INFECTION

Hypothesis: Vitamin A supplementation and childhood mortality: amplification of the non-specific effects of vaccines?

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Most areas of health research will have accepted data and a dominating interpretation. If the interpretation is not correct, contradictions will accumulate, and it will eventually become clear that the current interpretation is untenable. In this situation, the best hypothesis is the one that accounts for all of the known data as well as the apparent contradictions. The area of vitamin A supplementation and childhood mortality in developing countries is afflicted with many contradictions and there is a need for a new hypothesis. We propose that the effect of vitamin A supplementation may depend on the amplification of non-specific effects of vaccines on childhood mortality.

Keywords Vitamin A, childhood mortality, vaccines, hypothesis

Vitamin A supplementation to children above 6 months of age reduces all-cause mortality by 23%¹ to 30%²,³ in low-income countries. The beneficial effect of vitamin A supplementation is assumed to be due to the prevention of vitamin A deficiency.¹ The World Health Organization (WHO) therefore recommends administration of vitamin A at vaccination contacts in order to prevent vitamin A deficiency.⁴ Until recently, the policy has been to supplement 100 000 IU of vitamin A at the earliest possible opportunity after 6 months of age. However, it has recently been recommended that an additional 50 000 IU of vitamin A be administered with each of the diphtheria-tetanus-pertussis (DTP)/polio vaccinations, which are usually given at 6, 10, and 14 weeks of age.⁵

Contradictions in current knowledge

In the following, the contradictions in current knowledge are presented. The presentation deals with all the data currently available and not just anomalous results within an overall set of trial results.

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Age pattern

Though studies on vitamin A supplementation after 6 months of age consistently have revealed a beneficial effect on child survival, the studies in infants younger than 6 months of age have reported inconsistent effects (Table 1). The two studies of vitamin A supplementation at birth suggest significant reductions in infant mortality (Table 1).⁶⁷ However, all recent studies of vitamin A given between 1 and 5 months of age failed to demonstrate the expected reduction in all-cause mortality (Table 1). A meta-analysis of the two studies providing vitamin A at birth (Table 1) and the four studies providing vitamin A between 1 and 5 months of age (Table 1), and the studies providing vitamin A from 6 months of age (using the estimate obtained in Beaton’s meta-analysis¹) showed that the effect of vitamin A supplementation between 1 and 5 months of age differed significantly from the effect at birth (P for homogeneity of effects = 0.02) and the effect from 6 months of age (P for homogeneity = 0.009). The lack of beneficial effect between 1 and 5 months of age has been ascribed to breastfeeding covering the needs, whereas older children become increasingly deficient and therefore benefit more from vitamin A supplementation.⁸ However, several studies indicate that many of the young infants were vitamin A deficient,⁹,¹⁰ and the explanation would not account for the beneficial effect at birth.

No effect in low birthweight (LBW) neonates

A study of vitamin A supplementation of newborns conducted at the Hasan Sadikin General Hospital (HSGH), Bandung, Indonesia, revealed a significant mortality reduction only among newborns with a birthweight ≥2500 g (Table 1). The mortality
### Table 1  Effect of vitamin A supplementation on mortality in infants younger than 6 months (mo.) of age

<table>
<thead>
<tr>
<th>Study Location</th>
<th>Sample Size</th>
<th>Age at inclusion</th>
<th>Dosage (IU)</th>
<th>Age at end</th>
<th>Mortality ratio (95% CI)</th>
<th>Subgroup</th>
<th>Comments/ Vaccination status</th>
</tr>
</thead>
</table>
| **Supplementation at birth—at the time window of BCG vaccination**

- *Indonesia, 1996*<sup>b</sup>  
  - 2067 cases  
  - 1 day  
  - 50,000 IU  
  - 12 mo.  
  - BW >2500 g: 0.09 (0.01, 0.70)  
  - BW <2500 g: 0.74 (0.26, 2.02)  
  - Total: 0.36 (0.16, 0.87)  
  - PI <25th percentile: 0.60 (0.20, 1.87)  
  - PI 25th-75th: 0.12 (0.01, 2.23)  
  - $P$-trend = 0.03

- *India, 2003*<sup>7</sup>  
  - 1,619 cases  
  - 1-2 days  
  - 24,000 IU  
  - 6 mo.  
  - 0.78 (0.63, 0.97)  
  - A possible positive synergy with BCG vaccination was suggested.

<table>
<thead>
<tr>
<th>Study Location</th>
<th>Sample Size</th>
<th>Age at inclusion</th>
<th>Dosage (IU)</th>
<th>Age at end</th>
<th>Mortality ratio (95% CI)</th>
<th>Subgroup</th>
<th>Comments/ Vaccination status</th>
</tr>
</thead>
</table>
| **Supplementation between 1 and 5 months of age—at the time window of DTP vaccinations**

- *Nepal, 1992*<sup>5</sup>  
  - 1,058 cases  
  - 1-5 mo.  
  - 50,000 IU  
  - 5 mo. older  
  - 0.99 (0.41, 2.41)  
  - Significant effect in older children in the same study

- *Nepal, 1995*<sup>6</sup>  
  - 11,918 cases  
  - 0-6 mo.  
  - <1 mo: 50,000  
  - 1-6: 100,000  
  - 4 mo. older  
  - <1 mo: 1.07 (0.66, 1.72)  
  - 1-3 mo: 1.27 (0.89, 1.89)  
  - 4 mo: 0.92 (0.41, 2.03)  
  - 5 mo: 0.78 (0.37, 1.65)  
  - Total: 1.11 (0.86, 1.42)  
  - Generally the infants were not reached before 2-3 weeks post-partum.<sup>6</sup>  
  - MUAC<sub>c</sub> <105: 1.02 (0.73, 1.42)  
  - MUAC 105-124: 1.40 (0.78, 2.50)  
  - MUAC >125: 1.95 (0.88, 4.32)  
  - $P$-trend = 0.07<sup>f</sup>

- *Bangladesh, 1995*<sup>10</sup>  
  - 199 cases  
  - With DTP vaccines  
  - 25,000 IU  
  - 3 mo.  
  - 0.97 (0.29, 3.25)  
  - 87% of the children had serum retinol <20 ug/dl at the first dose

- *WHO multicenter, 1998*<sup>9</sup>  
  - 9,424 cases  
  - With DTP vaccines  
  - 25,000 IU  
  - 12 mo.  
  - 0.96 (0.73, 1.27)  
  - >60% of the children had serum retinol <20 ug/dl at the first dose

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<sup>a</sup> Age when follow-up ended.

<sup>b</sup> Preliminary data from a vitamin A supplementation trial in Zimbabwe suggested no effect of vitamin A supplementation at birth on the smaller subgroup of infant deaths that were unrelated to human immunodeficiency virus. However, the analyses have not yet been completed.<sup>44</sup>

<sup>c</sup> Birthweight (g).

<sup>d</sup> Ponderal index.

<sup>e</sup> Mid-upper-arm-circumference (mm).

<sup>f</sup> Based on Table 3 in the paper. We tested significance with a test for trend.

<sup>g</sup> Diphtheria-tetanus-pertussis.
ratio (MR) in normal birthweight (NBW) infants was 0.09 (95% CI: 0.01, 0.70) compared with 0.74 (95% CI: 0.26, 2.02) in LBW infants ($P$ for homogeneity = 0.068). Furthermore, the MR decreased with increasing ponderal index ($P = 0.032$). Since LBW infants have particularly low vitamin A status, the lack of effect for these children appears contradictory.

### Increased mortality with increasing nutritional status

In one Nepalese study of children aged 1–6 months, vitamin A compared with placebo was associated with increased mortality with increasing mid-upper-arm-circumference (MUAC); the MR for vitamin A supplementation being 1.01 (95% CI: 0.73, 1.42) for children with a MUAC of $<105$ mm, 1.40 (95% CI: 0.78, 2.50) for a MUAC at 105–124 mm, and 1.95 (95% CI: 0.88, 4.32) for a MUAC $\geq 125$ mm (age-adjusted test for trend, $P = 0.07$, Table 1). The result was characterized as ‘perplexing’ and no explanation was put forward. Furthermore, the results are in contrast with the Indonesian study of newborns; MR decreased with increasing birthweight when supplementation was given at birth but increased with another measure of malnutrition (MUAC) when vitamin A supplementation was given between 1 and 6 months of age. None of the other studies that supplemented at birth or between 1 and 5 months of age have reported data on any interaction with nutritional status.

### No association with degree of pre-existing deficiency

Even though the presence of xerophthalmia is a useful indicator of severe vitamin A deficiency, a meta-analysis reported that there was no association between the prevalence of xerophthalmia in a population and the effect of vitamin A supplementation in the population.

### No association between dose and effect

There is no clear evidence that a large dose is better than a small dose, the tendency being rather the opposite: in a WHO multicentre placebo-controlled study of vitamin A supplementation with routine immunizations in infancy, the children in the vitamin A group received 25 000 IU of vitamin A with each of the first three DTP/poliomyelitis vaccines at 6, 10, and 14 weeks of age. At the age of 9 months, with measles vaccination, infants in the vitamin A group were given a further dose of 25 000 IU, and those in the control group received 100 000 IU vitamin A. There was no difference in mortality after the first three doses of vitamin A between the two groups, and there was no difference in any measure of vitamin A status at 9 months of age, when the infants received measles vaccine and additional vitamin A supplementation (Table 2). However, we analysed the data from 9 to 12 months of age and found that the control children receiving 100 000 IU vitamin A with measles vaccine at 9 months of age had substantially higher mortality (MR = 2.36 [95% CI: 1.17, 4.77]) than the previously supplemented group, who only received 25 000 IU of vitamin A together with measles vaccine at 9 months of age (Table 2). The observation was dismissed as being unplanned and not consistent with other studies of the effect of vitamin A supplementation after 6 months of age.

In an Indonesian study of the effect on morbidity and growth of three doses of either 25 000 or 50 000 IU vitamin A, or placebo given with the three doses of DTP vaccines, the low dose had a significantly better effect on morbidity than the high dose.

### Hypothesis

The mortality age pattern following vitamin A supplementation is similar to the mortality pattern we observed after routine vaccinations. We have observed that many vaccines have non-specific effects; BCG usually given at birth and standard measles vaccine given after 6 months of age are associated with a larger than expected reduction in all-cause mortality, whereas DTP vaccine given with oral polio vaccine at 6, 10, and 14 weeks of age may be associated with increased mortality in an area with herd immunity to pertussis and diphtheria. Though these observations are controversial, no study has clearly contradicted these tendencies.

We hypothesize that the mortality effect of vitamin A supplementation may depend not only on the prevention of vitamin A deficiency, but also on vitamin A amplifying the non-specific immune modulation induced by routine vaccinations.

### Possible biological mechanisms

Little is known about how vaccines affect the immune system resulting in the observed non-specific effects. Early childhood is characterized by high susceptibility to infectious diseases, reflecting functional immaturity of cell-mediated immunity. Many of these deficiencies seem to be due to reduced capacity of neonatal antigen-presenting cells (APC) to deliver important Th1 polarizing signals to T-cells. The stimuli required to induce neonatal APC to synthesize adult levels of Th1 polarizing signals remain poorly understood. The BCG and measles vaccines induce a Th1 response in infants. In animal studies, other live vaccines like live respiratory syncitial virus (RSV) and influenza vaccines are likewise associated with a Th1 response. In contrast, priming with killed vaccines such as pertussis toxin, DTP, inactivated RSV, and inactivated influenza vaccine has been associated with a stronger Th2 response.

### Table 2: Mortality following supplementation of vitamin A with routine immunizations in infancy. WHO multicenter study

<table>
<thead>
<tr>
<th>Follow-up: 5–8 months</th>
<th>Follow-up: 9–12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dosage (IU)</strong></td>
<td><strong>Mortality (deaths/1000)</strong></td>
</tr>
<tr>
<td>Vaccine plus placebo</td>
<td>Placebo × 3</td>
</tr>
<tr>
<td>Vaccines plus vitamin A</td>
<td>25 000 × 3</td>
</tr>
<tr>
<td>Mortality ratio</td>
<td>1.02 (0.77, 1.35)</td>
</tr>
</tbody>
</table>

Note: We analysed data based on the trial profile presented in the paper. $p = 0.01$, $\chi^2$ test.
Although simplistic, a possible explanation for the observed mortality pattern after routine vaccinations is therefore that live and inactivated vaccines have different effects on the APC; live vaccines may strengthen the capacity for Th1-polarizing signals, resulting in maturation of this immature function, whereas killed vaccines may strengthen the capacity for Th2-polarizing signals, thus strengthening this already relatively stronger function.

Vitamin A acts as an adjuvant to vaccines, and vitamin A supplementation has, under different circumstances, been shown to enhance both cellular and humoral immune responses in animals as well as in humans.\(^{32,33}\) It was recently reported that in mice, the effect of vitamin A supplementation depends on the environment under which it is given; when vitamin A supplementation was given with cytokines that promote Th1 development, enhancement of the Th1 response was observed; when vitamin A was given with cytokines that promote Th2 development, enhancement of the Th2 response was observed.\(^{34}\) Vitamin A seemed to act directly on the APC.\(^{34}\) Hence, vitamin A may be a rather uncritical enhancer of ongoing processes in the immune system at the moment of supplementation. We find it biologically plausible that vitamin A supplementation may amplify ongoing immune reactions induced by vaccinations; when given with a live vaccine it may thus further enhance the capacity of the APC for Th1-polarizing signals, whereas when given with a killed vaccine it may further enhance the capacity of the APCs for Th2-polarizing signals. A synergistic stimulatory effect on the immune system of simultaneous administration of BCG and vitamin A is well known from experimental cancer treatment.\(^{35–37}\)

**Resolutions**

The hypothesis may resolve the existing contradictions:

**Age pattern**

The hypothesis would explain the beneficial effect of vitamin A supplementation when given at the time of BCG vaccination at birth and at the time of measles vaccination after 6 months of age. It would also explain the apparent lack of effect or even slightly negative effect when given in the time window of DTP vaccinations between 1 and 5 months of age in spite of pre-existing vitamin A deficiency.

**No effect in low birthweight (LBW) neonates**

In the Indonesian study, BCG vaccination was postponed in LBW or premature infants until they reached the gestational age of 9 months (personal communication, Dr Cissy B Kartasasmita, Department of Child Health, HSGH). LBW infants were therefore less likely to have received BCG and vitamin A at the same time, and this may explain why vitamin A given at birth had limited effect in these infants.

**Increased mortality with increasing nutritional status**

If vitamin A supplementation amplifies a possible negative mortality effect of the DPT vaccine, this could become more evident in infants with high MUAC and thus presumably better nutritional status, because they would not benefit in other ways from the extra vitamin A.

**No association with degree of pre-existing deficiency**

If the effect of vitamin A supplementation were partly due to amplification of the non-specific effects of vaccines, the effect of vitamin A supplementation would be expected to depend on the vaccines with which it is given and not just pre-existing deficiency.

**No association between dose and effect**

Compared to placebo, 100 000 IU given after 6 months of age reduces mortality. However, the effect of giving 25 000 versus 100 000 IU with measles vaccine has not been investigated elsewhere. It is possible that a small dose of 25 000 IU is even better than a large dose of 100 000 IU for producing a balanced and beneficial immune response to the measles vaccine.

**Addressing the hypothesis**

An optimal way to test the hypothesis would be in a two-by-two factorial design randomizing children to live or killed vaccine and vitamin A supplementation and placebo. In Guinea-Bissau we have previously done a small study of this kind, randomizing the children to a live or a killed vaccine and to vitamin A or placebo in order to investigate the effect of vitamin A supplementation on the immune response to measles vaccine.\(^{38,39}\) To address the present hypothesis, we re-analysed data from the trial even though the study had not been designed to answer this question.

Three hundred infants were randomly allocated to receive standard Schwarz measles vaccine (MV) or inactivated polio vaccine (IPV) at 6 months of age. Within each of the two vaccination groups, the infants were allocated randomly to receive 100 000 IU of vitamin A or placebo. At 9 months of age, all infants received MV and the same vitamin A supplement or placebo as given previously (Figure). A third group of infants (those being too old to be enrolled for the 6-month vaccination in the beginning of the study period, and those coming too late during the study period) received MV at 9 months of age. These infants were allocated randomly to 100 000 IU vitamin A or placebo given with the MV. There were no differences in mortality-related parameters between vitamin A and placebo recipients within each of the vaccination groups at baseline. The children were followed intensively to 18 months of age. By this age, the 227 (78 + 70 + 79) children, who had received vitamin A and MV (one or two doses) had a mortality ratio (MR) of 0.46 (95% CI: 0.14, 1.47) compared with the 235 (72 + 68 + 95) children, who had received placebo and MV (Figure).

Six infants died before they received the 9-month MV, three in the MV group (two had diarrhoea as a main symptom, one fever) and three in the IPV group (one had diarrhoea as the main symptom, one fever, and one child was said to have died of malnutrition) (Figure). In the MV group, all three deaths occurred in the placebo group (n = 72), whereas there were no deaths in the vitamin A group (n = 78); in the IPV group, all three deaths occurred in the vitamin A group (n = 76), whereas there were no deaths in the placebo group (n = 74). Hence, the effect of vitamin A was significantly different in the two vaccination groups (LogXact score test for homogeneity, P-mid = 0.02\(^{40}\)). Although our result is based on few deaths, the data suggest that the mortality-reducing effect of vitamin A was dependent on the type of vaccine given at the time of vitamin
A supplementation. Our data clearly do not prove the hypothesis, but add just another observation that is inconsistent with the current paradigm and consistent with the proposed hypothesis. It would seem indicated to test the hypothesis in a larger study with a two-by-two factorial design.

**Conclusion**

As suggested by Semba and colleagues, it is unclear why dosing with vitamin A at birth rather than through early childhood immunisation contacts is associated with a reduction in infant mortality. The present paper has pointed to other inconsistencies in the current interpretation of vitamin A supplementation. These observations have all been dismissed as unplanned or accidental. However, taken together, they question the common interpretation that the impact of vitamin A is due only to the prevention of vitamin A deficiency.

The proposed hypothesis accounts for all of these observations but should obviously be tested in future studies.

We have focused on the effect of vitamin A supplementation on mortality. However, the area of vitamin A supplementation and morbidity is also burdened by inconsistent observations. For instance, vitamin A used as therapy for measles and diarrhoea seems beneficial, whereas there seems to be no effect, or even a tendency for negative effects when vitamin A is given during respiratory diseases. It has been suggested that the effects of vitamin A supplementation used as therapy during infectious diseases are disease-specific because vitamin A acts not only by repleting tissue vitamin A, but also as an adjuvant. This suggestion is in line with our hypothesis; if vitamin A amplifies ongoing immune reactions, it is conceivable that this might in some instances be beneficial, in other instances harmful, depending on the type of immune response that the disease elicits.

**Relevance**

It has been recommended that 50 000 IU of vitamin A be administered with each of the DTP/polio vaccinations, which are usually given at 6, 10, and 14 weeks of age. Should our hypothesis be true, this may not contribute to the improvement of child health in developing countries.

**Acknowledgement**

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**KEY MESSAGES**

- The literature on vitamin A supplementation and childhood mortality in developing countries is contradictory.
- We hypothesize that the effect of vitamin A supplementation on childhood mortality may depend on amplification of non-specific effects of vaccines.
References


29. Moran TM, Park H, Fernandez-Sesma A, Schumlan JL. Th2 responses to inactivated influenza virus can be converted to Th1 responses and facilitate recovery from heterosubtypic virus infection. J Infect Dis 1999; 180:575–89.


Vitamin A supplements in community intervention trials seem to provide maximum protection against death from infectious diseases when provided at birth or after 6 months of age. Why this is so is not clear and part of the reason may be a lack of data in young infants. However, Benn and colleagues have other ideas and have proposed a hypothesis to explain this inconsistency. First, a little background information is in order.

The goals of vitamin A supplementation programmes in areas of the world where vitamin A deficiency is a public health problem are, of course, to treat and prevent vitamin A deficiency. The principal adverse consequences of vitamin A deficiency are xerophthalmia and increased risk of death from infectious diseases. Decreasing mortality risk has become the principal goal of vitamin A supplementation programmes in the past decade. Many large-scale community intervention trials have been conducted and a definitive conclusion is that vitamin A supplementation decreases mortality in infants and young children over 6 months of age. Fewer studies have been done in younger infants but the data support a similar benefit, at least if the supplements are given at birth. Speculation on the reason for a lesser effect when supplements are given between birth and 6 months has focused on differences in environmental influences on this age group (e.g. the protection of breastfeeding against malnutrition and infection) or on differences among the populations studied (e.g. different prevalence rates of infectious diseases among study sites may affect underlying mortality rates).

Although the principal benefits of vitamin A supplementation are well-established, the mechanisms underlying the decreased mortality risk are not understood in great detail. It is known that vitamin A deficiency impairs many aspects of both innate and adaptive immunity and it is presumed that preventing deficiency improves protection against development of severe disease, since total morbidity is little affected by supplements. However, the question of which improvements (e.g. improved mucosal integrity, enhanced T-cell mediated immunity) are causing the decreased mortality has not been answered. Some may argue that understanding how a thing happens is not important in a public health context as long as the desired result of decreased mortality is achieved. However, it is now quite clear that vitamin A improves recovery from some infections (e.g. some diarrhoeal diseases and measles) but not others (e.g. non-measles pneumonia). Better understanding of the mechanism of action of vitamin A on immune function would presumably explain these observations and better allow public health practitioners to target their interventions to maximize benefit and minimize risk.

Benn and colleagues propose that the effect of vitamin A supplementation on reducing mortality ‘may depend not only on the prevention of vitamin A deficiency, but also on vitamin A amplifying the non-specific immune modulation induced by routine vaccinations’. This same research group, have proposed that BCG and measles vaccines provide protection against death from infections other than tuberculosis and measles. They further propose that diphtheria, pertussis, tetanus (DPT) vaccine does not provide such protection. While (again) the underlying mechanism is not clear, the basic idea is that live vaccines may activate the innate immune system in such a way as to provide protection against a variety of pathogens in a way that killed vaccines with alum adjuvants do not. Increased activation of antigen-presenting cells, increased production of type I interferons by a variety of