Treatment Effects of Maternal Micronutrient Supplementation Vary by Percentiles of the Birth Weight Distribution in rural Nepal


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Previous Presentations of Data:

ABSTRACT

Certain antenatal micronutrient supplements have increased birth weight by 40-70 g in rural Nepal. The impact was estimated by calculating the mean difference in birth weight between control and treatment groups which assumes a constant treatment effect across the birth weight distribution. By estimating differences (and confidence intervals) in birth weight between treatment and control groups as a non-linear, smooth function of the percentiles of the birth weight distribution we can examine whether the shape of the birth weight distribution for a treatment group is different from that of the control group.

Supplementation groups were folic acid, folic acid and iron, folic acid and iron and zinc, and a multiple micronutrient supplement all with vitamin A, compared against the control group of vitamin A alone. The shape of the birth weight distribution in the multiple micronutrient group was the same as that of the control group, only the location of the distribution had shifted. The folic acid and iron group had fewer infants in the lower tail of its distribution but a similar proportion in the upper tail compared with the control group. The biologic pathways affecting intrauterine growth may vary by micronutrients such that some may confer a benefit among the most vulnerable infants, whereas others may have a more constant effect across the birth weight distribution. Future analytic approaches to estimating benefits of maternal supplementation on birth weight should examine whether there is a constant or variable treatment effect across the distribution of birth weight.

Key Words: Birth weight, micronutrients, pregnancy, infant mortality, Nepal
INTRODUCTION

Many studies use birth weight as an outcome of nutritional or other interventions in pregnancy since it is a measure of intrauterine growth retardation in the absence of gestational age data and is also a strong predictor of early infant survival. Usual analytic approaches to the estimation of treatment effects involve calculating the mean difference in birth weight between control and treatment groups. This assumes that treatment shifts the distribution of birth weights by a constant amount. Alternately, researchers look at the relative risk of low or very low birth weight but this only compares the left tails of the two distributions, and may not adequately capture changes in the middle or upper end of the distribution.

Previously, we published the effects of daily antenatal supplementation with different combinations of micronutrients on birth weight (1). In that community-based, randomized trial, despite the apparently smaller adjusted birth weight increase with folic acid plus iron alone (37 g) compared with a multiple micronutrient which included folic acid plus iron (64 g), the % reduction in low birth weight (<2500 g) was very similar with folic acid plus iron (16%) compared to the multiple micronutrients (14%), suggesting that the birth weight distributions of the treatment groups may have had different shapes from that of the control group, especially at the lower end of the distributions. Furthermore, we found that the multiple micronutrient supplement increased the proportion of babies with birth weight ≥3300 g (7.7% compared with 5.3% in the control group), an increase not observed with folic acid plus iron supplementation (6.0% compared with 5.3%) (1).

Thus, one issue that may be relevant to randomized trials of nutritional interventions is
whether treatments change the shape of the birth weight distribution in a way that may
not be reflected when comparing means of treatment and control distributions.

In this paper, we further explore these findings and describe an analytic approach
to addressing this issue in trials of nutritional interventions to improve birth weight. We
use data from our community-randomized trial that examined the impact of different
maternal micronutrient supplements on birth weight in rural Nepal to illustrate this
approach.

MATERIALS AND METHODS

The design, methods and results of the randomized trial have been described
previously (1-4). Briefly, 426 communities in Sarlahi district, Nepal, were randomized to
receive one of five different maternal supplements. From December 1998 through April
2001, all married women of childbearing age who were not already pregnant or
breastfeeding an infant less than nine months of age were visited every five weeks and
asked if they had experienced menses in the past five weeks. If they had not, they were
given a urine-based pregnancy test. If pregnant, they were enrolled in the trial and
supplemented daily with caplets of identical appearance containing either vitamin A
alone as the control group (1000 µg retinyl acetate), vitamin A plus folic acid (400 µg),
vitamin A plus folic acid plus iron (60 mg ferrous fumarate), vitamin A plus folic acid
plus iron plus zinc (30 mg zinc sulphate), or a multiple micronutrient supplement that
included the same quantities of vitamin A, iron folic acid and zinc, along with vitamin D
(10 µg cholecalciferol), vitamin E (10 mg) thiamin (1.6 mg), riboflavin (1.8 mg), niacin
(20 mg nicotinamide), vitamin B-6 (2.2 mg pyridoxine hydrochloride), vitamin B-12 (2.6 μg), vitamin C (100 mg ascorbic acid), vitamin K (65 μg menadione), copper (2.0 mg cupric oxide), and magnesium (100 mg magnesium oxide).

The trial received ethical approval from the Committee on Human Research of the Johns Hopkins Bloomberg School of Public Health and the Nepal Health Research Council.

Pregnant women were interviewed at the time of enrollment and maternal height, weight, age, date of last menstrual period, parity, smoking history, and other characteristics were recorded. The main outcomes of the study were birth weight and infant survival. Since 95% of births occurred in the home, a female supplement distributor resident in the village reported the birth to a supervisor who dispatched an anthropometrist to the home to obtain “birth weight”. The aim was to weigh the infant as soon after birth as possible. Weights were measured using a digital (Seca 727, Hamburg, Germany) infant weighing scale, accurate to 2 g. We enrolled a total of 4096 pregnancies that resulted in 4130 live born infants. About 80% of weights were obtained within 72 h.

Since weight is measured at variable times since birth, we wished to construct a statistical model that provided a better estimate of true birth weight using the age of the infant at the time of measurement. In addition, about 7% of infants were missing weight measurements, 34% of whom died very soon after birth (2.4% of all those missing birth weight), before the anthropometrist was able to reach the household. These infants are
likely to have been smaller at birth than those who survived beyond the time they were weighed. We developed a measurement error model that allowed us to estimate the weight of the baby at birth for measurements made at variable times after birth and to impute missing measurements. Exploratory analysis of the relationship between birth weight and time of the measurement indicated that birth weight dropped approximately 50 g between 24 and 48 h, was constant between 48 and 72 h, and increased linearly after 72 h. We assumed the expected birth weight for baby \( i \) at time \( t_i \) had a main effect for the treatment and the vital status, was modeled as a natural cubic spline with knots placed at 24, 48 and 72 h for weights taken between birth and 72 h, varied linearly with time after 72 h and with number of cigarettes smoked, but varied non-linearly with gestational age, and maternal age, weight and height (modeled as natural splines).

Specifically, let \( t_i \) and \( W_{it} \) be the time and the corresponding birth weight measurement for infant \( i \) and let \( N_{it} \) be an indicator of vital status (alive or died) at time \( t_i \). The following model was fit:

\[
E[W_{it} | t_i, N_{it}, Tr_i, x_i] = \beta_0 + \beta_1 Tr_i + \beta_2 N_{it} + s(t_i, \text{knots} = (24, 48, 72)) + \beta_3 \text{num.cig}_i + s(\text{gest.age}_i, 3) + s(\text{gest.age}_i, 3) + s(\text{maternal.height}_i, 3) + s(\text{maternal.age}_i, 3)
\]

where \( x_i \) is the vector of covariates for the \( i \)th woman (gestational age, maternal weight in the first trimester, maternal height, maternal age, and number of cigarettes smoked in the seven days prior to the first trimester interview). \( Tr_i \) was the type of supplement the ith infant’s mother received, and \( s(x, \lambda) \) was a cubic spline with \( \lambda \) d.f. (three in this case). Gestational age of the infant was based on date of last menstrual
period from the first trimester interview, or if not available, the date of first positive pregnancy test was used. The imputation assumes that those missing birth weight had similar weights to those with the same covariates who did have birth weights measured. Treatment group was one covariate in this model because it was known that treatment impacted birth weight. Including treatment in the model would prevent the imputed values from being biased by the treatment effect (5).

Missing birth weights were imputed using a multiple imputation method (6). Specifically, let $\hat{W}_{it_i} = E[W_{it_i} \mid t_i, N_{it_i}, Tr_i, x_i]$ be the predicted birth weight at time $t_i$ conditioned on the vital status of the infant, supplement group and maternal covariates. Let $\hat{\sigma}^2$ the estimated residual variance of the regression model. Fifty imputed data sets were created by sampling $W_{it_i}^{(j)}$ from a normal distribution with mean $\hat{W}_{it_i}$ and SD $\hat{\sigma}$ for $j = 1,\ldots, J = 50$. If the weight was taken after 72 h, then the birth weight was recalibrated by taking $\hat{W}_{it_i} + (W_{it_i} - \hat{W}_{it_i})$. A small percentage of infants (4%) have missing values for some of the mother’s covariates. We imputed missing data in the mother’s characteristics with the corresponding mean in the population. Figure 1 denotes the observed (circles), predicted (triangles) and imputed (squares) birth weights at time $t_i$ for the babies that have been measured before (A) and after (B) the 72 h ($\hat{W}_{it_i} + (W_{it_i} - \hat{W}_{it_i})$).

We developed a statistical approach for estimating the effect of different maternal nutritional supplements on birth weight as a smooth function of the percentiles of the...
birth weight distribution (7-9). We first calculated the treatment effect at each percentile by comparing the weight for the smallest infant in one of the treatment groups with that of the weight for the smallest infant in the control group. We continued this all the way across the percentiles through to the largest infants. This was done for each of the four treatment groups compared with the control group. We then smoothed these treatment effects across the percentiles and estimated the treatment effect by taking the mean of these smoothed values.

Specifically, we assumed that the treatment effect was a smooth function of the percentiles of the birth weight distribution:

$$ TE(p) = Q_T(p) - Q_C(p) = s(p, \lambda), $$

where $Q_T(p)$ and $Q_C(p)$ are the quantile functions of the birth weight distribution for treatment and control groups respectively, and $s$ is a natural cubic spline of the percentile $p$ with $\lambda$ degrees of freedom, which we have chosen to equal four. To estimate $TE(p)$, we:

1. calculated the percentiles $p_i = i/(n + 1)$ with $n = 750$ (the smallest number of infants across treatment groups);
2. calculated the corresponding empirical quantiles of the birth weights $q_{T_i}, q_{C_i}$ under the treatment and control groups, respectively;
3. smoothed the difference between quantiles $q_{T_i} - q_{C_i}$ across the percentiles $p_i$. 
Note that if we set $p=0.5$, then our approach reduces to the common method of estimating a treatment effect by comparing the central values of the birth weight distribution (means or medians) between the treatment and control groups. The smoothing of quantile differences across percentiles to improve estimation of the mean difference between two outcomes was introduced by Dominici and Cope (7) for continuous and skewed data. This approach was implemented for estimating mean medical expenditures between diseased and non diseased patients (8). In this paper we have modified this idea for a continuous and symmetric outcome to estimate percentile-specific treatment effects. More details of this approach are in Dominici et al (9).

To account for the uncertainty in the imputation of the missing values, we repeated steps 1-3 separately for 50 imputed data sets. We then calculated the percentile-specific treatment effect and its corresponding total statistical variance by using standard multiple imputation methods (6). More specifically, let $\hat{TE}^{(j)}(p)$ and $\hat{V}^{(j)}(p)$ be the point estimate and the bootstrap variance of $TE(p)$ for the j-imputed data set, respectively. For each $p$, the overall estimate of the treatment effect and its total variance, denoted by $\hat{TE}(p),\hat{TV}(p)$, were obtained as follows:

$$\hat{TE}(p) = (1/J) \sum_{j=1}^{J} \hat{TE}^{(j)}(p)$$

$$\hat{TV}(p) = W(V(p) + (1+1/J)BW(p)),$$ where

$$W(V(p) = (1/J) \sum_{j=1}^{J} V^{(j)}(p)$$
Birth weight distribution percentiles

\[ BW(p) = \frac{1}{(J - 1)} \sum_{j=1}^{J} (\hat{TE}^{(j)}(p) - \bar{TE}(p))^2 \text{ where } \bar{TE}(p) = \frac{1}{J} \sum_{j=1}^{J} \hat{TE}^{(j)}(p) \]

To test whether there was a constant treatment effect across the distribution, we performed a permutation test. Specifically, for \( m = 1, \ldots, 500 \), we randomly re-assigned the birth weights to the two treatment groups (each of the four active treatment groups against control) and calculated the test statistics \( T^m = \sum_{i=1}^{n} (\hat{s}^m(p_i, \lambda) - \bar{s}^m)^2 \text{ where } \bar{s}^m = \sum_{i=1}^{n} \hat{s}^m(p_i, \lambda). \) The one-sided P-value was calculated as the Probability that \( T^m \) exceeded the observed test statistics \( T_{obs} = \sum_{i=1}^{n} (\hat{s}(p_i, \lambda) - \bar{s})^2 \text{ where } \bar{s} = \sum_{i=1}^{n} \hat{s}(p_i, \lambda). \)

Because the correlation of birth weight within clusters that were randomized to different treatments was essentially zero, these analyses have not taken the cluster randomization into account (1).

RESULTS

Variables found to predict birth weight in a measurement error model were treatment group, infant survival, time of infant weight measurement, gestational age at birth, and maternal height, weight, and age, and number of cigarettes smoked in the seven days prior to the first trimester interview (Table 1, Figures 1A and B, Figures 2A-D). The \( r^2 \) for this model was 0.54. Infants who died were predicted to weigh 340 g less than those who survived. The predicted birth weight was 11.7 g lower for each cigarette smoked in the seven days prior to the first trimester interview. The predicted impact of
the multiple micronutrient supplement on birth weight was 75 g, and the iron plus folic acid group had an increase of 43 g compared with the vitamin A alone (Table 1).

The treatment effects were estimated as smooth functions of the percentiles of the birth weight distribution \( \hat{TE}(p) \) with the corresponding 95% bootstrap confidence bands for each treatment group (Figure 3A-D). The circles denote the difference in the empirical quantile functions between the two groups \( (q_{T} - q_{C}) \) as a function of the percentiles. As previously reported, there was a mean treatment effect for the folic acid plus iron and multiple micronutrient groups (1). The one-sided P-values of the permutation tests for the hypothesis of a constant treatment effect across percentiles of the birth weight distribution were 0.10 for folic acid plus iron, 0.96 for multiple nutrients, 0.04 for folic acid plus iron plus zinc, and 0.60 for folic acid. Therefore, for folic acid plus iron and for folic acid plus iron plus zinc, the treatment effect is significantly different across the distribution of birth weight at the 5 and 10% level, respectively.

More specifically, we found that: 1) folic acid plus iron increased birth weight for babies smaller than about 2800 g with a decline in the treatment effect for the larger babies; 2) the multiple micronutrients increase birth weight across the entire distribution of weights; 3) folic acid plus iron plus zinc increased birth weight only for babies in the middle of the birth weight distribution (between 2400 and 2900 g approximately); and 4) folic acid alone did not significantly increase the birth weight across the entire distribution.
DISCUSSION:

Our analysis demonstrates that the standard approach of estimating a mean difference in a continuous outcome between a treatment and control group may not adequately capture the impact of nutritional supplementation on birth weight. The ability to assess whether the treatment effect varies across the distribution of the outcome may provide insights into the mechanism by which the treatment affects the outcome, and ideas as to why a surrogate outcome (such as birth weight) may not reflect the effect of treatment on a primary outcome of interest (mortality). Such an approach could also be applied more generally to continuous outcomes in trials of nutritional interventions such as hemoglobin or z-scores for weight-for-height or height-for-age. From a public health perspective, this approach can also help identify whether a targeted, rather than universal supplementation program would be more effective and efficient in achieving a nutritional goal for a population.

Our data demonstrated that the mean treatment effect with iron folic acid was half that of the mean multiple micronutrient effect but in environments like rural Nepal, it may be more important to affect the lower than the upper part of the birth weight distribution. In fact, impacting the upper part of the distribution may be harmful to the mother and infant. In our study, we found the multiple micronutrient supplement to be associated with a slightly elevated risk of early infant mortality (Relative Risk (RR): 1.07, 95% CI: 0.75, 1.58), especially among term births (RR: 1.74, 95% CI: 1.00, 3.04) (2). This was despite the significant 14% reduction in low birth weight. The risk of birth asphyxia as a cause of neonatal mortality also appeared to be higher in the group
receiving the multiple micronutrient supplement. On the other hand, folic acid plus iron was associated with an overall 21% reduction (95% CI: 48% reduction to increase of 20%), and ~47% reduction in three month mortality (95% CI: 8, 70%) among preterm births. Given an improvement in birth weight at the lower end of the distribution, this intervention may have produced improved survival overall, while the multiple micronutrient appeared to have no impact on survival because deaths averted in the smaller infants were negated by higher mortality at the upper end of the distribution. A recent study examining a multiple micronutrient supplement relative to a control supplement of iron plus folic acid in Janakpur, Nepal, found a higher mean birth weight of 77 g (95% CI: 24, 130) in the multiple micronutrient group compared with the iron plus folic acid group, but a higher neonatal mortality in the multiple micronutrient group (RR: 1.53, 95% CI: 0.72, 3.2) (10). Pooled analyses of the Sarlahi and Janakpur studies found higher neonatal mortality (RR: 1.52 (1.03, 2.25) among those in the multiple micronutrient group relative to the folic acid and iron group (2, 11).

Our data also found evidence at a population level, when examined cross-sectionally, for a decline in weight of about 50 g between 24 and 48 h following birth. This is comparable to the drop in weight seen in a population of newborns in Bangladesh (12). The weights rise thereafter, and from 72 h through three months of age, weights can be modeled as a linear function of age in this population. Among those who died before a weight measurement could be obtained, or who survived but were missing weight measurements due to migration, birth weights can be imputed using a variety of maternal and other covariates that are strong predictors of birth weight. Using re-calibrated birth
weights for those measured at varying times after birth, and imputed weights for those with missing weights, the estimated impact of maternal supplementation was slightly larger for all treatment groups than when the treatment effects were estimated without imputation (Figure 3). The confidence intervals for the treatment effects using imputed values were wider, reflecting the uncertainty associated with the imputed birth weights. Although there is an assumption in imputation that the infants missing birth weight would have a similar weight to infants with similar covariates who were not missing weight, there are more infants missing weight because they died soon after birth, and such infants might have been smaller than those who survived long enough to be weighed. However, we do have birth weights on some infants who later died, and this imputation model does use those infants to help predict the birth weight of those who died before we could weigh them. The birth weight differential predicted from the model between those who died and those who survived is over 300 g, although the babies we did weigh who later died may not capture adequately this difference in birth weight. It is also possible that babies who were large relative to the size of their mothers may have died so soon after birth that they were also not weighed. This may be true given a reverse J shaped risk profile for mortality of these infants by birth weight (13). So there may be some bias, but the direction of the bias is uncertain. However, only 2.4% of missing birth weights were imputed because the child had died before weight could be obtained, so the bias from imputing weights for these infants on overall birth weight is likely small. Given the constraints in obtaining birth weight in settings where births occur at home, future studies or programs are likely to face the same situation with missing birth weight. Our measurement error model for imputing the missing birth weights and predicting the
weights “at birth” for measurements made after the 72 h may be relevant for future studies that examine intervention effects on birth weight.

Because of the strong association between birth weight and survival, which may be an inverse J-shape in this population (13), understanding the impact of a treatment on different parts of the birth weight distribution may provide more insight into how the treatment impacts both the surrogate outcome (birth weight) and the primary outcome (survival) (14). Such analyses can help explain contradictions in treatment effects on surrogate and primary outcomes, and may help identify the extent to which a treatment effect acts directly on survival or through its impact on birth weight. Direct analyses of treatment effects on mortality by birth weight distribution are currently underway (9).
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TABLE 1: Regression Coefficients for the Measurement Error Model of Birth Weight

<table>
<thead>
<tr>
<th>Variables*</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>t-statistic</th>
</tr>
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<tbody>
<tr>
<td>Intercept</td>
<td>768.86</td>
<td>239.91</td>
<td>3.20</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>-0.96</td>
<td>21.78</td>
<td>-0.04</td>
</tr>
<tr>
<td>Folic Acid + Iron</td>
<td>43.01</td>
<td>21.76</td>
<td>1.98</td>
</tr>
<tr>
<td>Folic acid + Iron + Zinc</td>
<td>8.84</td>
<td>21.47</td>
<td>0.41</td>
</tr>
<tr>
<td>Multiple micronutrients</td>
<td>74.65</td>
<td>21.00</td>
<td>3.55</td>
</tr>
<tr>
<td>Death</td>
<td>-340.39</td>
<td>34.81</td>
<td>-9.78</td>
</tr>
<tr>
<td>Number of cigarettes</td>
<td>-11.7</td>
<td>2.17</td>
<td>-5.39</td>
</tr>
<tr>
<td>Maternal height (cm)</td>
<td>2.83</td>
<td>1.53</td>
<td>1.85</td>
</tr>
</tbody>
</table>

* Also adjusted for time of measurement (h), gestational age (wk), maternal age (y) and maternal weight (kg).
TABLE 2. Crude and imputed mean difference and 95% CI in birth weight between treatment and control groups.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Crude mean difference (g)</th>
<th>Imputed mean difference (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic Acid</td>
<td>-7.1 (-61.0, 46.8)</td>
<td>16.9 (-54.5, 88.3)</td>
</tr>
<tr>
<td>Folic Acid + iron</td>
<td>60.0 (4.1, 115.9)</td>
<td>71.1 (0.4, 141.9)</td>
</tr>
<tr>
<td>Folic Acid + iron + zinc</td>
<td>0.3 (-57.1, 57.8)</td>
<td>26.3 (-42.4, 95.0)</td>
</tr>
<tr>
<td>Multiple micronutrients</td>
<td>63.5 (9.0, 118.0)</td>
<td>84.6 (15.6, 153.6)</td>
</tr>
</tbody>
</table>
Legends for Figures:

FIGURE 1: Scatter plot of observed, predicted and imputed weights of infants plotted against their times of measurement since birth through 3000 h (A) and within 72 h (B).

FIGURE 2: The predicted values of birth weight plotted against time of measurement using a spline with knots at 24, 48 and 72 hours (A). The deviations in predicted values from the average birth weight plotted against gestational age at birth (B), maternal age (C), and first trimester maternal weight (D), also using natural splines.

FIGURES 3: The estimated treatment effects shown as a function of the percentiles of the birth weights in the control group (A: iron plus folic acid, B: multiple micronutrients, C: folic acid plus iron plus zinc, D: folic acid). The x-axes show the quantiles of the birth weights in the control group. The zero line indicates no treatment effect and a negative treatment effect implies a reduction in birth weight associated with the treatment in that range of the birth weight distribution. The central solid black line is the smoothed treatment effect, with upper and lower 95% bootstrapped confidence bands. The dotted line on the right denotes the estimated crude treatment effects and 95% CI. The solid line on the right denotes the estimated treatment effect and 95% bootstrap CI obtained by averaging the treatment effect across the percentiles making use of the imputed values for birth weight.