Heterologous and sex differential effects of administering vitamin A supplementation with vaccines

Kristoffer J. Jensena,b, Jorjoh Ndurec, Magdalena Plebanskid and Katie L. Flanagand,*

aResearch Center for Vitamins & Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, DK-2300 Copenhagen S, Denmark; bProje´cto de Sau´de Bandim, Indepth Network, Apartado 861, Codex 1004, Bissau, Guinea-Bissau; cMRC Laboratories, Fajara, The Gambia; dVaccine and Infectious Diseases Laboratory, Department of Immunology, Monash University, 89 Commercial Road, Prahran, Victoria 3004, Australia

*Corresponding author: Tel: +61 3 6336 5081; E-mail: katie.flanagan@dhhs.tas.gov.au

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WHO recommends high-dose vitamin A supplementation (VAS) to children from 6 months to 5 years of age in low-income countries, in order to prevent and treat vitamin A deficiency-associated morbidity and mortality. The current policy does not discriminate this recommendation either by sex or vaccination status of the child. There is accumulating evidence that the effects of VAS on morbidity, mortality and immunological parameters depend on concomitant vaccination status. Moreover, these interactions may manifest differently in males and females. Certain vaccines administered through the Expanded Program on Immunization have been shown to alter all-cause mortality from infections other than the vaccine-targeted disease. This review summarizes the evidence from observational studies and randomized-controlled trials of the effects of VAS on these so-called heterologous or non-specific effects of vaccines, with a focus on sex differences. In general, VAS seems to enhance the heterologous effects of vaccines, particularly for diphtheria-tetanus-pertussis and live measles vaccines, where some studies, although not unanimously, show a stronger interaction between VAS and vaccination in females. We suggest that vaccination status and sex should be considered when evaluating the effects of VAS in early life.

Keywords: All-cause mortality, BCG vaccine, DTP vaccine, Measles vaccine, Sex, Vitamin A

Introduction

Vitamin A is derived from the diet either in the form of all-trans retinal, beta-carotene or retinyl esters, and is essential for the normal functioning of the immune system.1 It is estimated that 190 million children under the age of 5, worldwide, are at risk of vitamin A deficiency (VAD), especially in Africa and southeast Asia.2 VAD is principally associated with xerophthalmia and an increased risk of death from infectious diseases. It may be alleviated by vitamin A supplementation (VAS), usually as oral capsules of retinyl esters. WHO recommends VAS to children between 6 months and 5 years of age in low-income countries as a means of treating and preventing VAD;3 this policy is partly based on a meta-analysis of 17 randomized-controlled trials (RCT) in Asia (11 trials), Africa (5 trials) and Brazil (1 trial), which found that VAS can reduce all-cause mortality by 24% (95% CI 17%–31%) in this age group.4 The meta-analysis was an extension of a former review, reporting a similar effect size.5 VAS before 6 months of age is not currently recommended by WHO,6 due to a lack of convincing beneficial effects on mortality and morbidity as evaluated by several systematic reviews.7–9 However, studies from different regions of the world are conflicting (Table 1). The provision of 50 000 IU neonatal VAS significantly reduced mortality in Bangladesh,10 India11 and Indonesia,12 but not in Nepal.13 By contrast, trials in Africa failed to demonstrate any effect on overall mortality in the 12-month follow-up. In more than 4000 normal birth weight newborns in Guinea-Bissau, mortality was comparable at 12 months for 50 000 IU VAS or placebo groups.14 A smaller RCT in low-birth weight newborns of 25 000 IU versus placebo similarly showed no effect on mortality;15 and there was no mortality difference in Zimbabwean infants receiving 50 000 IU VAS or placebo.16
<table>
<thead>
<tr>
<th>Design</th>
<th>By vaccine</th>
<th>Mortality rate ratio (MRR)</th>
<th>Sex</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td></td>
<td></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>Sex/#V AS: NA</td>
</tr>
<tr>
<td></td>
<td></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>
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</tr>
<tr>
<td></td>
<td></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>
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</tr>
<tr>
<td></td>
<td></td>
<td>1.18 (0.76–1.83)</td>
<td>NA</td>
<td>Not analysed by sex</td>
<td>Zambia</td>
</tr>
<tr>
<td></td>
<td></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>Sex/#V AS: p=0.054</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.28 (0.91–1.81)</td>
<td>1.35 (0.84–2.16)</td>
<td>1.21 (0.73–2.01)</td>
<td>Sex/#V AS: NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.08 (0.79–1.47)</td>
<td>0.74 (0.45–1.22)</td>
<td>1.42 (0.94–2.15)</td>
<td>Sex/#V AS: p=0.046</td>
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<tr>
<td></td>
<td></td>
<td>1.14 (0.71–1.84)</td>
<td>NA</td>
<td>NA</td>
<td>Sex/#V AS: NA</td>
</tr>
<tr>
<td></td>
<td></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>Sex/#V AS: p=0.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.86 (0.48–1.54)</td>
<td>NA</td>
<td>NA</td>
<td>Sex/#V AS: p=0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.28 (0.72–2.29)</td>
<td>1.53 (0.69–3.40)</td>
<td>1.04 (0.45–2.41)</td>
<td>Sex/#V AS: NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.96 (0.73–1.27)</td>
<td>N/A</td>
<td>N/A</td>
<td>Not analysed by sex</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.43 (0.88–2.32)</td>
<td>0.90 (0.44–1.82)</td>
<td>2.19 (1.09–4.38)</td>
<td>Sex/#V AS: p=0.08</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.04 (0.62–1.72)</td>
<td>0.84 (0.42–1.69)</td>
<td>1.31 (0.61–2.81)</td>
<td>Sex/#V AS: NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.39 (1.62–17.99)</td>
<td>11.31 (1.50–85.47)</td>
<td>2.46 (0.51–11.85)</td>
<td>Sex/#V AS: NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.10 (0.64–1.90)</td>
<td>0.57 (0.23–1.42)</td>
<td>1.67 (0.81–3.42)</td>
<td>NVAS/#FUV AS: male: p=0.68, female: p=0.009; Sex/#NVAS/#FUV AS: p=0.035</td>
</tr>
</tbody>
</table>

The same paper may appear in more than one row. #: interaction analysis; 50 kIU: 50 000 international units of retinol; BCG: bacille Calmette-Guérin; DTP: diphtheria-tetanus-whole cell pertussis; FU: follow-up; FU-V AS: V AS at follow-up; mo: month(s); MRR: mortality rate ratio; MV: measles vaccination; NA: not analyzed; NS: not significant; NVAS: neonatal V AS; V AS: vitamin A supplementation; wk: weeks(s); yr: year(s).
A large multicentre trial of VAS with routine vaccines at 6–14 weeks in Ghana, India and Peru also failed to show an effect on mortality. This discrepancy is yet to be resolved and some of the potential underlying factors will be discussed here.

For logistic reasons, VAS is often delivered in combination with routine vaccines included in the Expanded Program on Immunization (EPI) schedule recommended by WHO. Several studies including RCTs suggest an interaction between VAS and vaccines in early childhood on mortality, and these will be discussed in this review. In particular, VAS may augment the heterologous effects of vaccines, the effects whereby prior vaccination alters morbidity and mortality from infections other than the vaccine-targeted diseases. A number of studies further suggest that the VAS effects and vaccine heterologous effects manifest differently in females and males. The administration of VAS with the EPI scheduled vaccines was not an evidence-based decision. Meanwhile, it is WHO opinion that the current evidence concerning the influence of sex or preceding vaccines on the effect of VAS are insufficient to lead to policy change.

This literature review explores the limited evidence for an effect of VAS in combination with childhood vaccines on morbidity and mortality or immunological endpoints, with an emphasis on sex differences. RCTs or observational studies of high-dose VAS in children <5 years of age reporting mortality or morbidity as an outcome available via PubMed were selected for the review. Additionally, immunological or anthropometric studies of VAS in children addressing sex and/or vaccination were included. The review will not cover animal studies, or studies in adults or children above 5 years of age.

The heterologous effects of vaccines on all-cause mortality

Accumulating data from observational studies and RCTs show that some vaccines affect resistance to infections other than the targeted diseases. These effects are termed heterologous or non-specific effects of vaccines. Live attenuated vaccines such as bacille Calmette-Guerin (BCG) and measles vaccine (MV) have been associated with a reduction in mortality, exceeding the possible specific protection against TB and measles, respectively, as recently acknowledged by WHO following a systematic review of the literature. In contrast, several studies have found that inactivated adjuvanted vaccines such as diphtheria-tetanus-whole cell pertussis (DTP) vaccine are associated with increased mortality. Hence, the heterologous effects of vaccines may be beneficial or detrimental. The heterologous effects may manifest differently in males and females; the beneficial heterologous effects of MV and the detrimental heterologous effects of DTP generally being greater in females. The immunological mechanisms behind the heterologous effects are not understood, although some mechanisms are beginning to emerge.

VAS interaction with BCG effects on all-cause mortality

In two RCTs of neonatal VAS conducted in Guinea-Bissau, VAS tended to be beneficial for males, but detrimental for females (interaction between VAS and sex: p=0.10 and p<0.05 for either trial). The latter trial also randomized low-birth weight infants to BCG at birth versus administration according to local practice, usually at age 6 weeks, but found no interaction between BCG and VAS (Table 1). Similarly, trials found no effect of neonatal VAS on mortality when given together with BCG or when the analysis was restricted to the time when BCG was the last vaccine; furthermore, no significant sex-differential effects of VAS were found (Figure 1).

In a RCT of a BCG booster at 19 months of age in Guinea-Bissau, a number of the participating children received VAS in national campaigns before or after randomization to BCG booster. Among infants not receiving VAS, there was no effect of the BCG booster on overall survival, whereas among VAS recipients, BCG was associated with a four times higher risk of dying than in the
controls (hazard ratio 4.14 [1.17–14.7]). The effects were not reported by sex.\textsuperscript{23}

### VAS interaction with DTP vaccine effects on all-cause mortality

Previously given alone, DTP is now administered as part of the Pentavalent vaccine (DTP, Hepatitis B, Haemophilus influenzae type b) in much of the developing world at 6, 10 and 14 weeks of age, with a booster DTP at 18 months. Sixty percent of the infants in the 19-months BCG booster study discussed above had not received a DTP booster before enrolment, and many would have received it with VAS or during follow-up. Among those that received a DTP booster before enrolment there was a significantly beneficial effect of BCG, whereas those who had no DTP before BCG were significantly more likely to die; the interaction between DTP booster status and BCG being highly significant (p=0.006). The study suggested that BCG followed by DTP and/or VAS may have unfavourable effects in the children, but may also indicate that a subsequent BCG vaccine may alleviate the putative negative effect of a DTP booster. The analyses were not reported by sex.\textsuperscript{23}

The above RCTs of neonatal VAS found that the interaction between sex and VAS was most evident after 3–4 months of age, during which infants usually receive multiple doses of DTP (Table 1).\textsuperscript{14,15} A subsequent re-analysis of a neonatal VAS trial indicated an interaction between sex and VAS (p=0.08) for children with DTP as the most recent vaccine, with VAS being associated with a significantly higher mortality in females (mortality rate ratio [MRR] 2.19 [1.09–4.38]), with no such effect in males (Table 1).\textsuperscript{24} A later trial of neonatal VAS in Guinea-Bissau did not find a differential effect of neonatal VAS for children having or not having received the first DTP vaccine; nor did the study find sex differences. However, the overall mortality was considerably lower than previous trials, thereby compromising the power of the subgroup analyses (Table 1).\textsuperscript{24}

An interaction between VAS and concomitant or subsequent DTP on mortality was indicated in two other observational studies from West Africa. To explore the hypothesis that the effect of VAS may be modulated by vaccination, Benn et al. reanalyzed data from a RCT providing VAS to 6–90-months old children in rural Ghana.\textsuperscript{25} Using health card information the authors found that VAS was associated with a beneficial effect in infants not vaccinated at the time of supplementation, with an overall MRR of 0.64 (0.47–0.88) for VAS versus placebo (Table 2). However, vaccination seemed to influence the VAS effect in females, which the authors ascribed to an effect of DTP. Those children who received MV before completing all the 3–4 scheduled doses of DTP were likely to receive additional doses of DTP during the 2-year follow-up; and among this group the effect of VAS was sex-differential, with a significantly negative impact on follow-up survival in females (MRR 2.60 [1.41–4.80]), but a borderline positive effect in males (MRR 0.50 [0.25–1.02]; p=0.0004 for interaction between VAS and sex). For children who had received all DTP doses before MV, there was no VAS effect overall or by sex (Table 2).\textsuperscript{26} Taking advantage of a national campaign providing VAS together with any missing vaccines to children >6 months of age, the effect of VAS on mortality in an urban community in Guinea-Bissau was studied up to 18 months of age. Receiving VAS together with any DTP was associated with a higher mortality rate compared with receiving VAS alone (MRR 3.43 [1.36–8.61]); this effect was significant in males but not in females, hence contrasting the sex-differential effect in the former study from Ghana. By contrast, there were no deaths among children receiving VAS with MV. The overall interaction between VAS and vaccine type was highly significant (<p=0.001), and evident in both males (p=0.007) and females (p=0.03), and suggests that combining MV and VAS is safe, whereas VAS with DTP may be harmful (Table 2).\textsuperscript{26}

### VAS interaction with measles vaccination effects on all-cause mortality

A reanalysis combining three RCTs of neonatal VAS\textsuperscript{16,15,22} and one RCT of an extra early dose of MV at age 4.5 months,\textsuperscript{27} all taking place in Guinea-Bissau, found that among recipients of early MV, neonatal VAS compared with placebo was associated with a significantly higher mortality at 9 months of age (p=0.01), with a stronger effect in males (p=0.05) than females (p=0.12). There was no significant effect of VAS among infants not receiving early MV, resulting in a significant interaction between VAS and MV (p=0.03). At 17-month follow-up, the interaction between VAS and MV sustained significance (p=0.008), with an overall effect of VAS in the early MV recipients of MRR 5.39 (1.62–17.99), and still strongest in males (MRR 11.31 [1.50–85.47]). This indicates a long-lasting priming effect of neonatal VAS affecting subsequent vaccine responses, possibly stronger in males.\textsuperscript{28}

The effect of neonatal VAS may differ from the effect exerted in older children. An observational study in Guinea-Bissau following children aged 6–35 months of age receiving VAS in campaigns found a significantly beneficial effect of VAS on mortality if the child had received MV as the last vaccine prior to the VAS campaign (MRR 0.34 [0.14–0.85]). The effect was significant for males but not for females, with no significant interaction between sex and VAS. The effect of VAS partially reverted, although not significantly, among children with DTP as the last vaccine, resulting in an interaction between vaccine type (MV vs DTP) and VAS (p=0.04).\textsuperscript{29} An analysis combining data from a trial in Guinea-Bissau of MV versus inactivated polio vaccine (IPV) at 6 months and a trial of VAS versus placebo at 6 and 9 months found that vaccine type and VAS interacted (p=0.02) on 18-month follow-up mortality. Among MV-recipient, deaths only occurred in the placebo group, whereas among IPV-recipient, deaths only occurred in the VAS group.\textsuperscript{30} The authors posited that the interaction with VAS might be ascribed to the nature of the vaccine as killed (IPV) or live (MV). However, overall the effect of VAS given together with or close to MV remains inconclusive, with studies indicating a detrimental effect of VAS given with MV on mortality in males\textsuperscript{28,31} and females\textsuperscript{25} in different studies.

### VAS interaction with oral polio vaccine (OPV) effects on all-cause mortality

A temporary lack of oral polio vaccine (OPV) in Guinea-Bissau facilitated a ‘natural experiment’ to observe the effect of OPV at birth on infant mortality; 22% of the infants did not receive OPV at birth. The study found an association between increased mortality and OPV at birth. All the infants were also enrolled in a trial of
Table 2. Summary of studies analyzing the effect of vitamin A supplementation to children from 6 months to 5 years of age

<table>
<thead>
<tr>
<th>Design</th>
<th>By intervention</th>
<th>Mortality rate ratio (MRR)</th>
<th>Sex#V AS:</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>No vaccine information or independent of vaccine type</td>
<td></td>
<td>200 kIU VAS vs placebo twice, 12–72 mo age, (6 mo interval). FU: 12 mo</td>
<td>Not reported</td>
<td>0.74 (0.85–0.99)</td>
<td>0.59 (0.39–0.88)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 kIU/200 kIU vs placebo, aged 6–72 mo. FU: 12 mo</td>
<td>Not reported</td>
<td>0.70 (0.56–0.88)</td>
<td>0.77 (0.55–1.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50/100/200 kIU vs placebo, aged 1–59 mo. FU: 5 mo</td>
<td>Not reported</td>
<td>0.74 (0.55–0.99)</td>
<td>0.72 (0.48–1.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200 kIU vs placebo, aged 9–72 mo. FU: 18 mo</td>
<td>Not reported</td>
<td>1.06 (0.82–1.37)</td>
<td>1.25 (0.85–1.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100/200 kIU vs placebo, aged 6–90 mo. FU: 2 yr</td>
<td>Not reported. Low vaccine coverage</td>
<td>0.81 (0.68–0.98)</td>
<td>0.73 (0.59–0.92)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo. FU: 6 mo</td>
<td>Irrespective of vaccine</td>
<td>0.91 (0.59–1.41)</td>
<td>1.92 (0.98–3.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 kIU/200 kIU vs placebo with missing vaccines, aged 6–90 mo. FU: 2 yr</td>
<td>Not vaccinated</td>
<td>0.64 (0.47–0.88)</td>
<td>0.68 (0.47–0.99)</td>
</tr>
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<td></td>
<td></td>
<td>100 kIU/200 kIU with missing vaccine, aged 6–60 mo. FU: aged 18 mo</td>
<td>VAS in campaign vs no VAS</td>
<td>1.11 (0.59–2.08)</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VAS + DTP vs nothing</td>
<td>3.04 (1.31–7.07)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All VAS: DTP vs no DTP</td>
<td>3.43 (1.36–8.61)</td>
<td>4.71 (1.37–16)</td>
<td>2.21 (0.52–9.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All VAS: MV vs DTP. Vaccine#V AS: p=0.0005</td>
<td>0.43 (among MV; 8/136 among DTP)</td>
<td>1.18 (0.84–1.67)</td>
<td>1.18 (0.84–1.67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DTP 100 kIU/200 kIU with missing vaccine, aged 6–60 mo. FU: aged 18 mo</td>
<td>DTP last vaccine</td>
<td>1.29 (0.52–3.22)</td>
<td>1.37 (0.39–4.93)</td>
</tr>
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<td></td>
<td></td>
<td>DTP 100 kIU/200 kIU in campaign vs not in campaign, aged 6–35 mo. FU: aged 3 yr</td>
<td>Given with DTP</td>
<td>1.06 (0.51–2.20)</td>
<td>2.16 (0.67–7.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 kIU/200 kIU vs placebo with missing vaccine, aged 6–23 mo. FU: 6 mo</td>
<td>Given with DTP</td>
<td>1.17 (0.84–1.64)</td>
<td>0.69 (0.41–1.16)</td>
</tr>
<tr>
<td>Study</td>
<td>Intervention</td>
<td>Comparator</td>
<td>Age</td>
<td>FU</td>
<td>Sex</td>
</tr>
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<td>-------</td>
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</tr>
<tr>
<td>Benn 2009</td>
<td>100 kIU/200 kIU vs placebo with missing vaccines, aged 6–90 mo.</td>
<td>Given with MV, incomplete DTP</td>
<td>NA</td>
<td>0.50 (0.25–1.02)</td>
<td>0.90 (0.45–1.81)</td>
</tr>
<tr>
<td>Benn 2009</td>
<td>Given with MV, complete DTP.</td>
<td></td>
<td></td>
<td>0.60 (0.25–1.41)</td>
<td>1.09 (0.54–2.20)</td>
</tr>
<tr>
<td>Guinea-Bissau Fisker 2012</td>
<td>100 kIU/200 kIU V AS in campaign vs not in campaign, aged 6–35 mo.</td>
<td>Given with MV</td>
<td>0.34 (0.14–0.85)</td>
<td>0.31 (0.10–0.96)</td>
<td>0.41 (0.09–1.85)</td>
</tr>
<tr>
<td>Guinea-Bissau Fisker 2014</td>
<td>Given with MV+DTP</td>
<td>1.29 (0.51–3.28)</td>
<td>2.40 (0.62–9.29)</td>
<td>0.62 (0.15–2.61)</td>
<td>Sex#VAS: p=0.18</td>
</tr>
<tr>
<td>Guinea-Bissau Benn 2003</td>
<td>100 kIU V AS vs placebo, aged 6 mo.</td>
<td>Given with MV or IPV</td>
<td>Deaths: Among MV: 0/78 in V AS, 3/72 in placebo;</td>
<td>0.34 (0.14–0.80)</td>
<td>0.59 (0.20–1.77)</td>
</tr>
<tr>
<td>Guinea-Bissau Fisker 2012</td>
<td>No previous V AS</td>
<td>0.80 (0.41–1.58)</td>
<td>5.98 (1.34–26.7)</td>
<td>0.18 (0.05–0.62)</td>
<td>Sex#VAS: p=0.0004</td>
</tr>
<tr>
<td>Guinea-Bissau Fisker 2014</td>
<td>Repeated V AS</td>
<td>Previous V AS</td>
<td>0.99 (0.57–1.77)</td>
<td>1.19 (0.53–2.65)</td>
<td>0.82 (0.35–1.89)</td>
</tr>
<tr>
<td>Guinea-Bissau Fisker 2012</td>
<td>100 kIU/200 kIU V AS vs placebo with missing vaccine, aged 6–23 mo, FU: 6 mo</td>
<td>V AS in previous campaign vs no V AS</td>
<td>0.34 (0.14–0.80)</td>
<td>0.59 (0.20–1.77)</td>
<td>0.14 (0.03–0.68)</td>
</tr>
</tbody>
</table>

The same paper may appear in more than one row. The column ‘By intervention’ refers to the health intervention status of the participants at the time of randomization or follow-up; usually vaccination status or other vitamin A supplements. #: interaction analysis; 50 kIU: 50 000 international units of retinol; DTP: diphtheria-tetanus-whole cell pertussis; FU: follow-up; FU-V AS: V AS at follow-up; IPV: inactivated polio vaccine; mo: month(s); MRR: mortality rate ratio; MV: measles vaccination; NA: not analyzed; NS: not significant; NV AS: neonatal V AS; V AS: vitamin A supplementation; wk: weeks(s); yr: year(s).
neonatal VAS administered together with BCG versus placebo and BCG. Neonatal VAS, however, did not modify the association between OPV and mortality; the combined effect of VAS and sex was not addressed, probably prohibited by the limited sample size.

Effect of different doses of VAS in early childhood

The dose of VAS recommended by WHO is 100 000 IU for children <1 year of age, and 200 000 IU for children above 1 year of age. This recommendation is somewhat arbitrary, but has been tested in a few studies. Taking advantage of a combined OPV and VAS national campaign in Guinea-Bissau, almost 5000 children between 6 months and 5 years of age were randomized to receive the recommended dose, or half the recommended dose. Overall, the study indicated improved survival in the lower dose group at 6-months follow-up (MRR 0.69 [0.36–1.35]). In females, the association was significant (MRR 0.19 [0.06–0.66]), and the interaction between dose and sex was significant as well (p=0.004), suggesting that females benefit more from this intervention. At 9-months follow-up, the effects were still significant, albeit less so. Two subsequent trials, one in neonates and the other in infants aged 6–59 months, did not find any difference in mortality at 12-months follow-up between the standard dose (50 000 IU for infants <6 months; 100 000 IU for infants 6–12 months; 200 000 IU for infants ≥12 months) and the halved dose, overall or by sex.

Effect of repeated doses of VAS on all-cause mortality

A study comparing the different size of VAS dose in children aged 6–59 months in Guinea-Bissau, of whom a large fraction had previously participated in a trial of neonatal VAS, did not find any significant interaction between dose of VAS (standard versus halved dose) and previous neonatal VAS, overall or in either sex; the MRR of low-dose versus high-dose campaign VAS was 1.50 (0.27–8.18) for those having previously received neonatal VAS and 0.18 (0.02–1.52) for neonatal placebo recipients. A subsequent study in Guinea-Bissau of a second VAS dose to children previously enrolled in a trial of neonatal VAS found that females who had received neonatal VAS benefited significantly from receiving high-dose VAS at age 1 year compared to females who received placebo at birth. This inferred a significant interaction between neonatal VAS and subsequent VAS in females (p=0.009), but not in males. An observational study was conducted in children 6–35 months of age living in an area of Guinea-Bissau targeted for consecutive VAS campaigns over 2 years. Those that participated in a prior VAS campaign had lower mortality after a second VAS campaign compared with participants in the second campaign only (MRR 0.34 [0.14–0.80]), the association being significant in females but not in males (interaction between repeated VAS and sex: p=0.14). These results suggest an immunological priming effect of neonatal VAS for the response to a secondary dose, and would imply that previous supplements should be considered in studies of VAS. The findings further imply a sex-differential priming effect, perhaps pertaining to differences in the early state of the immune system in males and females. Of note, the effect of VAS may depend on the vitamin A status. A differential effect of VAS on cellular responses to vaccine antigens was found in Bangladeshi children, as VAS increased the frequency of responders among children with serum retinol levels ≥0.7 μmol/l (considered adequate) at the time of testing, but had no effect in children with low serum retinol levels. Such differences in serum retinol levels, however, may not explain the effect of previous VAS, as the effects of VAS on serum retinol levels are only modest and rapidly wane.

Effects of VAS on morbidity

A few studies have investigated the effect of VAS on morbidity in respect to sex. A trial in South Indian pre-school children receiving repeated high-dose VAS every 4 months found no benefit of VAS on 12 morbidity indicators (including incidence, number of days ill, and duration of respiratory illness, diarrhoea, skin infection and measles infection) during the 1-year follow-up, but a longer duration of diarrhoea episodes in females. There was no effect of neonatal VAS on the measles incidence rate at 12-months follow-up during a measles epidemic in Guinea-Bissau among MV-naïve infants. However, before age 6 months, VAS tended to protect males from measles, but increased the risk in females (interaction between VAS and sex: p=0.04). The same interaction between VAS and sex was observed for severe measles cases leading to hospitalization or death, although it was not significant (p=0.08).

In Guinean infants <6 months of age, rotavirus infection and diarrhoea were more frequent in neonatal VAS recipients, with no difference by sex. The overall incidence rate ratio (IRR) for rotavirus infection was 1.72 (1.04–2.85), and 3.74 (1.40–9.98) for diarrhoea. Also in Guinea-Bissau, VAS was associated with a lower incidence of non-rotavirus infections in males <6 months (IRR 0.51 [0.27–0.95]), with no difference for <6-month-old females; while above 6 months, VAS tended to cause a higher incidence in females, with no effect in males (interaction between VAS and sex: p=0.03). In Indonesian pre-school children, VAS was associated with a significantly increased incidence of acute lower respiratory illness in females, but not in males (p=0.09 for interaction between sex and VAS). In Indian 2–24 months old children admitted to hospital, there was no effect overall or by sex of a double dose of 30 000 IU VAS for 4 consecutive days on recovery rate from severe acute lower respiratory infections.

Effect of VAS on immunological responses to vaccines

Antibody responses to vaccines

Savy et al. have comprehensively reviewed the literature addressing interactions between micronutrient status or supplementation and vaccine responses. Overall, the review found no evidence for an effect of VAS on specific responses to diphtheria and tetanus, cholera, influenza, Haemophilus influenzae type b and pneumococcal vaccines. For antibody responses to MV, the review concluded that VAS may reduce seroconversion rates in children with high baseline antibody levels, but have no effect in those with low baseline antibody levels; and there is a suggestion
that VAS impairs specific responses to BCG measured by skin test, particularly in males. There is, however, an almost complete lack of studies reporting the effects by sex. One exception is a study on the effect of VAS versus placebo together with MV at 6 and/or 9 months of age on measles-specific seroconversion rate in Guinea-Bissau. There was no overall effect on the antibody titres or seroconversion rate in infants receiving MV at 6 and 9 months; but for children receiving only a single MV at 9 months, VAS had an antibody enhancing effect in males, but not in females, leading to a significant interaction between VAS and sex (p = 0.02). It should be noted, however, that the seroconversion rates were >90% in all subgroups. When the infants were revisited at 6–8 years of age, the effect of VAS had waned as the frequency of protective titres was high in both supplemented and non-supplemented children, and sex differences were no longer apparent.

**Cellular responses to vaccines**

The available data support sex differences in the effect of VAS on the cell-mediated immune response to BCG vaccination. At age 2 months in Guinea-Bissau, neonatal VAS together with BCG was associated with a lower prevalence of tuberculin skin test responders in males (prevalence ratio 0.79 [0.67–0.92]), but no such effect in females. The association was transient and gone by 6 months. At 6 weeks, VAS was associated with significantly higher interferon-γ responses to purified protein derivative (PPD) in males (geometric mean ratio [GMR] 1.07 [1.00–1.15]), but not in females; however, subtracting the baseline responses of non-stimulated cells, there was no sex-difference, but the overall effect of VAS remained significant (stimulation index 1.40 [1.03–1.91]).

In Guinean infants, cytokine responses were sex-differentially affected 6 weeks after randomization to neonatal VAS. Baseline tumour necrosis factor (TNF) 24 hour-production by unstimulated cells (medium alone) was significantly lower in female VAS-recipients compared to non-supplemented females (GMR 0.41 [0.26–0.72]), with no significant differences in males. The TNF responses to lipopolysaccharide were lower in VAS males (GMR 0.68 [0.47–1.00]), but not females. Moreover, DTP-vaccinated VAS males had lower TNF and interleukin (IL)-10 released in unstimulated cells, as did non-DTP vaccinated VAS females, resulting in a significant three-way interaction between VAS, sex and DTP (p = 0.03 and p = 0.04 for TNF and IL-10, respectively). The VAS DTP-vaccinated males also had a higher IL-5 response to phytohaemagglutinin (PHA), and higher IL-13 to PPD suggesting enhanced type 2 immunity. Albeit the study was exploratory, the findings support a sex-differential interaction between VAS and DTP.

By combining data from two RCTs in Guinea-Bissau, early MV and neonatal VAS, neonatal VAS was associated with a differential effect of early MV on immunological markers. Among VAS recipients, MV females but not males had increased plasma IL-1 receptor agonist and IL-8, an effect not present in infants without neonatal VAS. The MV group had a higher ratio of in vitro TNF:IL-10 to antigenic stimulation among children who had received neonatal VAS, with the opposite effect in non-VAS recipients; for PHA stimulation, the interaction between VAS and MV was p = 0.02.

The study suggested long-term immunomodulation by neonatal VAS with effects sustained at least up to 5 months after administration.

Neonatal VAS was associated with increased monocytes at 6 weeks of age in females, but not in males, and only among those who received DTP before follow-up, but not in the DTP non-vaccinated. Lymphocytes, granulocytes or eosinophils were not significantly affected. There was no overall or sex-differential effect of VAS at 9 months of age on CD4 or CD8 T cell counts or the ratio between the two at 18 months of age if children had already received VAS at 6 months. In summary, VAS may modify heterologous immunological effects of vaccines, in a sex-specific manner, although current evidence is too limited to infer mechanistic hypotheses.

**Conclusions**

The literature is inconsistent as to the effect of VAS on morbidity and mortality and reactivity to vaccines in early childhood. Some of the variation may be explained by factors that could modify the effect of VAS, and which may differ between the studied populations. These include nutritional factors, for example VAS in Indonesia enhanced the incidence of acute lower respiratory infections particularly in children with normal growth status, but less so in stunted children, and the effect of VAS on cellular responses was differential in Bangladeshi children with adequate versus non-adequate serum retinol levels. Vaccination coverage may play a role since VAS effects seem to depend on vaccination status, or perhaps even genetic background, for example the effect of VAS on cytokine responses was only evident in homozygotic carriers of the G allele at the TNF-α locus -238. Moreover, a number of the trials are observational in nature and therefore prone to many confounding factors. Despite these caveats a series of sex-specific differences have been described, mostly, but not exclusively supporting a more beneficial effect of VAS in males than females (Figure 1). Hence, a null-effect may comprise differential effects in opposite directions in males and females, or perhaps vaccinated and un-vaccinated groups. Unfortunately, data analysis by sex is not yet commonplace in study design and dissemination. Many of the studies came from the same group in Guinea-Bissau, and there is an urgent need to extend these studies to other populations and geographical settings. In order to investigate this issue further, WHO has commissioned three large RCTs of neonatal VAS on all-cause mortality in India, Ghana and Tanzania. The studies will address potential effect-modifiers including sex and vaccines. Moreover, two smaller human immunological studies in The Gambia and Bangladesh are underway. Results from these studies will soon be available, and hopefully will resolve the question of the benefits or risks of neonatal VAS.

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References


