Should infant girls receive micronutrient supplements?

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Background We have proposed the hypothesis that the combination of vitamin A supplementation and diphtheria-tetanus-pertussis (DTP) vaccination may be associated with increased mortality in girls. Recent zinc/folic acid (FA) and iron supplementation trials did not find any beneficial effects on mortality. We reviewed the studies for evidence of a negative interaction between zinc/folic acid/iron and DTP vaccination in girls.

Methods Based on the published papers, we calculated age- and sex-specific mortality estimates. No vaccination status data were provided.

Results Both zinc/FA and iron seemed to have a sex- and age-differential effect, the effect being less beneficial in the youngest girls who are most likely to have DTP vaccine as their most recent vaccination.

Conclusions Like vitamin A, zinc/FA and iron may not benefit the youngest girls. The question is whether this is inherent in girls or due to an interaction with some environmental factor like DTP.

Keywords Vitamin A, zinc, iron, sex, DTP vaccine, child mortality

Background
Some years back IJE published our hypothesis that vitamin A supplementation (VAS) amplifies the non-specific effects of vaccines.1 The hypothesis was based on the observation that VAS was beneficial when given in the time windows of Bacille Calmette-Guérin (BCG) (at birth) and measles vaccine (MV) (after 9 months of age), but had no effect or even a slightly negative effect when administered in the time window of diphtheria-tetanus-pertussis vaccine (DTP) (between 1.5 to 5 months of age).1 Since the non-specific effects of vaccines are strongest in girls, the effect could be expected to be most pronounced in girls. Below we argue that subsequent studies have corroborated this hypothesis, especially the notion that the combination of DTP and VAS may be harmful for girls. Furthermore, new studies on iron/folic acid (FA) and zinc supplementation suggest that there might be similar negative interactions between DTP and other micronutrients in girls. Infant girls may not benefit from micronutrient supplements.

Negative interaction between VAS and DTP vaccine
We have consistently found DTP to be associated with non-specific negative effects on mortality and increased female-to-male mortality in low-income countries.2 Several types of studies support the possibility that VAS may amplify the effects of DTP.1 First, one randomized study administering DTP with VAS or placebo reported the results by sex. Among 200 children discharged from a paediatric ward in Bangladesh, the girls receiving DTP and VAS tended to have higher mortality than placebo recipients, the relative risk (RR) being 1.40 [95% confidence interval (CI) 0.59–3.34], whereas the RR was 0.70 (0.21–2.36) for boys.3 Second, we conducted a randomized trial of different doses of VAS to children aged 6–60 months during a VAS and oral polio vaccine campaign in Guinea-Bissau. As hypothesized, half the
recommended dose was even better than the standard dose of VAS recommended by WHO—but only in girls [mortality rate ratio (MRR) for WHO dose vs half that dose = 5.26 (1.52–16.7)]. This was particularly strong among girls who had received DTP as their last vaccine before the campaign [MRR for WHO dose vs half that dose = 10.0 (1.25–100)] (unpublished data) supporting the possibility that the standard dose of VAS may amplify the negative effect of DTP in girls. Third, during national immunization days in Guinea-Bissau, we conducted an observational study of the effect of providing VAS with missing vaccines; as hypothesized the effect of VAS was negative when given with DTP but not when given with MV. There was significantly higher mortality among those who received VAS with any DTP compared with children receiving VAS alone, the MRR being 3.43 (1.36–8.61). None of the children who received VAS + MV died (P = 0.0005 for interaction between VAS and vaccine type). VAS + DTP recipients also had higher mortality than non-participants, who traditionally have the highest mortality [MRR = 3.04 (1.31–7.07)]. It should be noted though, that these negative effects were seen in boys as well as girls. Fourth, surprisingly we found no overall beneficial effect on infant mortality in a randomized trial of giving VAS with BCG vaccine at birth in Guinea-Bissau. However, the effect differed between boys and girls, with a tendency for a beneficial effect in boys and a negative effect in girls. Female VAS recipients had the same mortality as female placebo recipients as long as BCG was the last vaccine received. However, after receiving DTP, female VAS recipients had 2-fold increased mortality compared with female placebo recipients [MRR = 2.19 (1.09–4.38)] (CS Benn et al., submitted for publication). Furthermore, in the same trial, although vitamin A status generally improved from 6 weeks of age to 4 months of age, the increase correlated inversely with the number of doses of DTP received in the interval (P = 0.009), particularly in girls (P = 0.01 for interaction between number of DTP and sex) and in VAS recipients (P = 0.01 for interaction between number of DTP and VAS). Fifth, to test whether VAS interacted with vaccinations, we reanalysed data from one of the major vitamin A trials from Ghana, which found that VAS reduced child mortality by 19%. The reanalysis does indicate interaction between VAS and vaccinations. Among children with no vaccination card, the reduction was 36% (12–53%); whereas there was no effect among children who had a vaccination card. Among children with a vaccination card, VAS tended to be beneficial for boys but slightly negative for girls, the test of interaction between VAS and sex being significant. The negative effect of VAS for girls with a vaccination card was mostly found among girls who received DTP after MV (Benn et al., unpublished data).

Hence, these data suggest that while DTP is the predominant vaccine the impact of VAS may be negative, particularly for girls. Since DTP is the predominant vaccine in infancy, the effect of VAS is likely to be least beneficial among the youngest girls.

**Effect of iron/FA and zinc on mortality**

The situation may be similar for other micronutrients. Over the last years, The Lancet has published four papers presenting the results of two large randomized trials in Nepal and Zanzibar conducted by researchers at Johns Hopkins University. The two trials had several features in common. They enrolled more than 40,000 children aged 1–35 months in populations with iron and zinc deficiency. They addressed the effect of daily iron plus FA supplementation and daily zinc supplementation on mortality and morbidity in a two-by-two factorial design. Results were presented separately for iron plus FA and for zinc. Unexpectedly, the trials found no beneficial effects, except a slight effect of zinc in older children. Both studies stopped the iron/FA arm early; in Zanzibar due to the detection of excess serious adverse events (deaths and hospitalizations) in the iron/FA arm, and in Nepal because an interim analysis showed no beneficial effect of iron/FA and the statistical power to detect significant differences would be too small with the planned sample size. The overall conclusions on mortality were: first, daily supplementation with iron/FA had no effect on mortality in Nepal, but was associated with increased risk of serious adverse events in Zanzibar, and second, daily supplementation with zinc was associated with a tendency for reduced mortality after 12 months of age in both settings.

**Sex- and age-specific effects on mortality of micronutrients**

These papers share another important feature: sex- and age-differential effects of preventive treatment with micronutrients.

First, iron/FA and zinc supplementation both tended to be associated with decreased mortality in boys but less effect in girls (Table 1). Based on the two zinc papers, we calculated that zinc was associated with a 15% reduction of overall mortality in boys [MRR = 0.85 (0.73–1.00)]. Iron was also associated with a 16% reduction in mortality among boys in Nepal; the effect of iron was not reported by sex in Zanzibar. In contrast, there was no effect of micronutrients among girls; based on the two zinc papers the MRR for girls was 1.00 (0.86–1.16), in particular it was 1.15 (0.94–1.40) for infant girls. Iron was associated with 18% increased mortality among girls in Nepal. Had the trials been independent, the meta-analysis estimate of the mortality effect of iron/FA
and/or zinc among boys would have been 0.85 (0.73–0.98) compared with 1.03 (0.91–1.18) among girls, resulting in an interaction between iron/FA/zinc and sex (P = 0.051). However, since the trials had partly overlapping control groups, the CIs should probably be slightly wider. A potential sex-differential effect was noted in the zinc study from Zanzibar11 but seems to have been more general.

Second, in all four papers the effect was worse in the youngest age groups compared with older children (Table 1). Had the trials been independent, the meta-analysis estimate of the mortality effect of iron/FA and/or zinc among infants would have been 1.07 (0.95–1.22) compared with 0.89 (0.79–1.01) among children aged 1–3 years (P = 0.039). Again, since the trials had partly overlapping control groups, the CI should probably be slightly wider.

Third, unfortunately only the two zinc papers reported the effect by both sex and age group. The results in these papers suggest that the shift in effect with age is mostly due to a shift among girls. Based on the estimates presented in the two papers, the effect of zinc differed for infant girls and older girls (P = 0.049); whereas, there was no noticeable difference between age groups in boys.

Among VAS studies, only Sommer’s original trial13 reported the effect of VAS by both sex and age. There was a similar pattern of the effect of VAS on survival improving with age for girls, but not changing for boys (Table 1). As seen in Table 2, with two exceptions the eight trials providing VAS in the first year of life found a better effect for boys than for girls, whereas there was no consistent sex difference among older children. Hence, common sex and age differences may exist in the response to iron/FA, zinc and VAS.

Possible explanations for sex and age differential effects of micronutrients

Sex and age differences in the mortality response to micronutrients could be expected if infant boys were more micronutrient deficient than infant girls. Indeed there have been some indications that boys are more vitamin A deficient at birth,22 and more iron-deficient in infancy.23 However, the Zanzibar study assessed zinc and iron status in a subgroup and found no differences between boys and girls,11 and we found slightly better vitamin A status among boys than girls at 4 months of age in Guinea-Bissau.7 Alternatively, micronutrient supplementation might affect the immune system differently in boys and girls, as also suggested in one of the zinc papers.11
We propose that iron/FA and zinc supplementation like VAS may amplify the negative effect of DTP in girls. Infants below 1 year of age are most likely to have DTP as their last vaccine, since MV is only administered after 9 months of age. While DTP is the last vaccine, girls have higher mortality than boys.2 Following MV, mortality among girls decline, and hence the relative mortality of girls decrease from 6–11 months of age. 2 Thus, a negative interaction with DTP vaccine would explain the observed lack of benefit of micronutrients in infant girls, and the subsequent shift in effect with age.

There are several potential explanations for sex-differential effects and for age-differential effects of micronutrients on mortality.9–12 However, for iron/FA/zinc as well as for vitamin A1 an interaction with vaccines would explain both effects at the same time.

**Reporting of micronutrient data**

The consistent limited or negative effect on mortality of micronutrient supplementation among the youngest girls—in spite of micronutrient deficiencies—deserves attention. It should become standard that micronutrient trial data are reported separately for boys and girls within all age groups (e.g. 1–5, 6–8, 9–11, 12–17, 18–23 and 24+ months) and we hope the authors of the *Lancet* papers will also present their data this way. The four papers do not present data on vaccination status or vaccination coverage. If vaccination status data are available, data should also be presented by vaccination status to explore possible interactions between micronutrient supplementation and vaccines. If vaccination status is not available, but vaccination coverage is high in the recommended age

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**Table 2** Estimates of the effect of VAS on mortality by sex

<table>
<thead>
<tr>
<th>Author, country and year of publication</th>
<th>Overall effect</th>
<th>Boys</th>
<th>Girls</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td><strong>At-birth supplementation</strong></td>
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<tr>
<td>Humphrey,14 Indonesia, 1996</td>
<td>0.36 (0.16–0.87)</td>
<td>0.15 (0.03–0.68)</td>
<td>0.84 (0.26–2.77)</td>
<td>Hospital deliveries. No vaccination information</td>
</tr>
<tr>
<td>Rahmatullah,15 India, 2003</td>
<td>0.78 (0.63–0.96)</td>
<td>0.70 (0.52–0.94)</td>
<td>0.87 (0.65–1.17)</td>
<td>Low vaccination coverage</td>
</tr>
<tr>
<td>Benn,6 Guinea-Bissau, 2007</td>
<td>1.07 (0.79–1.44)</td>
<td>0.84 (0.55–1.27)</td>
<td>1.39 (0.90–2.14)</td>
<td>Negative effect of VAS in girls only seen after DTP (CS Benn et al., submitted for publication)</td>
</tr>
<tr>
<td>Klemm,16 Bangladesh, 2008</td>
<td>0.85 (0.73–1.00)</td>
<td>0.89 (0.72–1.10)</td>
<td>0.81 (0.65–1.00)</td>
<td>Low vaccination coverage</td>
</tr>
<tr>
<td><strong>0–11 months</strong></td>
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<td></td>
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<tr>
<td>Pathwardhan,17 Jordan, 1966</td>
<td>0.50 (0.13–1.94)</td>
<td>0 (one placebo death)</td>
<td>0.59 (0.15–2.30)</td>
<td>1–5 month old. Pre-vaccination era</td>
</tr>
<tr>
<td>Sommer,13 Indonesia, 1986</td>
<td>0.83 (0.51–1.37)</td>
<td>0.59 (0.33–1.06)</td>
<td>1.06 (0.62–1.81)</td>
<td>0–11 month old. No vaccination information</td>
</tr>
<tr>
<td>West,18 Nepal, 1995</td>
<td>1.11 (0.86–1.42)</td>
<td>1.24 (0.86–1.78)</td>
<td>0.98 (0.68–1.42)</td>
<td>0–5 month old. No vaccination information</td>
</tr>
<tr>
<td>Mahalanabis,3 Bangladesh, 1997</td>
<td>1.06 (0.52–2.18)</td>
<td>0.70 (0.21–2.36)</td>
<td>1.40 (0.59–3.34)</td>
<td>6–17 weeks. VAS given with each of the three doses of DTP</td>
</tr>
<tr>
<td><strong>12 months+</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Sommer,13 Indonesia, 1986</td>
<td>0.66 (0.44–0.97)</td>
<td>0.59 (0.37–0.95)</td>
<td>0.80 (0.46–1.40)</td>
<td>12–71 month old</td>
</tr>
<tr>
<td>West,19 Nepal, 1991</td>
<td>0.70 (0.56–0.88)</td>
<td>0.77 (0.55–1.09)</td>
<td>0.65 (0.48–0.89)</td>
<td>6–60 month old (~90% of the children were between 12 months and 60 months)</td>
</tr>
<tr>
<td>Daulaire,20 Nepal, 1992</td>
<td>0.74 (0.55–0.99)</td>
<td>0.72 (0.48–1.08)</td>
<td>0.76 (0.48–1.19)</td>
<td>1–59 month old (~85% of the children were between 12 months and 59 months)</td>
</tr>
<tr>
<td>Herrera,21 Sudan, 1992</td>
<td>1.06 (0.82–1.37)</td>
<td>1.25 (0.85–1.83)</td>
<td>0.93 (0.66–1.31)</td>
<td>9–72-month old</td>
</tr>
<tr>
<td>Ghana Vast Study Team,9 Ghana, 1993</td>
<td>0.81 (0.68–0.98)</td>
<td>0.73 (0.59–0.92)</td>
<td>0.90 (0.71–1.15)</td>
<td>6–90-month old</td>
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</table>

**Note:** All major studies which have addressed the effect of VAS by sex. Some of the studies of children aged 12 months and older have also included younger children, but apart from Sommer’s study it is not possible to extract the sex-specific mortality ratios for separate age groups. Values in bold indicates a better effect in boys than in girls.

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groups, the mortality effect in boys and girls in the age groups 1.5–8 months (DTP is the predominant vaccine) and 9–17 months (MV is the predominant vaccine) could indicate whether there were micronutrient/vaccine interactions or not.

Conclusions
Irrespective of the underlying explanation(s) for the sex- and age-dependent mortality patterns after micronutrient supplementation, they are potentially of large public health importance. The authors of the Lancet papers discuss whether zinc should be given in a higher dose or only to older children. However, micronutrient supplementation policies might have to differ for boys and girls. The data from the zinc papers and the vitamin A trials (Table 2) suggest that it would be a shame to disregard early zinc supplementation and early VAS for boys just because there was no overall beneficial effect. On the other hand, all evidence suggests that currently infant girls have little to gain from micronutrients. The question is whether this is inherent in girls or due to an interaction with some environmental factor like DTP.

References