’ [19].
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>No. of trials</th>
<th>Description</th>
<th>Outcome</th>
<th>Effect</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vitamin A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Katz et al. [12], 2000</td>
<td>1</td>
<td>Nepal, placebo vs. vitamin A or β-carotene (n = 11,720)</td>
<td>Infant mortality</td>
<td>1.04 (0.88, 1.20)</td>
<td>Large trial, high rates of vitamin A deficiency</td>
</tr>
<tr>
<td>Fawzi et al. [5], 1998</td>
<td>1</td>
<td>HIV-1-infected Tanzania, 2 × 2 factorial of vitamin A and multivitamins (n = 1,075)</td>
<td>LBW</td>
<td>0.89 (0.61, 1.29)</td>
<td>Implications for non-HIV women not clear. Multiples of RDA used</td>
</tr>
<tr>
<td>Coutsoudis et al. [13], 1999</td>
<td>1</td>
<td>HIV-1-infected, South Africa, placebo vs. vitamin A (n = 689)</td>
<td>LBW</td>
<td>0.85 (0.54, 1.32)</td>
<td>Large effect on preterm birth without any impact on birth weight</td>
</tr>
<tr>
<td>Kumwenda et al. [14], 2002</td>
<td>1</td>
<td>HIV-1-infected, Malawi, iron-folate vs. iron-folate + vitamin A (n = 697)</td>
<td>LBW</td>
<td>21 vs. 14% (p = 0.03)</td>
<td></td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahomed and Gulmezoglu [15], 2002</td>
<td>2</td>
<td>Placebo vs. vitamin D (n = 232)</td>
<td>LBW</td>
<td>No impact</td>
<td>Not enough data</td>
</tr>
<tr>
<td><strong>Folic acid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahomed [16], 2002</td>
<td>5</td>
<td>Majority of the data from 2 trials in Australia and UK (n = 1,479)</td>
<td>LBW</td>
<td>0.73 (0.47, 1.13)</td>
<td>Not enough evidence to evaluate whether folic acid has any effect on these clinical outcomes</td>
</tr>
<tr>
<td>Lunley et al. [17], 2002</td>
<td>3</td>
<td>Periconceptional supplementation studies</td>
<td>Stillbirth/Neonatal death</td>
<td>1.14 (0.55, 2.35)</td>
<td>Small reduction</td>
</tr>
<tr>
<td><strong>Vitamin B₆</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mahomed and Gulmezoglu [18], 2002</td>
<td>1</td>
<td>Placebo vs. pyridoxine (n = 371)</td>
<td>–</td>
<td>–</td>
<td>No data on infants, decline in dental decay</td>
</tr>
</tbody>
</table>
### Iron

<table>
<thead>
<tr>
<th>Study</th>
<th>intervention</th>
<th>Outcome</th>
<th>Effect Size</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preziosi et al. [21], 1997</td>
<td>Placebo vs. iron, Niger (n = 197)</td>
<td>Birth weight</td>
<td>3,016 g vs. 3,046 g, NS</td>
<td>Small sample size</td>
</tr>
<tr>
<td>Mahomed [20], 2002</td>
<td>Placebo vs. iron</td>
<td>–</td>
<td>–</td>
<td>Little information on pregnancy outcome. Few data from iron-deficient populations</td>
</tr>
<tr>
<td>1 Selective vs. routine supplementation, Finland (n = 2,694)</td>
<td>Perinatal mortality</td>
<td>0.33 (0.11, 0.99)</td>
<td>One study, small number of deaths (n = 13)</td>
<td></td>
</tr>
</tbody>
</table>

### Zinc

<table>
<thead>
<tr>
<th>Study</th>
<th>intervention</th>
<th>Outcome</th>
<th>Effect Size</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahomed [23], 2002</td>
<td>Placebo vs. zinc (n = 1,472) studies done in UK and US</td>
<td>Preterm</td>
<td>0.73 (0.54, 0.98)</td>
<td>Populations may not be zinc deficient</td>
</tr>
<tr>
<td>Osendarp et al. [24], 2000</td>
<td>Placebo vs. zinc, Bangladesh (n = 559)</td>
<td>LBW</td>
<td>0.75 (0.52, 1.07)</td>
<td>20% loss to follow-up</td>
</tr>
<tr>
<td>Caulfield et al. [25], 1999</td>
<td>Iron-folate vs. iron-folate + zinc, Peru (n = 1,016)</td>
<td>LBW</td>
<td>45.9 vs. 40.3%, NS</td>
<td>Incidence of low birth weight was low</td>
</tr>
<tr>
<td></td>
<td>Preterm</td>
<td>3.6 vs. 3.5%, NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preterm</td>
<td>6.1 vs. 5.5%, NS</td>
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</table>

### Magnesium

<table>
<thead>
<tr>
<th>Study</th>
<th>intervention</th>
<th>Outcome</th>
<th>Effect Size</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makridges and Crowther [28], 2002</td>
<td>Placebo vs. magnesium treatment trial for preeclampsia (n = 2,689)</td>
<td>LBW</td>
<td>0.67 (0.46, 0.96)</td>
<td>Of the 7 trials, only 1 was deemed to have high quality</td>
</tr>
<tr>
<td></td>
<td>SGA</td>
<td>0.70 (0.53, 0.93)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preterm</td>
<td>0.73 (0.57, 0.94)</td>
<td></td>
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</tbody>
</table>

### Calcium

<table>
<thead>
<tr>
<th>Study</th>
<th>intervention</th>
<th>Outcome</th>
<th>Effect Size</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hofmeyr et al. [30], 2002</td>
<td>Placebo vs. calcium</td>
<td>LBW</td>
<td>0.83 (0.71, 0.98)</td>
<td>Most studies were conducted among populations with high risk of hypertension and adequate intake of dietary calcium</td>
</tr>
<tr>
<td></td>
<td>Preterm</td>
<td>0.66 (0.43, 1.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stillbirth/death</td>
<td>1.04 (0.65, 1.66)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**LBW** = Low birth weight (<2,500 g); **SGA** = small-for-gestational age; **Preterm** = gestational age <37 weeks at birth; **NS** = not significant.
Among 23 intervention trials that were reviewed, many of which were not randomized, placebo-controlled or double-masked, there were serious problems of confounding, bias, poor design and low sample size. Similarly, the Cochrane review [20] which analyzed 20 trials found ‘no detectable effect on any substantive measure of either maternal or fetal outcome’. Only 1 trial of selective versus routine iron supplementation showed a lower perinatal mortality rate due to iron supplementation, although the number of deaths was only 13. A randomized, double-masked trial of iron supplementation (100 mg elemental iron) during the third trimester of pregnancy in Niger showed no effect on the birth weight of infants although the average birth length of infants of iron-supplemented mothers was higher compared to those receiving a placebo [21]. In the same study maternal iron supplementation resulted in a significant reduction in fetal/neonatal death (7.1 vs. 1%; p = 0.03) [22], although, again, the number of deaths was small.

**Zinc**

The Cochrane review that includes 5 trials from the UK and the US found a significant reduction in preterm delivery, and a small potential reduction in low birth weight due to maternal zinc supplementation [23], but this has not been confirmed in other trials in developing countries. A randomized, placebo-controlled trial from Bangladesh found that antenatal zinc supplementation (30 mg daily) had no effect on birth weight, length or incidence of prematurity [24]. Similarly, in Peru, birth weight among women receiving 15 mg daily zinc with iron-folate supplementation during pregnancy was no different from those receiving iron-folate alone [25]. In the study in Bangladesh, low birth weight infants experienced a reduction in the incidence of acute diarrhea, dysentery and impetigo due to maternal zinc supplementation [26], suggesting a potential positive impact on their immune system despite the lack of impact on birth weight per se. In the same cohort, however, maternal zinc supplementation was associated with lower scores on mental and psychomotor development indices in infants [27], suggesting that prenatal zinc supplementation may have both a beneficial and harmful effect on infants, depending on the outcome that was measured.

**Magnesium**

Although the Cochrane review [28] suggests significant reductions in preterm delivery, low birth weight and small-for-gestational age incidence due to supplementation with magnesium during pregnancy, these results were influenced strongly by 1 trial, the exclusion of which rendered all relative risk estimates nonsignificant. The quality of most of the trials included in the analysis was questionable, as a result of which the authors conclude that ‘there is not enough high quality evidence to show that dietary magnesium supplementation during pregnancy is beneficial’. Recently a large multicenter trial of 10,141 pre-eclamptic women in 33 countries showed that magnesium
Maternal Micronutrient Supplementation

sulfate halved the risk of eclampsia and reduced the risk of maternal death (RR = 0.55, 95% CL 0.26, 1.14), although the risk of infant death was not altered (RR = 1.02) [29].

**Calcium**

The effect of maternal calcium supplementation on birth outcomes has been examined in the context of preventing hypertensive disorders and related problems of pregnancy. Calcium supplementation does not appear to have an effect on preterm delivery or stillbirth or infant death rate [30]. There was a small reduction (RR = 0.83, 95% CL 0.71–0.98) on the incidence of low birth weight, although this needs confirmation by studies in developing countries. Many of these studies used 1–2 g of daily calcium, but were conducted among populations with adequate dietary intakes of calcium. Future studies should include communities with a low dietary intake of calcium using lower dosages of calcium for supplementation during pregnancy.

This brief overview of the efficacy of single, daily prenatal micronutrient supplements reveals mostly weak effects on infant outcomes largely due to inappropriately designed studies and lack of studies in populations with nutritional deficiencies. The study in Nepal, described below in some detail, is one of the first trials to examine the effects of alternative combinations of prenatal micronutrients on birth weight and infant survival in a rural, malnourished population in South Asia.

**The Nepal Study**

*Study Design and Methods*

The Nepal study [31, 32] was a double-masked, cluster-randomized, community-based trial to assess the impact of alternative combinations of micronutrients given during pregnancy and up to 3 months postpartum on birth weight, preterm delivery and infant mortality in rural Nepal. Previously, in this population we had shown no impact of maternal vitamin A or β-carotene supplementation on birth weight (unpublished, West et al.) or infant mortality [12]. Yet, both these nutrients reduced pregnancy-related mortality by 44% (RR = 0.56, 95% CL 0.37, 0.84) [11]. We randomized 426 communities to the following 5 supplement regimens: C = vitamin A (1,000 μg retinol equivalents, control); FA = vitamin A + folic acid (400 μg); FAFe = vitamin A + folic acid + iron (60 mg); FAFeZn = vitamin A + folic acid + iron + zinc (30 mg), and MN = vitamin A + folic acid + iron + zinc + other micronutrients1.

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1 Vitamin D (10 μg), vitamin E (10 mg), vitamin B1 (1.6 mg), vitamin B2 (1.8 mg), niacin (20 mg), vitamin B6 (2.2 mg), vitamin B12 (2.6 μg), vitamin C (100 mg), vitamin K (65 μg), copper (2.0 mg) and magnesium (100 mg).
Vitamin A alone was the control group. Each nutrient approximated a US RDA for a pregnant or lactating woman [33], except for iron and zinc which were given at a level twice the RDA for pregnancy. The amount of iron (60 mg) is the WHO recommended dosage for an antenatal supplement in countries where anemia prevalence is >40% [2]. Because a competitive inhibition of zinc uptake by iron has been reported to occur at ratios of >2:1 [34], the amount of zinc in the supplement was set at 30 mg (twice the RDA) to achieve an iron:zinc ratio of 2:1.

Women of reproductive age were first screened for their eligibility to become pregnant over the course of a year by 426 local female workers hired on the project. Women currently pregnant, breast-feeding an infant <9 months of age, sterilized, widowed or menopausal were excluded. The remaining women were visited once every 5 weeks for a year and asked if they had menstruated in the past 30 days. Amenstrual women were administered a human chorionic gonadotropin-based urine test for detection of pregnancy. Using this method, almost 50% were enrolled within 9 weeks and 90% with 16 weeks of gestation. Upon enrolment, women were provided with a bottle containing 15 supplements according to their allocation code, with instructions to take one each night before going to bed. The local female workers visited the participants twice each week to monitor compliance, ascertain pregnancy status and outcome, and to replenish the supplements. Daily supplementation was continued through 12 weeks postpartum in the event of a live birth, and for at least 5 weeks following a miscarriage or stillbirth.

Almost 97% of births in the study area occur at home, assisted by traditional birth attendants, relatives and neighbors [11]. Every effort was made to obtain birth weight and other anthropometric measurements as soon after birth as possible. Once birth was reported, the anthropometrist went to the home to perform ‘day-of-birth’ assessment, which included weight, length, and head and chest circumference measurements. Low birth weight was defined as weight <2.5 kg taken within 72 h of birth. Small-for-gestational age was defined as weight below the 10th percentile of the gestational age, sex-specific US reference for fetal growth [35]. Gestational age was calculated using the reported 1st day of the last menstrual period at the initial pregnancy interview. Preterm delivery was defined as a birth occurring before 37 weeks of gestation.

Over a year, 4,096 pregnancies ended in at least 1 live birth, with a total of 4,130 children being born alive. Approximately 80% of these were measured within 72 h and 70% within 24 h of birth. Median compliance was high at 88% and comparable across treatment groups.

**Impact on Low Birth Weight, Preterm Delivery and Small-for-Gestational Age**

The incidence of low birth weight in the control group was high at 43%. The combinations of folic acid + iron and multiple micronutrients reduced
the incidence of low birth weight by a modest 16% (RR = 0.84, 95% CL 0.72, 0.99) and 14% (RR = 0.86, 95% CL 0.74, 0.99), respectively (table 2). The combinations of folic acid alone or folic acid + iron + zinc did not have any impact on birth weight. The mean effect size in the folic acid + iron group was 37 g (95% CL -16, 90) and that in the multiple micronutrient group was 64 g (95% CL 12, 115). Both folic acid + iron and multiple micronutrients increased newborn head and chest circumference, but not length.

The incidence of small-for-gestational age was somewhat reduced with folic acid + iron supplementation, compared to controls (RR = 0.91, 95% CL 0.83, 1.00). There was no effect on preterm birth; all RR compared to controls were approximately 1.0. Folic acid + iron and multiple micronutrient effects may have differed in the distribution of the added weight, with multiple micronutrients increasing weight disproportionately at the upper end. The proportion of infants with weights of ≥3.3 kg was 50% higher (RR = 1.5, 95% CL 0.96, 2.2) in the multiple micronutrient group but similar to controls (RR = 1.1, 95% CL 0.72, 1.8) in the folic acid + iron group. Although modest, the magnitude of the effect of micronutrient supplementation is well within the range reported for maternal food supplementation: a 25- to 84-gram increase in birth weight for every additional 10,000 kcal consumed during pregnancy [36]. In the present setting where the mean maternal weight at baseline was low (~43.5 kg), largely due to energy and protein malnutrition, micronutrient supplementation alone, in the absence of food supplementation, was able to ameliorate this huge burden of low birth weight by 14–16%.

Adding zinc appeared to prevent the beneficial effect on birth weight and other growth parameters observed with iron and folic acid alone. Birth weight was higher by 53 g (95% CL 00, 108) among newborns of mothers who received folic acid + iron over those who received the same supplement with added zinc. Competition between bivalent iron and zinc for mucosal uptake in the gut may result in one interfering with the absorption of the other [38].

Table 2. Effect of maternal micronutrient supplementation on low birth weight (<2,500 g) in Nepal

<table>
<thead>
<tr>
<th>Low birth weight</th>
<th>n</th>
<th>%</th>
<th>RR 1</th>
<th>95% CL 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>685</td>
<td>43.4</td>
<td>1.0</td>
<td>–</td>
</tr>
<tr>
<td>Folic acid</td>
<td>628</td>
<td>41.7</td>
<td>1.00</td>
<td>0.88, 1.15</td>
</tr>
<tr>
<td>Folic acid + iron</td>
<td>635</td>
<td>34.3</td>
<td>0.84</td>
<td>0.72, 0.99</td>
</tr>
<tr>
<td>Folic acid + iron + zinc</td>
<td>672</td>
<td>39.4</td>
<td>0.96</td>
<td>0.83, 1.11</td>
</tr>
<tr>
<td>Multiple micronutrients</td>
<td>705</td>
<td>35.3</td>
<td>0.86</td>
<td>0.74, 0.99</td>
</tr>
</tbody>
</table>

p = 0.0103.

1 Adjusted for design effect using Generalized Estimating Equations [36] and maternal weight at baseline.
Maternal Micronutrient Supplementation

Previously we have shown that improvement in the hemoglobin (Hb) concentration due to iron-folate treatment, although not significant, was lower (4.8 g/l) among pregnant Nepalese women receiving supplemental zinc compared to those not receiving zinc (7.8 g/l) [39]. In the present study, the Hb concentration improved by 13.8 g/l in the folic acid + iron group compared to only 10.0 g/l in the folic acid + iron + zinc group (data not shown). Although this difference was not statistically significant, it is suggestive of an inhibitory effect of zinc on iron. This potential inhibitory action of zinc may have contributed to the lack of birth weight gain as seen among those who were supplemented with iron-folic acid alone.

The full complement of micronutrients (including folic acid, iron and zinc), however, did increase birth weight and other measures of growth, suggesting mechanisms other than those mediated by iron or Hb. The efficacy of a multiple micronutrient supplement that excluded zinc was not tested in this trial. If we assume that zinc in the multiple micronutrient continued to reverse the effect of iron on birth weight, it can be postulated that the other nutrients in the supplement (mostly B-complex vitamins) on their own may have had a larger effect than iron alone. For example, we know that B-complex vitamins participate in numerous aspects of intermediary protein, lipid and carbohydrate metabolism that regulate energy and nitrogen utilization, which could affect fetal growth [39]. The study among HIV-1-infected women in Tanzania found that multivitamin supplementation (that did not include any zinc) decreased the risk of low birth weight by 44% [5]. Our results suggest that, for this rural Nepalese population, an optimal multiple micronutrient supplement for reducing low birth weight and anemia should probably include less zinc.

Impact on Fetal Loss, Perinatal, Neonatal, and 3-Month Infant Mortality

There is little evidence on the impact of maternal micronutrient supplementation on infant health and survival. Studies of maternal zinc supplementation in Bangladesh and Peru have shown an improvement in the health [24] and fetal neurodevelopment [41] without any impact on birth weight. In Nepal, we examined the impact of the four combinations of micronutrient supplements on miscarriage, and 3-month infant mortality [32]. Rates of miscarriage did not differ by treatment group and ranged between 12 and 15% (data not shown). Kaplan-Meier analysis in the 1st 6 months of life showed higher survival among infants of mothers receiving folic acid with or without added iron or iron + zinc compared to controls (fig. 1). However, the individual treatment-control differences were not statistically significant. The mortality reduction was most apparent through the first 3 months of life, after which the curves tended to parallel each other. Relative risks of 3-month mortality ranged from 0.78 to 0.89 in the 3 groups compared to controls, with confidence intervals including 1. The mortality rate among infants in the
multiple micronutrient group was similar, and consistently, even slightly worse than that of infants in the control group, such that the highest infant mortality in each period of life was observed in the multiple micronutrient group. This was surprising as multiple micronutrients were noted to have the highest mean increase in birth weight. When treatment effects were stratified by preterm and term delivery, a significant interaction was revealed (table 3). Among preterm births, supplementation with folic acid and folic acid + iron was associated with a 45–50% reduction in 3-month infant mortality. Adding zinc or other micronutrients to folic acid + iron did not show any additional benefit and perhaps even attenuated the protective effect, as reflected by a lower relative risk and confidence intervals that included $1$. In contrast, there appeared to be little survival benefit due to supplementation among term infants. In fact, term infants of mothers who were supplemented experienced a slightly higher mortality relative to the controls, with the highest risk of mortality being observed among those who received the multiple micronutrient supplement ($RR = 1.74, 95\% \text{ CL } 1.00–3.04$).

**Birth Weight and Infant Mortality: A Role of Preterm Birth**

Folic acid supplementation appeared to reduce mortality without improving birth weight. On the other hand multiple micronutrient

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**Fig. 1.** Kaplan-Meier curves depicting the cumulative mortality rate of infants through 6 months of life by treatment group (the Nepal Study). $p > 0.05$ using log rank test.
supplementation, which had a significant impact on birth weight, failed to show any survival benefit. These seemingly contradictory results suggest that improvement in infant survival observed with maternal nutritional supplementation might not always be mediated through increases in birth weight, as suggested previously [42]. It is argued that preterm birth and not birth size per se increases mortality risk. Birth weight is good in as much as it captures small preterm babies, but direct measurement of prematurity is much better to have [43]. In the present study, while the risk of preterm itself remained unchanged, the elevated risk of poor survival among these infants was ameliorated through maternal supplementation. Excess perinatal and neonatal mortality may operate through several mechanisms mediated by preterm birth and severe intrauterine growth retardation or independent of these mediators (fig. 3). For instance preterm infants may be at an increased risk of oxidative stress and free radical damage, especially in the presence of a deficient maternal antioxidant state [44]. A lowered immune status due to preterm birth or fetal growth retardation can result in increased risk of early infant morbidity and mortality. Congenital abnormalities caused by maternal nutritional deficiencies also contribute to perinatal mortality. Finally, perinatal and neonatal deaths may occur due to complications of pregnancy and delivery such as fetal malpresentation, eclampsia, intrapartum bleeding, multiple births, prolonged labor, placentia previa and premature rupture of the membranes [45]. Birth weight may or may not influence these mechanisms.

Table 3. Treatment effects of maternal supplementation on risk of mortality at 0–3 months in infants stratified by preterm and term birth (the Nepal Study)

<table>
<thead>
<tr>
<th></th>
<th>RR</th>
<th>95% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preterm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>0.4</td>
<td>0.2–0.8</td>
</tr>
<tr>
<td>FAFe</td>
<td>0.5</td>
<td>0.3–0.9</td>
</tr>
<tr>
<td>FAFeZn</td>
<td>0.8</td>
<td>0.4–1.3</td>
</tr>
<tr>
<td>MN</td>
<td>0.7</td>
<td>0.4–1.2</td>
</tr>
<tr>
<td>Term</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FA</td>
<td>1.6</td>
<td>0.9–2.8</td>
</tr>
<tr>
<td>FAFe</td>
<td>1.6</td>
<td>0.6–2.2</td>
</tr>
<tr>
<td>FAFeZn</td>
<td>1.1</td>
<td>0.6–2.0</td>
</tr>
<tr>
<td>MN</td>
<td>1.7</td>
<td>1.0–3.0</td>
</tr>
</tbody>
</table>

The p-values for the main effects and interaction effects for FA, FAFe, FAFeZn and MN were 0.001 and 0.001, 0.03 and 0.069, 0.34 and 0.39 and 0.03 and 0.015, respectively.
Maternal Micronutrient Supplementation

Fig. 3. Possible mechanisms by which maternal micronutrient status may influence perinatal mortality.

Hypotheses Regarding Adverse Effect of Multiple Micronutrient Supplement on Infant Mortality

It is not clear why multiple micronutrient supplementation failed to show the same overall reduction in mortality observed with folic acid or with folic acid + iron. We examined the relationship between birth weight and infant mortality by treatment group (fig. 4). There appeared to be a linear relationship between birth weight and infant mortality in each of the treatment groups, except the multiple micronutrient group. In this group, there was a suggested trend of increasing mortality at the upper end of the birth weight categories. The excess mortality risk due to multiple micronutrient supplementation, as shown before, was apparent only in the term infants (fig. 2). When treatment effects on high birth weight (≥3.3 kg) were examined by term birth, we found that while none of the supplements increased high birth weight in preterm births, multiple micronutrients significantly increased the risk of high birth weight relative to controls in term births (RR = 1.71, 95% CL 1.10–2.65). High birth weight was associated with symptoms of birth asphyxia. The risk of birth asphyxia was also higher among term infants whose mothers received multiple micronutrients relative to controls. Thus it is likely that among term infants, multiple micronutrient supplementation resulted in high birth weight,
Fig. 4. Relationship between birth weight (g) and infant mortality by treatment group (the Nepal Study).
which consequently may have increased the risk of birth asphyxia and infant mortality. Multiple micronutrients may have lead to a protective survival effect among preterm infants as shown in figure 2, but it also increased the risk of mortality among term infants. These countervailing influences may have cancelled each other out which resulted in multiple micronutrients seemingly having no overall benefit on mortality.

The South Asian Context

The findings of this study may be most relevant to rural South and, possibly, Southeast Asia where maternal malnutrition, including wasting and stunting are highly prevalent. Indeed, it was surprising to find increased problems of asphyxia among newborns who were above 3.2 kg weight at birth, a birth weight which would be considered normal in most other regions of the world. While prepregnancy nutritional status and weight gain during pregnancy influences birth weight, gestational age at birth is a stronger determinant of birth size as well as subsequent infant health and survival. Our analysis suggested that the micronutrients most benefited infant survival of short mothers (height <150 cm) who were preterm and may have had the most detrimental effect on infants of term mothers who had a height of ≥150 cm. It is likely that the components of fetal weight gain due to micronutrient supplementation among the better nourished women who carried their fetus to term may be quite different from those who were poorly nourished and had a shorter gestational duration. The mechanisms of these differences in intrauterine growth are not clear, yet our data suggest that while correcting iron deficiency may have affected low birth weight in the group receiving folic acid + iron, other micronutrients such as the B-complex vitamins may have caused the birth weight differences in the multiple micronutrient supplement.

Conclusions and Future Research

One other study which was conducted in a Mexico semi-rural population found that daily maternal multiple micronutrient supplementation had no additional impact on birth weight and other aspects of birth size compared to iron alone [46]. These findings corroborate the results of the study in Nepal which showed that multiple micronutrients conferred no additional benefit over iron folate in reducing low birth weight. The preliminary evidence generated from these trials, combined with a potential harm caused by multiple micronutrients is adequate to give a pause to the use and promotion of such a supplement in the developing world. Nutrient-nutrient interactions may be more complex than previously thought, especially in the context of multiple micronutrient deficiencies, some of which may be more limiting for
Maternal Micronutrient Supplementation

birth weight and infant survival than others. The Nepal study suggests that, for this rural Nepalese population, an optimal multiple micronutrient supplement for reducing low birth weight and anemia should probably include less zinc. Birth weight is strongly associated with infant mortality. Yet, the Nepal study indicates that improvements in survival may or may not be mediated by birth weight. Thus future trials testing maternal micronutrient supplementation should include, beyond birth weight, infant health and mortality as outcomes. The Nepal study questions conventional knowledge and views on maternal micronutrient supplementation and its use for improving infant survival and health in the developing world. While it goes far in supporting the use of folic acid alone or with iron, it gives a basis for ‘pause’ before the world rushes forward toward multiple micronutrient supplementation without understanding its potential public health impact. Many research questions need to be urgently addressed in this regard. What is the optimum combination of prenatal micronutrients that will result in a health benefit? For which outcomes and which population? What is the etiologic fraction of maternal micronutrient deficiency in early infant mortality? More research is clearly needed in these areas to better inform policy and programs in the developing world.

References

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19 Rasmussen KM: Is there a causal relationship between iron deficiency or iron deficiency anaemia and weight at birth, length of gestation and perinatal mortality? J Nutr 2001;131(suppl):590S–603S.
Discussion

Dr. Al Saeid: Was there a difference in the incidence of congenital anomalies between the groups of babies born to the different mothers?

Dr. Christian: There were few congenital malformations but the numbers were really small, so we have not looked at them separately by treatment group.

Dr. Al Faouri: My question is about the 5,000 women enrolled in your study from more than 400 communities. Are there any differences in the dietary habits and high altitude because in Nepal there are variations in altitude. Does this affect the results?

Dr. Christian: In Nepal the study was conducted in the southern region which is flat, the altitude is not a factor in this study population. I would be guessing if I made any statement about how a change in altitude would alter it.

Dr. Pettifor: The issues of the mortality in neonates above 3.3 kg that you used as your cutoff point, were they similar in all groups or was there an increase in mortality in those over 3.3 kg that were getting the micronutrient supplement?

Dr. Christian: In the graph in which I looked at birth weight against infant mortality separated by treatment group, what I showed was in fact that it was only in the multiple micronutrient group that there was this increased risk of mortality of high birth weight.

Dr. Moukarzel: Thank you for this wonderful presentation and we appreciate the work on 5,000 pregnant women. This is a very large number, so statistically you can make many things out of them, for instance when you give us the relative risk on increased birth weight, the p value is <0.05. Statistically when you have large
numbers, even if it is statistically significant, it is not really very relevant to have a p value of only <0.05. The other comment I have is that your population is presumed to be malnourished, the average weight of the pregnant women was 42 kg. Therefore this study will not be applicable to a normal healthy population, and also the conclusion. Is that correct?

Dr. Christian: That is why I stated with my concluding slide that these results are probably most applicable to populations in south Asia and south-east Asia. Regarding your first comment, I agree with you completely with the sample size issue. And despite having 5,000 women we did not have adequate power to look at the mortality differences, which is a problem. But you are right that with this sample size, we were able to pick up small birth weight differences by treatment group.

Dr. Moukarzel: What is the confidence interval in this relative issue?

Dr. Christian: They were significant.

Dr. Moukarzel: It crosses by 1?

Dr. Christian: The upper limit did not cross 1.0.

Dr. Fawzi: Thank you for this very well-analyzed paper. Touching on the same point of sample size, it seems that, as you said, there was limited power, we didn't see the number of cases. How many deaths were due to asphyxia? Do you think that these findings could be a potential explanation for those adverse effects? The second question, could the supplement dose that you used play a role in the overall lack of effect of the multiple micronutrients with respect to birth weight or any other outcomes?

Dr. Christian: With regard to your first comment/question, I think that yes, this could be purely a chance finding and therefore it needs to be examined again in other populations. Dr. Bates started the first presentation by putting up a quote that said you could be either completely wrong if you came up with some different kind of finding, or you have made a new discovery. I think that with the results of this study we are standing in the middle ground somewhere and we do not really know if this is a fluke finding or whether we have made a new discovery. The point you made about the absolute difference: I showed you relative risks and what we infer with regard to the magnitude of the increased risk and how we weigh that against the magnitude of the protective effect in survival. I think those are very good points. The problem of high birth weight was quite low and so the absolute change in increased mortality was quite low. I am not quite sure about the dosage. We have collected serum samples from 1,000 women across 200 in each group at a baseline and follow-up, and we are going to look carefully at the serum indicators to see if they have an impact on status or not. With regard to iron we were able to show a significant impact on hemoglobin concentrations, and serum ferritin, serum iron and transferrin receptor.

Dr. Saadah: Did you eliminate confounding factors like maternal diabetes which might adversely influence birth weight?

Dr. Christian: No, we did not, they were all included in the study, and because antenatal care in this population is very low, almost nonexistent, they weren't screened on a regular basis for diabetes.

Dr. Verhoef: I continue to be puzzled by the apparent lack of effect of zinc supplementation in pregnancy on growth in children, especially regarding the dramatic effects that have been achieved in infancy in for example the trials that our group has carried out in Ethiopia, and I would like to exchange my views about that. From your trial it seems that at baseline the groups are fairly comparable regarding maternal weight, which seems to indicate that it is unlikely to be a major confounder and yet in the analysis you adjusted for that. That seems to indicate that you have considered that nevertheless to be a potential confounder, whereas I would think that if zinc is supplemented, it is very likely that maternal weight would increase and that
could indicate a mediating factor in the relationship between zinc supplementation and birth weight of children. If you adjust for that, as you seem to have done in your analysis, it seems that the effect that you were looking for might have inadvertently been eliminated. Could you comment on that and why didn’t you adjust for maternal height instead of weight because zinc supplementation obviously wouldn’t have an effect on maternal height.

**Dr. Christian:** We adjusted for baseline weight and that was before supplementation. Secondly the reason we adjusted for maternal weight was, even though the groups were highly comparable, because of our large sample size the small differences between the treatment groups were statistically significantly different, and so I have done both adjusted and unadjusted analysis of the results.

**Dr. Verhoef:** So how do you interpret the lack of effect there? Is that possibly because zinc supplementation would inhibit absorption of iron, is that the issue?

**Dr. Christian:** Yes, that is what we think and that is also suggested when you look at the hemoglobin indicators because the increase in hemoglobin was not as high with added zinc.

**Dr. Verhoef:** On the basis of these data would you advise against zinc supplementation in pregnancy or do you think that would be a premature conclusion?

**Dr. Christian:** Our colleagues have looked at all the trials of maternal zinc supplementation that have been conducted in developing countries to look at its impact on birth weight, and their finding is that zinc supplementation has no impact on birth weight [1]. There is one study from Bangladesh by Osendarp [2] which found that while zinc supplementation in women during pregnancy had no impact on the birth weight of the infants, the low birth weight infants in that study, when followed up through 6 months of life, had a lower incidence of acute lower respiratory infections and diarrheal diseases. But again in the same study, published recently in the *Lancet*, by McGregor and Osendarp [3] found that maternal zinc supplementation was associated with a significantly lower Bayley score. But with regard to antenatal zinc supplementation. I am not quite sure if there is any bottom line because based on this study some outcomes are positively affected and some are not affected at all and then others are negatively affected.

**Dr. Rao:** In our country we have a program of child survival and safe motherhood in which an iron folic acid supplement is given to all antenatal mothers expecting a good fetal outcome. However, in a control area apart from iron folic acid supplementation, zinc and also extra protein supplementations were given to the mothers in the last two trimesters of pregnancy, and it was found that the ultimate birth weight of the babies increased by 250 g and more. Also there were no perinatal complications like asphyxia. So apart from the micronutrient supplementation, there is also a need to supplement protein to these antenatal mothers.

**Dr. Al Saeid:** I just wanted to mention a similar point. Did you make sure that those mothers, especially since they were apparently malnourished, received enough calories, enough protein and enough carbohydrates? Maybe the effect of micronutrients will not be apparent if someone is already malnourished.

**Dr. Christian:** I agree with you completely. I think that what is limiting in the diet is energy, calories, and whatever the micronutrient did was small. They need more food during pregnancy.

**Dr. Delange:** What was the rationale for including copper in the micronutrient group? Secondly, I was impressed by the extremely high birth weight of some of the babies born in Norway, which suggests that perinatal prenatal monitoring in this highly sophisticated country was perhaps a bit questionable. How many of these mothers were diabetic, do you know that?
Dr. Christian: Those data were taken from a paper [4] and I don’t really know the details. But with regard to copper, I think the combination of micronutrients that were induced ultimately in that cocktail was based on taking an educated guess, trying to be inclusive. So we had a wide range of micronutrients, and copper was added specifically to address the issue of zinc causing copper deficiency.

Dr. West: I just thought it is useful to keep in mind that what we seem to be coming up with in our infants in this trial is that different populations may have different nutritional needs and may respond differently to the same type of nutritional supplement. You would not expect these supplements to have these effects in Baltimore or in Dubai. Therefore there is value in defining a population that is large enough that the infants can stick with. It is true that these women are malnourished but the population in this area of Nepal is not unlike those women who live throughout the rest of Nepal, Bihar, Uttar Pradesh, Bangladesh. It captures a population of several hundred million people in the world, and it may be that we have to be very circumspect in looking at what the micronutrient supplement needs are for that population which may not have the same needs as in northern Africa or southern Africa or Europe. So it is a very typical malnourished southern Asian female population.

References