Vitamin A Supplementation Does Not Affect Infants’ Immune Responses to Polio and Tetanus Vaccines

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ABSTRACT It has been suggested that administering vitamin A with the measles vaccine may reduce the vaccine’s immunogenicity. This trial examined the effect of supplementing vitamin A during the early months of life on infants’ immune responses to tetanus and polio vaccines. Young infants (n = 1085) were enrolled and individually randomized into 1 of 4 groups in a factorial, double-blind, placebo-controlled trial. Three vitamin A supplementation strategies were investigated: 1) supplementation of breast-feeding mothers with 60 mg retinol equivalent (RE) vitamin A within 4 wk of delivery; 2) Expanded Program on Immunization (EPI)-linked supplementation of infants with 7.5 mg RE vitamin A at 6, 10, and 14 wk; and 3) combined mother and child supplementations. A 4th group in which mother and child were given placebos served as controls. Blood samples were collected from each child at 6 wk and 6 mo of age to measure antipolio antibody titer, antitetanus toxoid antibodies, and avidity of antibodies to tetanus. Of the infants randomized into the 4 arms of the study, 767 (71%) completed follow-up at 6 mo of age. Follow-up rates were similar in all 4 arms (69–72%, P = 0.8). Antibody titers were relatively high in all 4 groups at both 6 wk and 6 mo of age, with no differences among the groups. We found no evidence that vitamin A supplementation affects infants’ antibody responses to tetanus toxoid or oral polio vaccine delivered at EPI contacts.

KEY WORDS: • vitamin A • poliomyelitis • immunization • tetanus toxoid • immune response

Vitamin A supplementation can substantially reduce mortality (1) and severe morbidity (2,3) in children 1 to 5 y of age in less developed countries. The benefits of improving vitamin A status in this age group are clear. Many children, however, are born with low vitamin A stores (4) and depend on their mother’s breast milk as their only source of vitamin A. If the mother herself has low vitamin A levels, she may not be able to provide enough to boost the child’s liver stores before the introduction of complementary foods. In addition, infections may adversely affect the infant’s vitamin A status (5).

The current WHO recommendation is for vitamin A supplementation to be linked to existing immunization contacts under the Expanded Program of Immunization (EPI)3 taking advantage of these contacts with health services. However, if there are undesirable consequences of administering vitamin A to infants at the time of routine childhood immunizations, the EPI program could be jeopardized because mothers would be unlikely to use the services of the EPI clinics. On the other hand, if maternal supplementation alone can achieve adequate infant vitamin A status (6), there would be no need for infant supplementation. Adequate vitamin A status is required for optimal antibody responses and other immune functions (7). In addition, large doses of vitamin A can act as an adjuvant and might therefore improve responses to vaccines given at the same time. In a study of supplemented children 3–6 y old, antibody responses to tetanus toxoid were increased (8). However, it was reported that responses to measles vaccines (given at 6 mo when maternal antibodies tend to be high, rather than at the usual 9 mo) are decreased in children with high maternal antibodies and supplemented with vitamin A (9). The investigators hypothesized that vitamin A supplementation in the presence of maternal antibodies might enhance the child’s immunity to the extent of limiting the ability of a live vaccine such as measles to establish an immune response. This raised concerns about the strategy of linking vitamin A supplementation of young children to the EPI.

A subsequent study in Bangladesh (10) reported that seroconversion rates to oral polio vaccine, which is a live virus vaccine like measles, were unaffected by vitamin A supplementation; another study in Indonesia (8) showed that vitamin A supplementation with oral poliovirus vaccine (OPV) immunization at the 6-, 10-, and 14-wk visits to the EPI did not interfere with seroconversion to any of the 3 polio types. We sought to replicate that finding and to assess 3 different strategies for improving the vitamin A status of young infants.
SUBJECTS AND METHODS

Study site. The trial was carried out in Kintampo, a small town (population 28,000) in a rural area of Brong Ahafo Region, Ghana, where breast-feeding is almost universal and oral polio vaccine is routinely given after delivery in health facilities and at 6, 10, and 14 wk of age in health facilities. Previous studies carried out around Kintampo indicated severe vitamin A deficiency in the area, with 51% of children < 5 y old having serum retinol concentrations < 0.70 μmol/L (11). Anthropometric data indicated prevalences of stunting and wasting of 32 and 4%, respectively, among 12-mo-old children. At the time of this trial, vitamin A was not routinely provided to infants < 9 mo old. Kintampo was also one of the field sites of the WHO-funded multicenter trial of maternal and child supplementation.

Study interventions. The trial was an individually randomized, double-blind, placebo-controlled trial. Trained field workers identified newborn infants through active weekly surveillance in the community. Mothers who gave written informed consent and intended to stay in the study area for the duration of the trial were enrolled. Mothers and infants were allocated to 1 of 4 treatment groups, using a blocked randomization scheme.

Group 1 mothers received 60 mg retinol equivalent (RE) vitamin A (as retinol palmitate), 3–4 wk postdelivery, and their infants received 3 oral doses of 7.5 mg RE vitamin A, at 6, 10, and 14 wk of age, at the time they received their diphtheria pertussis tetanus (DPT)/polio immunizations. At the end of the study (6 mo of age), infants received a 4th dose of 7.5 mg RE vitamin A.

Group 2 mothers received a placebo and their infants received 3 doses of 7.5 mg RE vitamin A, at 6, 10, and 14 wk of age, at the time they received their DPT/polio immunizations. At the end of the study (6 mo of age), infants received 3 oral doses of 7.5 mg RE vitamin A.

Group 3 mothers received 60 mg RE vitamin A, 3–4 wk post delivery, and their infants received a placebo at 6, 10, and 14 wk of age at the times they received DPT/polio immunizations. At the end of the study (6 mo of age), infants received 30 mg RE of vitamin A.

Group 4 mothers and their infants received placebo capsules rather than vitamin A, but at 6 mo of age, the infants received 30 mg RE in a single dose, providing them with the same total dose as the infants in the other groups.

The test and placebo capsules were identical in size color and shape. The active ingredient in the vitamin A capsules was retinol palmitate, 7.5 mg (RE) and 60 mg (RE). We verified that the oral polio vaccine, which was used in this study, met the WHO requirements in terms of the cold chain, which was monitored to ensure that the potency of the vaccines was not compromised. At age 6, 10, and 14 wk, infants received 2 drops of Trivalent oral polio vaccine (Polio Smithkline Beecham Biological Rixensart, lot S2785AA) directly into the mouth and three 0.5-mL doses of DPT vaccine i.m. (DPT Serum Institute of India Limited. Lot E-39975-A). Vaccines were administered by community health nurses.

Sample size. Using standard formulas (12), without adjustment for multiple comparisons (13), a sample size of 200 infants completing follow-up in each treatment group (800 in total) was required to provide the study with 90% power to detect a decrease of 12.5% in the seroconversion rate to polio type 2 and a decrease of 16% for polio type 3. Assuming that ~16% of infants would be lost to follow-up, we aimed to recruit 960 infants. As the study progressed, it became clear that the proportion of infants lost to follow-up was >16% and the period of recruitment was extended.

Study procedure. Trained field workers living locally identified new births through weekly active surveillance in the community. Consent was obtained when the birth was first identified but enrollment took place only when the maternal dose was to be delivered, 3–4 wk postpartum. Infants were scheduled to receive vitamin A or placebo at 6, 10, and 14 wk to coincide with their routine immunization contacts with EPI. Each week, listings of infants due to be immunized were generated from the computerized database of enrolled infants. Field workers notified mothers in advance of these visits. Study infants were identified with the help of their ID cards by trained staff who administered the vitamin A or placebo before the immunizations. At the first EPI contact (i.e., 6 wk) and again 12–13 wk after the 3rd immunization at ~6 mo, blood samples (1 mL) were collected by heel prick for the measurement of the antibodies to polio and tetanus. At the 6-mo visit, the child received the last dose of vitamin A or placebo. Blood samples were centrifuged (500 × g for 5 min) in Kintampo and stored at −20°C before being transported to the Noguchi Memorial Institute for Medical Research (NMIMR) at the University of Ghana for analysis.

Sample analyses. Enzyme-linked immunosorbent assay (ELISA) assays were carried out for the tetanus affinity.

ELISA for anti tetanus antibody determination. Briefly, the method included coating 96-well plates (Maxisorp Nunc) with tetanus toxoid at a concentration of 2.5 Lf (flocculation units)/mL in 0.1 M bicarbonate buffer, pH 9.5. After coating and washing with PBS containing 0.05% Tween 80, the plates were blocked with 2% bovine serum albumin (BSA) in PBS, and washed with PBS containing 0.05% Tween 80; then, samples diluted in PBS-Tween 80 with 0.5% BSA were applied to the wells. Test sera were diluted 1:10 and a positive control (reference serum) was diluted 1:250 (40 IU/L). After incubation and washing, anti-human IgG (Fc fraction) coupled to horseradish peroxidase (1:40,000 dilution) was added; after incuba-

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*60 mg retinol equivalent (200,000 IU), 7.5 mg retinol equivalent (25,000 IU).*
tion, plates were washed again and incubated with substrate solution containing 3',3',5',5'-tetramethylbenzidine substrate (TMB; Sigma). The color reaction was stopped with 2 mol/L H₂SO₄ and the absorbance was read using a plate-reading spectrophotometer.

The assessment for polio antibodies was based on actual seroconversion. This was defined as a 4-fold rise in neutralizing antibodies after immunization. These assays were established at NMIMR, which is a reference laboratory for polio in Africa (14). At the time of the study, we tried to develop an ELISA assay for the detection of polio antibody and the measurement of antibody avidity but we were unsuccessful. Other groups have since developed an ELISA for the detection of polio antibodies (15).

Data management and statistical analyses. Field supervisors checked all forms manually for completeness and consistency. These were then double entered on computers; range and consistency checks were performed and discrepancies resolved by reference to the form. A simple comparison of baseline measures (demographic, socioeconomic, biochemical) was performed across the treatment groups to confirm their comparability; 95% CI and geometric mean concentrations or titers of the outcome measures were compared among the groups initially on an intent-to-treat basis. FoxPro data management software was used for data entry and the analyses were done using Stata version 7. The analysis of continuous outcomes was based on ANOVA of the log (titers).

Outcome measures. The effect of the different supplementation strategies was assessed by comparing the infants' total serum antibody titers for polio and tetanus at 6 mo among the 4 groups. This age was chosen as the moment at which outcomes were evaluated because this is the age at which it is presently recommended that vitamin A supplementation begin for young infants. The primary purpose of this study was to investigate the effect of vitamin A on individual protective responses.

Ethical issues. Written informed consent was sought from all mothers for participation in the study after a detailed explanation of the purpose of the study. The ethical review committee of the Ministry of Health of Ghana and the ethics committee of the London School of Hygiene and Tropical Medicine approved the study. An independent treatment effect monitoring committee body was set up to monitor serious side effects that arose. All serious side effects were reported to the committee within 7 d.

RESULTS

Enrollment. Enrollment started in November 1996 and the study was completed in January 1999; 1170 mother-child pairs meeting the enrollment criteria were identified and initially consented to take part in the study. A total of 85

![Figure 1](https://i.imgur.com/3Q5Q5Q5.png)

**FIGURE 1** Profile of mother/infant pairs in the immune response trial at recruitment, randomization, and the reasons for losses to follow-up at different stages of the trial, by treatment group. A = mother and child supplements; B = child supplements only; C = mother supplements only; D = control (placebo) group.
Infants’ antibody levels at 6 wk of age after the mothers had received vitamin A or placebo but before the infants received vitamin A or placebo

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Test 1</th>
<th>Group 1 (n = 194)</th>
<th>Group 2 (n = 191)</th>
<th>Group 3 (n = 182)</th>
<th>Group 4 (n = 193)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus</td>
<td>GMC</td>
<td>0.96 (0.7, 1.3)</td>
<td>0.96 (0.7, 1.3)</td>
<td>1.15 (0.9, 1.5)</td>
<td>0.99 (0.8, 1.3)</td>
<td>0.75</td>
</tr>
<tr>
<td>Polio Type 1</td>
<td>GMT</td>
<td>31.6 (26, 39)</td>
<td>33.3 (26, 42)</td>
<td>31.7 (24, 41)</td>
<td>34.9 (28, 44)</td>
<td>0.92</td>
</tr>
<tr>
<td>Polio Type 2</td>
<td>GMT</td>
<td>37.9 (31, 47)</td>
<td>38.7 (31, 48)</td>
<td>44.8 (35, 57)</td>
<td>42.0 (33, 53)</td>
<td>0.72</td>
</tr>
<tr>
<td>Polio Type 3</td>
<td>GMT</td>
<td>31.0 (25, 38)</td>
<td>35.0 (28, 44)</td>
<td>33.0 (27, 41)</td>
<td>31.2 (25, 39)</td>
<td>0.85</td>
</tr>
</tbody>
</table>

1 GMC, geometric mean concentration; an arbitrary value of 0.001 IU/L was assigned to samples below detection limits. GMT, geometric mean titer; an arbitrary value of 1 was assigned to samples below detection limits.

DISCUSSION

We found no evidence that vitamin A supplementation of recently delivered mothers and/or their infants at the routine EPI contacts at 6, 10, and 14 wk of age interfered with infants’ immune responses to current polio and tetanus vaccines. It should be noted that this trial was conducted in a population with high rates of coverage with tetanus toxoid vaccine to pregnant women and of oral polio at birth to newborns. Our tetanus results are consistent with the findings of a study conducted in infants in rural Bangladesh (16), which found no evidence of an altered antibody response to tetanus vaccine when it was administered with vitamin A. In a study in Indonesian children (8), increased antibody responses to tetanus were observed in children receiving vitamin A, but that study recruited children who were 3–6 y old compared with the infants in this study. An additional difference between the studies is that we administered vitamin A together with the vaccines at 6, 10, and 14 wk, whereas the Indonesian study administered vitamin A 2 wk before the immunization. Our results for oral polio vaccine (Table 3) are similar to those from a study in Bogor District, West Java, Indonesia (17) in which antibody responses to polio types 1, 2, and 3 were measured by neutralization assay at enrollment at 6 wk and at 9 mo of age. That study found no evidence that oral vitamin A altered infants’ antibody responses to any of the 3 types of polio viruses when administered as part of EPI. Similar results were also obtained in a study conducted in India by Bhaskaram and Balakrishna (18), which investigated the effect of maternal supplementation with vitamin A on infants’ immune response to oral polio vaccine. Mothers were supplemented only with 60 mg RE of vitamin A within 24 h of delivery and the

Infants’ antibody levels at 6 mo of age after supplementation with vitamin A for both mothers and infants (Group 1), infants only (Group 2), mothers only (Group 3), or no supplementation (Group 4)

<table>
<thead>
<tr>
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<th>Test 1</th>
<th>Group 1 (n = 196)</th>
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<th>Group 3 (n = 185)</th>
<th>Group 4 (n = 194)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetanus</td>
<td>% protected IU/L 100%</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.70</td>
</tr>
<tr>
<td>Polio Type 1</td>
<td>% with titer ≥1:8 91.2</td>
<td>91.6</td>
<td>90.6</td>
<td>91.9</td>
<td>93.3</td>
<td>0.79</td>
</tr>
<tr>
<td>Polio Type 2</td>
<td>% with titer ≥1:8 91.2</td>
<td>94.2</td>
<td>94.6</td>
<td>94.3</td>
<td>95.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Polio Type 3</td>
<td>% with titer ≥1:8 94.5</td>
<td>89.0</td>
<td>88.6</td>
<td>88.1</td>
<td>85.1</td>
<td>0.51</td>
</tr>
<tr>
<td>Polio Type 3</td>
<td>GMC (95% CI) 55.2 (43.7, 69.7)</td>
<td>47.0 (38.3, 57.5)</td>
<td>52.3 (42.1, 64.9)</td>
<td>50.2 (40.5, 62.3)</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

1 GMC, geometric mean concentration; an arbitrary value of 0.001 IU/L was assigned to samples below detection limits. GMT, geometric mean titer; an arbitrary value of 1 was assigned to samples below detection limits.
2 Antibody titer ≥1:8 for polio.
geometric mean titers of antibody to the 3 types of polio virus in the infants at 6 wk of age were comparable to those in our study. In a study with a design similar to ours carried out in South Delhi (19), vitamin A supplementation to mothers in the postpartum period and to their infants with OPV was associated with an increased proportion of infants with protective antibody titer against polio virus type 1 after immunization. There was no evidence of a difference between the treatment and the placebo groups with respect to polio type 2 or 3. We observed a high refusal rate at 6 mo of age due to sensitivities of some mothers to a blood draw, which arose from a rumor in the community that the blood drawn was for sale! We also examined the characteristics of those who were lost to follow-up and there were still no significant differences among the groups. We found no evidence that vitamin A supplementation of recently delivered mothers and/or their infants at routine EPI vaccine contacts at 6, 10, and 14 wk of age altered the infants' antibody responses to tetanus or oral polio vaccine delivered at the time of those contacts.

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LITERATURE CITED