

# Combining Vitamin A and Vaccines: Convenience or Conflict?

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**This thesis is based on the following 11 papers, referred to in the text by their Roman numerals:**

- I. **Benn CS**, Whittle H, Aaby P, Balé C, Michaelsen KF, Olsen J. Vitamin A and measles. *Lancet* 1995; 346: 503-504
- II. **Benn CS**, Aaby P, Balé C, Olsen J, Michaelsen KF, George E, Whittle H. Randomised trial of effect of vitamin A supplementation on antibody response to measles vaccine in Guinea-Bissau, West Africa. *Lancet* 1997; 350: 101-105
- III. **Benn CS**, Balde A, George E, Kidd M, Whittle H, Lisse IM, Aaby P. Long-term effect of vitamin A supplementation with measles vaccine in infancy on measles-specific antibody levels in Guinea-Bissau, West Africa. *Lancet* 2002; 359: 1313-14.
- IV. **Benn CS**, Balé C, Sommerfelt H, Friis H, Aaby P. Hypothesis: Vitamin A supplementation and childhood mortality: Amplification of the non-specific effects of vaccines? *Int J Epidemiol* 2003; 32:822-8.
- V. **Benn CS**, Martins C, Rodrigues A, Jensen H, Lisse IM, Aaby P. Randomised study of the impact of different doses of vitamin A on childhood morbidity and mortality. *BMJ* 2005; 331: 1428-32.
- VI. **Benn CS**, Martins C, Rodrigues A, Fisker AB, Christoffersen D, Aaby P. The effect of vitamin A supplementation administered with missing vaccines during national immunisation days in Guinea-Bissau *Int J Epidemiol* 2009; 38: 304-11.
- VII. **Benn CS**, Diness BR, Roth A, Nante E, Fisker AB, Lisse IM, Whittle H, Rodrigues A, Yazdanbakhsh M, Aaby P. Randomised trial of the effect on mortality of 50,000 IU vitamin A given with BCG vaccine to infants in Guinea-Bissau, West-Africa *BMJ* 2008; 336: 1416-20.
- VIII. **Benn CS**, Rodrigues A, Yazdanbakhsh M, Fisker AB, Ravn H, Whittle H, Aaby P. The effect of high-dose vitamin A supplementation administered with BCG vaccine at birth may be modified by subsequent DTP vaccination. *Vaccine* 2009; 27: 2891-98
- IX. **Benn CS**, Fisker AB, Napirna BM, Roth A, Diness BR, Lausch KR, Ravn H, Yazdanbakhsh M, Rodrigues A, Whittle H, Aaby P. Vitamin A supplementation and BCG vaccination at birth in low birthweight neonates: two by two factorial randomised controlled trial. *BMJ* 2010; 340:c1101. doi: 10.1136/bmj.c1101.
- X. **Benn CS**, Aaby P, Nielsen J, Binka FN, Ross DA. Does vitamin A supplementation interact with routine vaccinations? An analysis of the Ghana Vitamin A Supplementation Trial. *Am J Clin Nutr* 2009; 90: 629-39.
- XI. **Benn CS**, Fisker AB, Diness BR, Aaby P. Sex-differential effects of neonatal vitamin A supplementation on mortality? *J Infect Dis* 2006; 194: 719.

## 1. BACKGROUND

### GLOBAL BURDEN OF VITAMIN A DEFICIENCY

Vitamin A deficiency is widespread among children in low-income countries. It is estimated that 4 million children under 5 years of age, mostly in South Asia and Sub-Saharan Africa, are affected by xerophthalmia, the clinical eye manifestation of vitamin A deficiency, which can lead to blindness. Far greater numbers of children (estimated 127 million) show no external signs of vitamin A deficiency, but have dangerously low stores, which lead to increased risk of infection and death, making vitamin A deficiency a major contributor to child mortality (1).

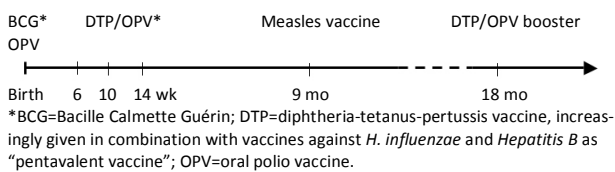
### VITAMIN A SUPPLEMENTATION AGAINST CHILD MORTALITY

Vitamin A supplementation is considered among the most important tools to reduce child mortality in low-income countries. Recently a series of papers on maternal and child under nutrition were published in *The Lancet*. It was concluded that "Of available interventions, counselling about breastfeeding and fortification or supplementation with vitamin A and zinc have the greatest potential to reduce the burden of child morbidity and mortality" (2). The Copenhagen Consensus 2008 aimed to set priorities among a series of proposals to confront great global challenges. Vitamin A and zinc supplements for children were ranked the top priority (3).

## THE CURRENT VITAMIN A SUPPLEMENTATION POLICY

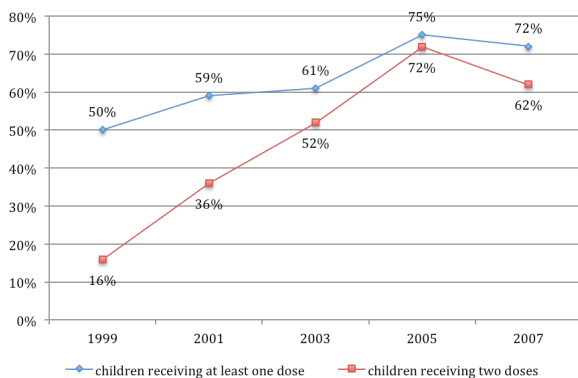
For the last two and a half decades the World Health Organization (WHO) has recommended that children between 6 months and 5 years of age receive an oral high-dose vitamin A supplement every 4-6 months in areas of vitamin A deficiency (4-5). Currently, 103 countries are considered priority countries for vitamin A supplementation (1). To increase vitamin A supplementation coverage, WHO recommends integration of vitamin A supplementation with the Expanded Programme on Immunization (EPI) (4-8). Two main strategies to reach children are pursued. *First*, it is recommended to provide vitamin A supplementation at routine vaccination contacts. A typical vaccination schedule in low-income countries is shown in **Figure 1**.

**Figure 1. Routine vaccination schedule in many low-income countries**



Vitamin A can be provided together with measles vaccine, delayed primary vaccination doses or booster doses. *Second*, vitamin A can be provided at national immunization days or campaigns together with vaccines and other health interventions. The two strategies can be combined as long as the coverage through routine vaccination contacts does not exceed 80% (8). During the last decade the coverage of vitamin A supplementation has increased dramatically (**Figure 2**). Every year, roughly 200 million children in the 103 priority countries receive one or more vitamin A supplements, almost always in combination with other health services such as vaccines (1, 9).

**Figure 2. Progress with vitamin A supplementation for children aged 6-59 months**



Source: UNICEF Global Database, 2008.

## JUSTIFICATION FOR THE CURRENT POLICY

The WHO vitamin A supplementation policy picked up steam after a number of randomised community-based trials in the late eighties and early nineties (10-18) had shown that high-dose vitamin A supplementation to children between 6 months and 5 years of age reduced overall mortality. Several meta-analyses estimated that vitamin A supplementation could decrease mortality by an impressive 23-30 % (19-21).

The effect of vitamin A supplementation is ascribed to the prevention and treatment of vitamin A deficiency. Supplementation is considered safe; as stated in the WHO guidelines "When vitamin A is administered in recommended doses, there are no serious or permanent adverse effects, such side-effects as may occasionally occur (e.g. for infants, a tense or bulging fontanelle or vomiting) are minor and transitory and do not require specific treatment. As adequate vitamin A status is achieved through other means, supplementation becomes less necessary, although its continuation is not harmful" (5).

None of the original trials had linked vitamin A supplements with vaccinations or studied the effect of vitamin A supplementation according to vaccination status. When they were conducted, the implementation of the EPI was still in its youth, and vaccination coverage was low. Hence, the current WHO policy of providing vitamin A supplements with vaccines has never been tested in randomised trials. In other words, though the world increasingly demands evidence-based medicine, one of the major policies to reduce child mortality has never been evaluated for its overall effect on child mortality.

## INCONSISTENT FINDINGS

In fact, two of the eight original vitamin A trials did not find a beneficial effect of vitamin A supplementation (12, 15). Importantly, several recent trials have shown that the effect of vitamin A may not always be beneficial. A large trial of 1 million Indian children showed no effect on mortality of vitamin A supplements of children aged 1 to 6 years. This trial was presented at a conference in 2007, but has not yet been published (22). Furthermore, all trials which have tested the effect of providing vitamin A to children between 1 and 5 months of age have failed to show a beneficial effect (14, 23-29), and though such a policy at one stage had many advocates (30-32), it has now been abandoned. Lately, several trials providing vitamin A to neonates, have showed worrying tendencies for negative effects, as will be discussed later.

## TWO MAIN HYPOTHESES

In this thesis, I will argue that vitamin A supplements and vaccines interact, and such interactions may explain divergent effects of vitamin A supplementation on child mortality. I will also argue that vitamin A supplements may have sex-differential effects. If these observations are taken into account, the WHO vitamin A supplementation policy can be optimised, resulting in decreased child mortality without additional costs.

The main hypotheses are:

Vitamin A supplementation and routine vaccinations interact with consequences for child mortality (The *Vitamin A-vaccine-interaction hypothesis*) (IV)

The effect of vitamin A supplementation differs between the two sexes (XI)

## 2. THE RESEARCH PROCESS

### AN IDEA

In 1992, as a medical student, I decided to do research with the aim of reducing the high child mortality in low-income countries. I would attack the major problems of infectious diseases and malnutrition. I searched Medline for abstracts on infectious diseases, vaccines, and nutrition interventions, and became interested in

vitamin A supplementation – several recent trials reported that prophylactic vitamin A supplementation reduced all cause mortality substantially. A recent paper reported a beneficial effect of vitamin A in the treatment of measles cases in New York. The abstract ended “Additional studies of vitamin A in measles and other infectious diseases, and in vaccine efficacy trials, should be done” (33). I searched for papers on the effect of vitamin A supplementation on the immune response to measles vaccine and found no such papers. However, in the process I discovered that it was already WHO policy to provide vitamin A supplementation with measles vaccine in low-income countries (4-5). This policy had been instituted by the WHO for logistic reasons. Nobody had studied whether providing vitamin A at the same time as measles vaccine would influence the formation of measles specific antibodies. I had identified my research topic.

#### A RESEARCH YEAR

I approached Peter Aaby at the Bandim Health Project in Guinea-Bissau with the idea to study the impact of vitamin A supplementation given with measles vaccine on measles-specific antibody titres. I initiated my research year in Guinea-Bissau in 1993. The results were published in 1995 (I, 34), 1997 (II), and 2000 (35). The main findings were that vitamin A supplementation given with measles vaccine at 9 months of age was associated with increased antibody titres at 18 months of age. Intriguingly, this was only seen in boys (II).

#### TH1/TH2, AND A FOR ATOPIC?

In 2000 I went back to Guinea-Bissau to do a follow-up study of the previous trial participants. Atopic patients had been found to have a T-helper (Th)-2-deviated immune system (36). I speculated that since vitamin A deficiency had been associated with Th1 deviation of the immune system (37), vitamin A deficiency might be part of the explanation why low-income countries have less atopic diseases. Hence, vitamin A supplementation in childhood – though associated with lower overall mortality and increased antibody titres – could have the side effect that more children became atopic. We found no large effect of vitamin A in infancy on the risk of being skin prick test-positive 6-7 years later (38). Importantly, the children who had received vitamin A with measles vaccine at 9 months of age still had higher antibody levels and were more protected against measles (III).

#### A HYPOTHESIS: VITAMIN A-VACCINE INTERACTIONS

In 2000 several different lines of thoughts converged into a hypothesis. *First*, my interest in the Th1/Th2 balance of the immune system and the impact of vitamin A on this balance. *Second*, Peter Aaby's research on vaccines, which showed that vaccines, apart from protecting against the targeted disease, also had important so-called “non-specific effects” on overall mortality. The live measles vaccine given after 6-9 months of age and the live BCG vaccine given at birth reduced mortality from other causes than merely measles and tuberculosis - i.e. had beneficial non-specific effects on overall mortality (39-44). In contrast, the inactivated diphtheria-tetanus-pertussis (DTP) vaccine given between 1 and 5 months of age was associated with increased child mortality in areas with herd immunity to pertussis (39-40, 45-46). *Third*, immunological studies had mainly associated live vaccines with Th1 deviation (47-51) and inactivated vaccines with Th2 deviation (49-54). Fourth, the surprising fact that though vitamin A supplementation was beneficial after 6 months of age (19-21) and at birth (55-56), it had no effect between 1 and 5 months of age (14, 23-

29), even though many children in the age groups were vitamin A-deficient (25, 26). One evening it converged into a hypothesis: that vitamin A supplementation amplified the non-specific effects of routine vaccines, being beneficial when provided with BCG and measles vaccine, but potentially harmful when given with DTP vaccine, perhaps due to excessive Th2 deviation. According to conventional understanding, the effect on mortality of vitamin A supplementation was due to prevention and treatment of vitamin A deficiency, the *Prevention-of-deficiency* hypothesis. However, that interpretation was challenged by a number of findings, which did not fit, for instance the mortality-age pattern (IV). My hypothesis, the *Vitamin A-vaccine-interaction* hypothesis, seemed to fit the existing data on vitamin A supplementation better than the *Prevention-of-deficiency* hypothesis. The hypothesis was published in 2003 (IV).

#### TESTING THE HYPOTHESIS

Since I formulated the hypothesis my work has focused on testing the hypothesis, continuously comparing the consistency of existing and evolving data with my hypothesis and the *Prevention-of-deficiency* hypothesis. This has led to three trials and one observational study in Guinea-Bissau plus a reanalysis of an existing data-set from Ghana. The vitamin A studies have resulted in a number of papers (I-XI, 34-35, 57-69), of which the most important constitute the basis for the present thesis (I-XI).

#### A NEW HYPOTHESIS: VITAMIN A HAS SEX-DIFFERENTIAL EFFECTS

Our first vitamin A trials had one thing in common – sex-differential effects of vitamin A supplementation (II, V). Tendencies for sex differences in response to vitamin A supplementation had also been observed in the two first trials of neonatal vitamin A supplementation (55-56), and I put forward the hypothesis that the effect of neonatal vitamin A supplementation is sex-differential (XI). This observation might be linked to the hypothesis of vitamin A and vaccine interactions. While the above trials were undertaken it had become more and more clear that the negative non-specific effect of DTP vaccine was strongest in girls (70-75). Hence, vitamin A amplification of a negative non-specific effect of DTP vaccine could be the real explanation for the sex-differential effects of neonatal vitamin A supplements. However, there may also be other explanations for the sex differences – such as underlying differences in vitamin A status or underlying immunological differences between boys and girls – which determine two independent events in girls: a negative interaction with DTP vaccine and a less positive or even negative response to vitamin A supplementation. Hence, so far I see the two hypotheses as independent.

In conclusion, the initial idea to study the effect of vitamin A supplementation on the specific immune response to measles vaccine led to the formulation of two new hypotheses.

In the following three Chapters 3-5, I will summarise existing evidence regarding the *specific vitamin A-vaccine interactions* on the immune response to routine vaccines, the *non-specific vitamin A-vaccine interactions* influencing mortality, and the *sex-differential effects of vitamin A supplementation*. In Chapter 6, I will briefly review the potential immunological mechanisms behind the specific and non-specific vitamin A-vaccine interactions and the sex-differences. Chapters 7 and 8 are devoted to conclusions, and discussion of further research questions as well as the implications for public health.

### 3. VITAMIN A AND THE IMMUNE RESPONSE TO VACCINES

The policy of providing high-dose vitamin A supplements together with routine vaccinations was introduced for logistical reasons. However, it is plausible that vitamin A could affect the immune response to vaccines; vitamin A deficiency has been associated with compromised immune function and vitamin A has been shown to affect almost all functions of the immune system (76-77). Vitamin A has been suggested as a useful adjuvant to vaccines (78-79) and animal studies have provided evidence for a negative effect of vitamin A deficiency on antibody responses to different vaccines, and for an antibody-enhancing effect of vitamin A supplements in both deficient and normal animals (80).

When we initiated our study of the effect of vitamin A supplementation on the immune response to measles vaccine in 1993, only a few studies had addressed the effect of vitamin A on the immune response to routine vaccines in childhood (81-83). Since then, several trials have been carried out, mostly on measles vaccine, but the immune response to other routine vaccines such as DTP and oral polio vaccine has also been studied. The studies, which have tested the effect of high-dose oral vitamin A supplementation on the immune response to routine vaccines, are presented in **Table 1** (I-III, 27, 62, 83-95). Below, I summarise the evidence for an effect of vitamin A supplementation on the immune response to each of the vaccines.

#### MEASLES VACCINE

When we set out to do our study we had the *a priori* hypothesis that vitamin A would increase the antibody response to measles vaccine, because we believed it would strengthen the immune system and its capacity to respond adequately. However, another scenario could be that vitamin A made the immune system clear the virus more rapidly, perhaps too rapidly to establish an adequate immune response. This fear was strengthened when an Indonesian trial providing vitamin A or placebo with measles vaccine at 6 months of age was published, showing significantly reduced seroconversion after vitamin A (84).

To date a total of six studies have been conducted (I-III, 84-88). Our own study produced several observations for vitamin A given with measles vaccine at 6 and 9 months of age (I, II), and was the only one which presented data on long-term effects (III).

*Measles vaccine at age 6 months.* Among children, who received vitamin A with measles vaccine at age 6 months, the study from Indonesia reported a negative effect of vitamin A on seroconversion to measles vaccine (84). The results were not presented by sex, but based on the estimates for vitamin A and for sex, the group which had the lowest seroconversion was girls who received vitamin A (84). In our study, we found no negative effect of vitamin A supplementation at age 6 months on antibody titres and seroconversion at age 9 months (I). Furthermore, we found no negative effect of vitamin A given with measles vaccine at 6 and 9 months of age on antibody titres and seroconversion at age 18 months (II). Boys tended to benefit more from vitamin A than girls, but the study population was small and none of the effect estimates reached statistical significance.

*Measles vaccine at age 9 months.* Among children who received vitamin A with measles vaccine at age 9 months, two studies reported significant beneficial overall effects on the antibody response (II, 85) and one study reported significant beneficial

effect in malnourished children (87). One study found no effect (88), whereas one found no overall effect, but a negative effect in the 14% of the children who had preimmunisation titres (86).

Our study has been the only one to date to study long-term effects of vitamin A on antibody titres. We found that vitamin A provided with measles vaccine at age 9 months was associated with significantly higher probability of having protective antibody levels against measles at 6-8 years of age. In the placebo group, 7/79 (9%) had non-protective antibody levels against measles compared with 0/73 (0%) in the vitamin A group ( $p=0.0095$ ) (III).

In our study the beneficial effect on antibody titres at 18 months of age was significantly stronger in boys than in girls. Another study reported data for boys only; there was a tendency for a better response to vitamin A after 1 month but an opposite tendency after 6 months (88). The other studies have unfortunately not reported data by sex.

Our interpretation of the existing data on vitamin A and measles vaccine is as follows: vitamin A supplementation may impair seroconversion at age 6 months and at least does not seem to be beneficial. In contrast, vitamin A given with measles vaccine at age 9 months may improve the antibody response to measles vaccine, perhaps most pronounced in boys, non-breastfed and/or malnourished children. One interesting observation, also noted by Savy (96), is that all studies, which found a positive effect of vitamin A supplementation, had used Haemagglutination Inhibition (HI) assays or ELISA assays to assess antibody titres, whereas the studies which found no effect all had used Plaque Reduction Neutralization (PRN) assays.

#### DIPHTHERIA-TETANUS-PERTUSSIS VACCINE

The five studies testing the effect of vitamin A supplementation on the immune response to DTP vaccine or DTP components have been of quite different design. Two were 2-by-2 factorial trials with vitamin E (91) and maternal supplementation (27), respectively. One supplemented older children 14 days before vaccination (83). The outcomes and the time of assessment also differed. Hence, it is difficult to reach any overall conclusion. Three of the studies suggested that vitamin A may increase the immune response (83, 89-90), none show the opposite. None of the studies reported data by sex.

#### ORAL POLIO VACCINE

Four studies on oral polio vaccine have been conducted (27, 92-94). One found a beneficial effect of vitamin A supplementation on antibody titres to poliovirus type 1 (94). The others found no effect. It is noteworthy that the study, which did find an effect, was the study with the lowest seroconversion rate. In situations with very high seroconversion rates, the response may be difficult to improve further. None of the studies reported data by sex.

#### PENTAVALENT VACCINE

Only one study linking vitamin A supplementation with the new pentavalent vaccines has been carried out (95). It found no overall effect of vitamin A on the antibody response to *H. Influenzae* type b vaccine, but vitamin A significantly improved the antibody response to hepatitis B vaccine. The authors did not present the data by sex, but according to the PhD thesis based on the trial the beneficial effect was only significant in boys (97).

**Table 1. Studies of the effect of oral high-dose vitamin A supplementation given with routine vaccines in childhood on the immune response to the vaccine(s)**

Vaccine type	N	Intervention	Outcome	Main effect of vitamin A supplementation	Vitamin A effects presented by sex	Seroconversion rates	Assay used
<b>Measles vaccine, 6 months</b>							
Indonesia (84)	336	100,000 IU vitamin A + measles vaccine at 6 mo	Seroconversion / titres after 1 and 6 mo	Significantly reduced seroconversion: Odds ratio=0.40 (0.19-0.88)	No. The majority of children who did not seroconvert were girls who received vitamin A	82% seroconverted	PRN
Guinea-Bissau (I)	150	100,000 IU vitamin A + measles vaccine at 6 mo	Seroconversion / titres at 9 mo of age	No overall effect on titres or seroconversion. Relative risk=1.14 (0.91-1.43) adjusted for sex	Seroconversion: Boys: 1.20 (0.87-1.66) Girls: 1.08 (0.79-1.47)	70% seroconverted	HI
Guinea-Bissau (II)	150	100,000 IU vitamin A + measles vaccine at 6/9 mo	Seroconversion / titres at 18 mo of age	No overall effect. GMT ratio=0.81 (0.50-1.30)	GMT ratio: Boys: 0.99 (0.50-1.95) Girls: 0.66 (0.34-1.27)	Almost all seroconverted after two doses	HI
<b>Measles vaccine, 9 months</b>							
Guinea-Bissau (II)	312	100,000 IU vitamin A + measles vaccine at 9 mo	Seroconversion / titres at 18 mo of age	Significantly higher antibody titres: GMT ratio= 1.52 (1.22-1.88)	Effect only seen in boys GMT ratio: Boys: 2.04 (1.53-2.72) Girls: 1.16 (0.85-1.58)	Almost all seroconverted	HI
India (85)	100	100,000 IU vitamin A + measles vaccine at 9 mo	Seroconversion / titres after 4 w	Significantly higher seroconversion, p<0.01	No	Appr. 70% seroconverted	HI
Indonesia (86)	394	100,000 IU vitamin A + measles vaccine at 9 mo - also at 6, 10, 14 w	Seroconversion / titres after 1 and 6 mo	No overall effect. Lower titres after 6 months in the 14% of the children with pre-immunization titres, p=0.03	No	Almost all seroconverted	PRN
India (87)	618	100,000 IU vitamin A + measles vaccine at 9 mo	Seroconversion / titres after 12 w	No overall effect: GMT ratio=1.19 (0.97-1.46). Significantly higher GMT ratio in malnourished children: 1.57 (1.18-2.08)	No	Appr. 88% seroconverted	ELISA
India (88)	395	100,000 IU vitamin A + measles vac at 9-12 mo	Seroconversion / titres after 1 and 6 mo	No significant findings	After 1 month: Tendency for higher GMT boys (p=0.29). After 6 months: Tendency for less protection boys (p=0.10).	Almost all seroconverted	PRN
Guinea-Bissau (III)	278	100,000 IU vitamin A + measles vaccine at 9 mo	Protective levels /titres at age 6-8 years	Significantly more children with antibody levels above protective level (p=0.0095)	No sex differences observed	Note: only trial with long follow-up	HI
<b>DTP*</b>							
Indonesia (83)	236	200,000 IU vitamin A 2 w before DTP	IgG to tetanus after 3 w	Significantly higher IgG levels to tetanus (p<0.05)	No. 72% of participants were boys	No information Note: 50% were xerophthalmic	ELISA
Bangladesh (89)	120	50,000 IU vitamin A + DTP/OPV at 6, 10, 14 w	Cell-mediated immunity to tetanus and pertussis after 1 month	No overall effect. Significantly more positive responses in children with adequate retinol levels at the time of measuring (p=0.008)	No	N/A	
Bangladesh (90)	56	50,000 IU vitamin A + DTP/OPV at 6, 10, 14 w	IgG to diphtheria, tetanus, pertussis after 1 mo	Significantly more IgG to diphtheria toxin (p=0.029)	No	No information	ELISA
Turkey (91)	89	2*2 factorial design 1) 30,000 IU vitamin A for three days 2) vitamin E + DTP at 2/3/4 mo	IgG to tetanus after 1 month and at 16-18 mo of age	No	No	No information	ELISA
Ghana (27)	1085	2*2 factorial design 1) Maternal vitamin A 2) 25,000 IU vitamin A + DTP/OPV at 6, 10, 14 w	IgG to tetanus at 6 mo of age	No	No	No information	ELISA
<b>Oral Polio Vaccine</b>							
Bangladesh (92)	57	50,000 IU vitamin A + DTP/OPV at 6, 10, 14 w	Seroconversion / titres poliovirus 1-3 1 month after	No	No	Seroconversion 81, 86, and 84% for type 1-3, respectively	Neutralis.
Indonesia (93)	467	25,000 IU, 50,000 IU, or placebo + DTP/OPV at 6, 10, 14 w	Seroconversion / titres poliovirus 1-3 at 9 months of age	No	No	Almost all seroconverted	Neutralis.
India (94)	399	25,000 IU vitamin A + DTP/OPV at 6, 10, 14 w - also vitamin A to mothers	Protective levels /titres poliovirus 1-3 at 26 weeks of age	Significantly increased GMT to poliovirus type 1 GNT ratio=1.55 (1.03-2.31). No effect on poliovirus type 2+3	No	Protective titres: 76, 93, and 82% for type 1-3, respectively	Neutralis.
Ghana (27)	1085	2*2 factorial design. 1) Maternal vitamin A 2) 25,000 IU vitamin A + DTP/OPV at 6, 10, 14 w	Seroconversion / titres poliovirus 1-3 at 6 months of age	No	No	Seroconversion approx. 92, 93, and 87% for type 1-3, respectively	Neutralis.
<b>H. influenzae type B / Hepatitis B vaccine</b>							
Ghana (95)	1077	50,000 IU vitamin A + DTP/Hib/HepB/OPV at 6, 10, 14 w	Protective levels /titres Hib and HepB at 18 weeks of age	Hib: No effect. HepB: Significantly more children with antibody levels above protective level hepatitis B: RR=1.05 (1.01-1.09) (97)	Boys: 1.07 (1.01-1.14) Girls: 1.01 (0.96-1.05)	No information	ELISA
<b>BCG</b>							
Guinea-Bissau (62)	2710 **	50,000 IU vitamin A + BCG vaccine at birth	PPD response at 2 and 6 months of age. Ex-vivo cytokines to PPD at 6 weeks of age	No overall effect; RR for positive PPD reaction at 2 months=0.90 (0.80-1.02). Significantly increased ex-vivo IFN-γ to PPD.	PPD response 2 months: Boys: 0.81 (0.69-0.95) Girls: 1.04 (0.86-1.26)	N/A	PPD test/ Luminex

\*Study by Brown (81) excluded due to use of injected water-miscible vitamin A. Study by Bhaskaram (82) excluded due to lack of direct comparison of vitamin A recipients and controls. GMT=Geometric mean titre. PRN=Plaque Reduction Neutralization. HI=Haemagglutination Inhibition. \*\*607 for cytokines

## BCG VACCINE

We are the only ones to date who have studied the effect of vitamin A supplementation on the immune response to BCG vaccine. We examined the effect of simultaneous vitamin A supplementation and BCG vaccine on the *in vivo* delayed type hypersensitivity response to purified protein derivative of *Mycobacterium tuberculosis* (PPD). In the placebo group, more boys than girls mounted a positive PPD response at 2 months of age, as also previously observed. However, vitamin A supplementation at birth significantly diminished the proportion of boys, who had a positive PPD reaction at 2 months of age. In contrast, vitamin A was associated with significantly increased *in vitro* IFN- $\gamma$  production to PPD stimulation in both sexes at 6 weeks of age. Vitamin A supplementation was not associated with either PPD response or BCG scarification at 6 months of age (62).

## DISCUSSION

Overall, 14 different studies investigated the effect of high-dose oral vitamin A supplementation on the *antibody response* to one or more vaccines. One found a significantly negative effect (on the antibody response to measles vaccine at age 6 months (84)), six found a significantly positive effect (II, 83, 85, 90, 94-95), whereas seven found no overall effect (27, 86-88, 91-93). Most of the "no-effect" studies were small, and the confidence intervals allow for rather large effects. One exception is the Ghana trial, which investigated the response to DTP and oral polio vaccine in more than 1000 individuals, and found no effect (27).

Only two studies had *cell-mediated immunity* as an outcome (62, 89), one found a beneficial effect of vitamin A on delayed type hypersensitivity (DTH) responses to pertussis and tetanus in children with adequate vitamin A levels (89), the other, our own, found a temporarily negative effect of vitamin A on the DTH response to PPD at age 2 months in boys. Paradoxically, vitamin A seemed to increase the IFN- $\gamma$  response to PPD *in vitro* (62).

When assessing the overall evidence for an effect of vitamin A supplementation on the immune response to routine vaccinations in childhood, publication bias has to be considered, both in terms of overall effects and in terms of potential sex-differential effects. Publication bias resulting in less publication of "no-effect" trials does not seem plausible. In situations in which vitamin A is recommended given with vaccines, it is important to make sure that there is no negative effects of combining the two interventions. The possibility that vitamin A could enhance protection is of secondary interest. Hence, especially after the first study reporting a negative effect of vitamin A with measles vaccine at age 6 months, it has been important to report "no-negative-effect". It may be that some studies did not report non-significant results of sex-stratified analyses, though it would seem natural in response to our observation of sex-differential effects of vitamin A on the antibody response to measles vaccine. However, surprisingly few outside our group seriously consider that preadolescent boys and girls may differ immunologically, and the lack of sex-stratified results is likely due to the fact that stratification by sex has not been considered.

Hence, the current evidence suggests that vitamin A supplementation may enhance humoral responses to measles vaccine and potentially also other vaccines. There are weak indications that this could be most pronounced in settings with low seroconversion rates and thereby room for improvement, and in boys, in non-breastfed children, as well as in malnourished children.

It should be considered whether assay type influences the assessment of vitamin A effects on antibody responses.

Vitamin A may also affect cell-mediated responses, but more studies are needed. Our study suggested that it may temporarily dampen cell-mediated immune responses in boys. The consequences of a temporarily dampened cellular immune response to PPD for tuberculosis immunity are unknown, and should be more thoroughly investigated before vitamin A was recommended given with BCG vaccine.

The potential biological mechanisms are discussed in Chapter 6.

Weaknesses include that very few studies assessed vitamin A status, and many of the studies, including our own, were conducted in areas with potentially limited vitamin A deficiency. We found the best effect in non-breastfed children who were presumably most vitamin A-deficient (II). Another study found the best effect in malnourished children (87). Since numerous animal studies have confirmed a beneficial role of vitamin A on antibody production (80, 98), the more inconsistent results in human studies could be due to lack of vitamin A deficiency. It would be recommendable if future studies included an assessment of vitamin A status, and were powered to detect more subtle effects, of course to the extent that the effects should still be of clinical relevance.

If vitamin A supplementation can enhance the antibody response to measles vaccine and prolong the period during which children are protected against measles, as we found (II, III), it would be of public health importance.

The observation that vitamin A provided together with vaccines may amplify the specific antibody response to the vaccines adds plausibility to the hypothesis that vitamin A could also amplify the non-specific effects of vaccines. The non-specific vitamin A-vaccine interactions are discussed in the following chapter.

## 4. VITAMIN A-VACCINE INTERACTIONS AND MORTALITY

It is currently assumed that vitamin A exerts its effect on overall mortality by preventing and treating vitamin A deficiency, which would otherwise lead to increased risk of infections and mortality – the *Prevention-of-deficiency* hypothesis. We have questioned that assumption and proposed the hypothesis that vitamin A is also an immuno-modulator, and its effects on morbidity and mortality are dependent on the state of the immune system. In particular, we have proposed that the effect may depend on the type of vaccine(s) given around the time of supplementation – the *Vitamin A-vaccine-interaction* hypothesis (IV).

A key difference between the two hypotheses is whether vitamin A has only beneficial specific effects related to prevention of vitamin A deficiency and its consequences, or whether it may also have non-specific effects on the immune system, which may be beneficial, but also potentially harmful.

According to the *Prevention-of-deficiency* hypothesis, vitamin A supplementation would always be beneficial if a child was vitamin A-deficient, or have no effects if a child was vitamin A replete – except for acute toxic adverse events such as bulging fontanelles or vomiting as a consequence of transient intracranial pressure. This is considered to be without significant consequence, as the symptoms usually resolve quickly, and there

seems to be no long-term developmental consequences (5, 30, 99-100). According to the *Vitamin A-vaccine-interaction* hypothesis, the effect of vitamin A supplementation would depend on what was going on in the immune system; it could be beneficial but also potentially harmful. A deficient child would of course benefit from having the deficiency treated – but it could also gain additional survival benefit, if vitamin A was provided under favourable conditions – or the benefit from treating the deficiency could be counterbalanced by negative non-specific effects due to amplification of inappropriate activities in the immune system.

The hypothesis of vitamin A-vaccine interactions was founded on contradictions within the *Prevention-of-deficiency* hypothesis (IV). The most important contradictions were *first*, that there apparently was no association between the effect of vitamin A supplementation on one hand and the baseline mortality and the degree of underlying vitamin A deficiency on the other hand. In other words the best effect was not seen in areas with the highest mortality and the highest prevalence of vitamin A deficiency (19). *Second*, there was a peculiar mortality-age pattern; there was a beneficial effect of vitamin A on mortality when vitamin A was given at birth or after 6 months of age, but not between 1 and 5 months of age in spite of vitamin A deficiency in this age group (IV). Since the formulation of the hypothesis more trials have been published. We have published two trials of vitamin A at birth showing no overall effect (VII, IX). Two other trials of vitamin A supplementation at birth found no effect in Zimbabwe (101-102) and a significant beneficial effect in Bangladesh (103). Several smaller trials designed to study other outcomes than mortality have reported mortality data pointing towards a negative effect of vitamin A given with DTP/pentavalent vaccine (27, 29). One large, yet unpublished, trial of vitamin A supplementation to more than 1 million children above 6 months of age in India found no effect (22). Hence, the mortality-age pattern is not as clear as when we formulated the hypothesis (IV). An overview of the existing trials presenting mortality data is presented in **Figure 3**, stratified by age group.

Apart from the lack of an association between vitamin A supplementation effects and vitamin A deficiency, and the strange mortality-age pattern, the hypothesis was based on several additional contradictions, which have been summarised in the hypothesis paper (IV).

We have conducted studies with the specific aim to explore vitamin A-vaccine interactions in terms of mortality and continuously compare the evidence for our hypothesis against the evidence for the *Prevention-of-deficiency* hypothesis. Since it would be unethical to randomise children to most vaccines, and to randomise children above 6 months of age to vitamin A supplementation, we have had to be pragmatic when designing the trials. Hence, our studies have taken many different forms.

#### A SMALLER DOSE MAY BE EVEN BETTER THAN A HIGH DOSE

One of the observations, which were contradictory according to the *Prevention-of-deficiency* hypothesis, was made in a WHO multicenter trial published in 1998 (26). Almost 10,000 children were randomised to 25,000 IU vitamin A or placebo with the three DTP vaccines. At 9 months of age, at the time of measles vaccine, the children, who had received vitamin A received another 25,000 IU vitamin A, whereas those, who had received

placebo, received 100,000 IU vitamin A. According to the mortality curves in the paper, mortality was slightly higher among vitamin A compared with placebo recipients during the first 6 months of life (**Figure 4**). However, the curves subsequently crossed, and based on the flow chart in the paper we calculated that from 9 months of age to the end of follow-up at 12 months of age, mortality was significantly higher in the group that had received 100,000 IU than in the group that had received 25,000 IU with measles vaccine (Paper IV, **Table 2**). If vitamin A supplementation worked only by preventing vitamin A deficiency, it was an implausible finding that less should be better than more. We reasoned that if vitamin A supplementation interacted with vaccines and their non-specific effects, a smaller dose of vitamin A might be even better than a large dose. Hence, when a national campaign providing oral polio vaccine and vitamin A supplementation to children aged 6 months-5 years was due in Guinea-Bissau in November 2002, we obtained ethical permission to randomise children to the WHO-recommended dose of vitamin A or half that dose. We hypothesised that the smaller dose would be even more beneficial than the recommended dose. We did not formulate any sex-specific hypotheses. As hypothesised, we found a tendency for a better effect of a smaller dose (V). This was due to a significantly beneficial effect in girls. Overall mortality was lower among trial participants than among non-participants, and there was no indication that the high dose of vitamin A was associated with increased mortality - a smaller dose just seemed even more beneficial in girls (V). Numbers were small in the subgroup analyses, but the beneficial effect of the low dose tended to be most apparent in girls who had a DTP vaccine as their last vaccine prior to the campaign. If girls had received DTP, the mortality rate ratio for the high dose versus the low dose was 9.9 (1.3-78), whereas it was 3.1 (0.6-15.5) if this was not the case (unpublished data). The finding corroborated the finding from the WHO multicenter trial (which did not present data by sex) – and hence both studies were incompatible with the *Prevention-of-deficiency* hypothesis.

#### Discussion

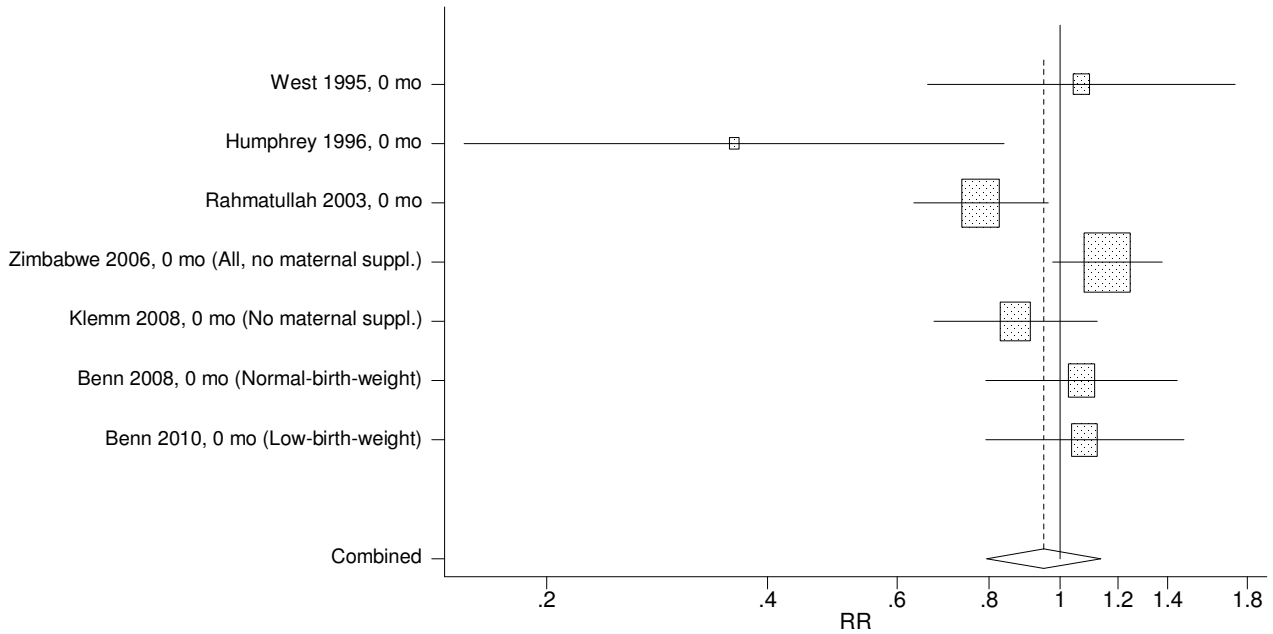
One of the first vitamin A trials to be conducted used a small weekly dose instead of the high dose, and that study found the most beneficial effect on mortality of all the trials conducted at that time, the relative risk being 0.46 (0.30-0.71); 0.41 for girls ( $p<0.01$ ) and 0.52 for boys ( $p<0.05$ ) (17). To our knowledge, no other trials than the two above have compared the mortality effect of two different doses of vitamin A in community studies.

A few smaller studies with morbidity and growth as the primary outcome have generally supported that a smaller dose was more beneficial than a large dose (108-112). Only one study showed the opposite tendency, and in that study the high-dose supplement degraded and subsequent analyses showed that the actual dosing was lower in the high-dose than in the low-dose supplements (113). Hence, the evidence so far suggests that a lower dose is more beneficial in terms of mortality and also morbidity than a high dose. Our study suggests that this is most pronounced in girls; the other studies did not report data by sex. The results support that vitamin A supplementation exerts its effects on mortality by other mechanisms than merely prevention and treatment of vitamin A deficiency.

**Figure 3. An overview of the trials reporting mortality-effects of vitamin A supplementation. Presented by age group**

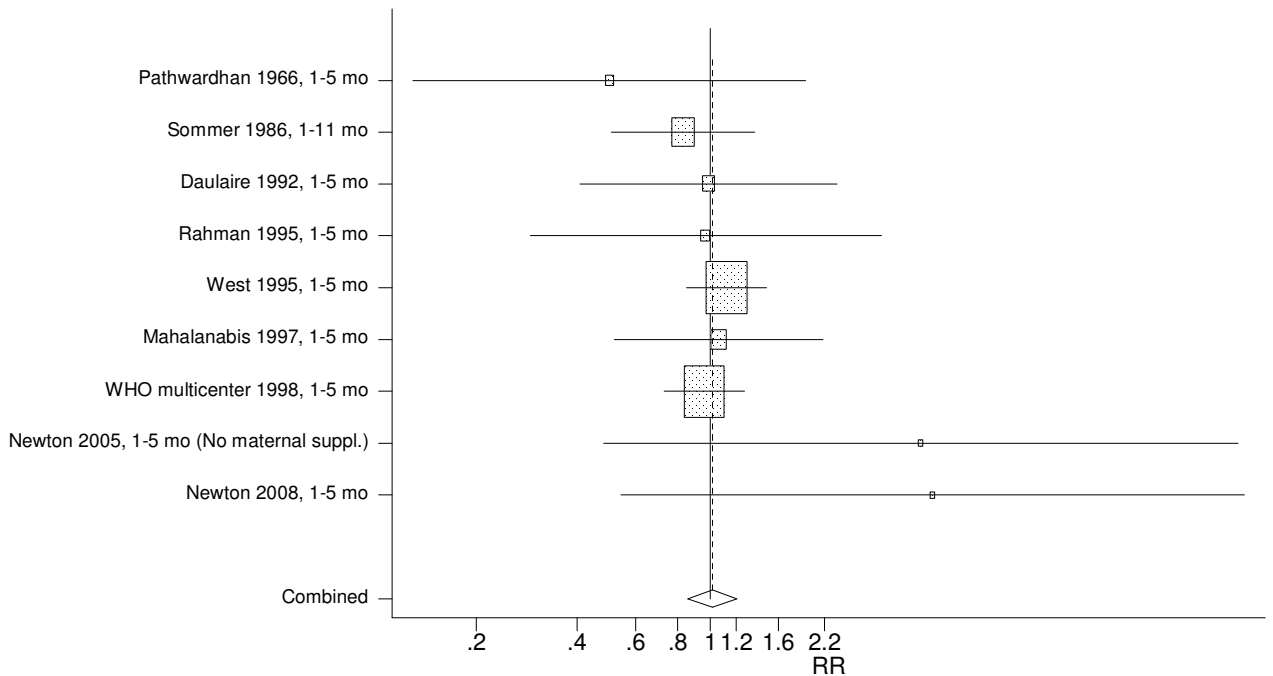
Notes: Only trials reporting more than 5 deaths have been included. In some studies it has not been possible to extract information on the age groups 0 months, 1-5 months and above 6 months; such studies have been presented in the predominant age group. A few small studies with broad age groups and little information on the effect within age groups have been left out (105, 107)

**A. 0-month-old children**



Test for homogeneity:  $p=0.02$ . Combined random effects meta-analysis relative risk=0.95 (0.80-1.14)  
References (in order of appearance): 24, 55, 56, 102, VII, 103, IX

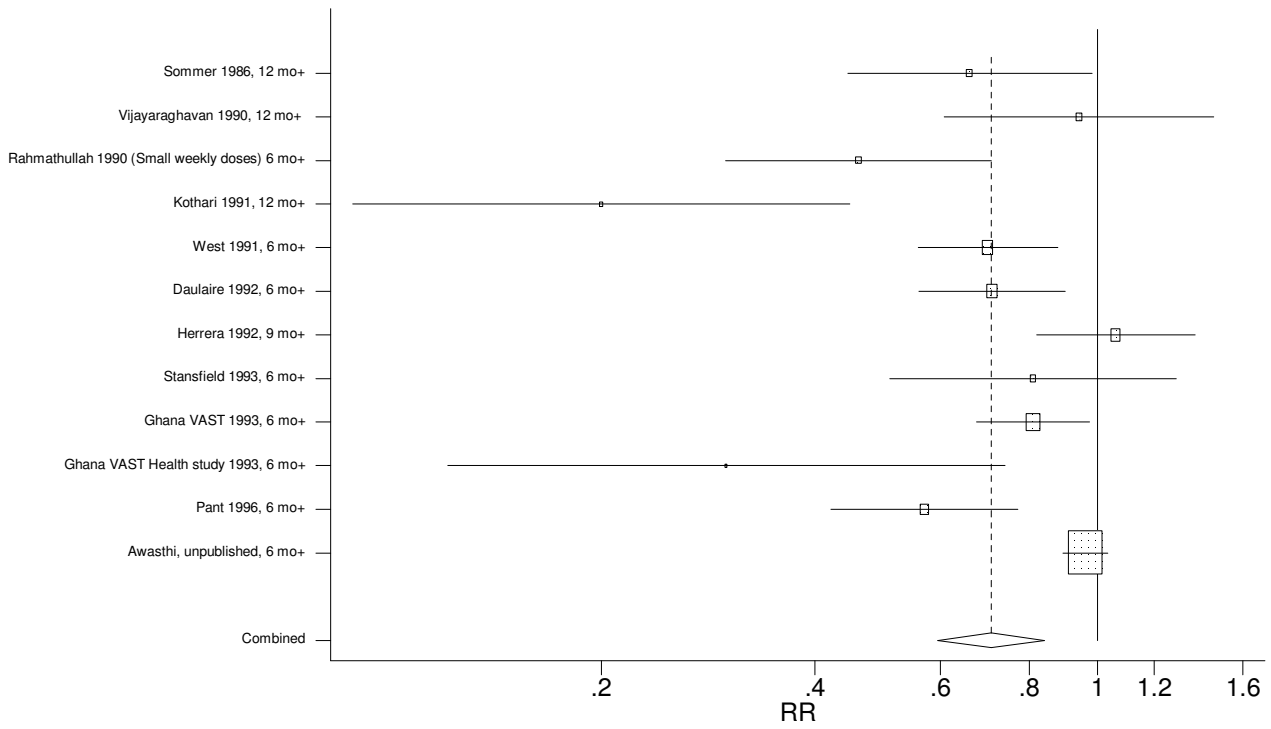
**B. 1-5-months-old children**



Test for homogeneity:  $p=0.65$ . Combined fixed effects meta-analysis relative risk=1.02 (0.86-1.20)  
References (in order of appearance): 23, 10, 14, 25, 24, 28, 26, 27, 29



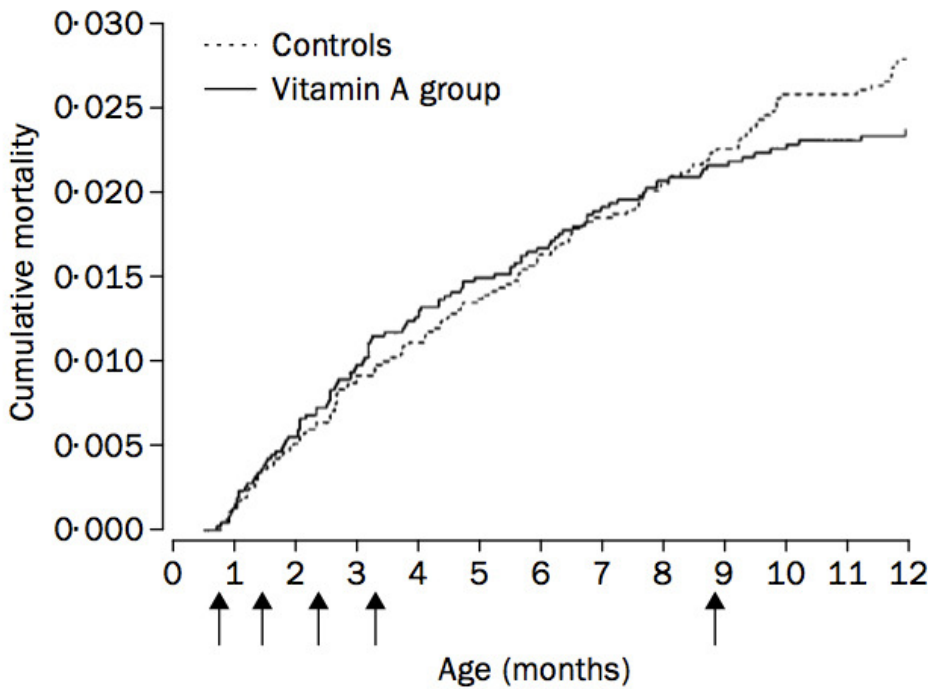
C. Children above 6 months of age



Test for homogeneity:  $p < 0.0001$ . Combined random effects meta-analysis relative risk=0.71 (0.60-0.84)

References (in order of appearance): 10, 12, 17, 18, 13, 14, 15, 104, 16, 16, 106, 22

Figure 4. WHO multicentre study curves for vitamin A and controls. Vitamin A recipients received three times 25,000 IU vitamin A with each of the three DTP vaccines, and 25,000 IU vitamin A with measles vaccine. Controls received 100,000 IU vitamin A with measles vaccine



#### VITAMIN A SUPPLEMENTATION WITH MISSING VACCINES

Guinea-Bissau has frequent national immunization days. In 2003, vitamin A was provided together with missing vaccines to all children above 6 months of age, who came to the health posts. We registered all participating children along with their treatment. This provided an opportunity to test the hypothesis that vitamin A supplementation would be beneficial when given with the live measles vaccine compared with when given with inactivated DTP vaccine. This proved the case (VI). The effect of vitamin A supplementation differed significantly depending on the type of vaccine with which it was given. Furthermore, receiving vitamin A with DTP compared with only vitamin A was associated with significantly increased mortality. Also, the mortality among children, who received vitamin A with DTP, was higher than among non-participants, though non-participants in such campaigns normally have higher mortality than participants (VI).

#### Discussion

Numbers were admittedly small and it was an observational study. In particular we cannot exclude that children who were missing DTP vaccines had a higher risk of dying *a priori* compared with children, who were missing measles vaccine, or who did not miss any vaccines. However, control for a number of background factors did not change the conclusions. The results provided support for our main hypothesis. Combining vitamin A with measles vaccine seemed more beneficial than combining vitamin A with DTP. In this study there was no sex-differences, the combination of vitamin A and DTP seemed equally bad for boys and girls. No other group has studied this hypothesis.

#### VITAMIN A SUPPLEMENTATION WITH BCG AT BIRTH

We conducted two trials of neonatal vitamin A supplementation in Guinea-Bissau. The trials were based on the fact that the two first trials designed to test the effect of neonatal vitamin A supplementation on mortality, both conducted in Asia, had found significantly beneficial effects on mortality (55, 56). The Indonesian study reported 64% reduction in mortality (55), the Indian study 22% reduction (56). In both trials the effect of vitamin A supplementation was only seen during the first 3-4 months. None of the trials provided information on whether vitamin A was given with vaccines. Intriguingly, the Indonesian trial found the most beneficial effect in normal-birth-weight infants ( $\geq 2500$  g) whereas the opposite was the case in India. The data did not make sense from the *Prevention-of-deficiency* hypothesis perspective, since low-birth-weight infants are more vitamin A-deficient (114). However, according to our hypothesis vitamin A supplementation would potentiate the beneficial non-specific effects of BCG vaccine (IV). While the Indonesian trial was carried out, it was policy to postpone BCG vaccination in low-birth-weight infants – a policy which is implemented in many low-income countries (IV). In India, there was no special policy for low-birth-weight infants (IV). Hence, the data fitted well with our hypothesis; the most beneficial effect was seen among normal-birth-weight Indonesian children, who presumably received vitamin A supplementation with BCG vaccine (IV).

Based on this information, we initiated two neonatal vitamin A trials; a trial in normal-birth-weight infants who came for their BCG vaccine after the delivery, and a 2-by-2 factorial trial with vitamin A supplementation and early BCG vaccination to low-birth-weight neonates who would normally not receive BCG at birth. All children were randomised to vitamin A (50,000 IU to normal-birth-weight infants and 25,000 IU to low-birth-weight

infants) or placebo, and were followed to 12 months of age. Our main hypothesis was that vitamin A supplementation would reduce overall mortality within the first year of life by 25-30%.

When we wrote the protocols, we did not formulate any hypotheses regarding sex-differential effects. However, during the conduct of the trials we became increasingly aware that vitamin A supplementation may have sex-differential effects. The previous two trials had both observed tendencies for a better effect of neonatal vitamin A in boys (55, 56). Furthermore, we had observed that boys responded better to vitamin A in terms of measles-specific antibody titres (II) and we subsequently found that girls had lower mortality after receiving a low than a high dose (V). Hence, before the trials ended we had formulated another hypothesis – that the effect of neonatal vitamin A supplementation would be particularly beneficial for boys (XI).

#### Overall mortality

Unexpectedly, we found no effect of neonatal vitamin A supplementation on overall mortality. However, both trials showed remarkably similar results when stratified by sex; boys tended to have a beneficial effect of vitamin A throughout the first year of life, whereas girls had a negative effect, particularly after the first months of life (VII, IX). In a combined analysis of the two trials, the interaction between vitamin A and sex was statistically significant, as was the negative effect of vitamin A in girls (IX).

We speculated that vitamin A given with BCG at birth had interacted negatively with subsequent DTP vaccine in girls. A *post hoc* analysis of the normal-birth-weight trial revealed that, indeed, there was a tendency for a beneficial effect of neonatal vitamin A supplementation as long as BCG vaccine was the last vaccine to be received, but once DTP vaccine was received there was a significantly negative effect of having received neonatal vitamin A supplementation in girls (VI). Though much less pronounced, the same tendency was seen in the low-birth-weight trial. In a combined analysis of the two trials, vitamin A was associated with 75 % (9-183%) increased mortality, when DTP was the most recent vaccine (Table 2).

**Table 2. The effect of neonatal vitamin A supplementation in children who had DTP as their most recent vaccine**

	Overall	Boys	Girls	P for interaction vitamin A and sex
Guinea-Bissau (VII)	1.43	0.90	2.19	0.08
Normal-birth-weight	(0.88-2.32)	(0.44-1.82)	(1.09-4.38)	
Guinea-Bissau (IX)	1.09	0.66	1.44	0.16
Low-birth-weight	(0.65-1.82)	(0.27-1.59)	(0.75-2.78)	
Combined analysis	1.26	0.80	1.75	0.04
	(0.88-1.79)	(0.46-1.39)	(1.09-2.83)	

It should be noted that the low-birth-weight trial was a 2-by-2 factorial trial with vitamin A and early BCG vaccine. For funding reasons, the trial was not sized to look for statistical interactions between the two treatments, but only to look at vitamin A effects in the combined population. We found no evidence of interaction between BCG and vitamin A, and hence the two groups were combined (IX). This lack of interaction did not support our hypothesis that the combination of vitamin A and the live BCG vaccine would be particularly beneficial. This could perhaps be partly due to the fact that many of the children randomised to no early BCG received BCG within the next weeks at the health centres.

With regard to causes of death, the negative effect of vitamin A supplementation in girls was seen for all causes of death, but may have been particularly evident for diarrhoea among normal-birth-weight girls (VII) whereas it was most prominent for fever, septicaemia, and malaria, as well as malnutrition among low-birth-weight girls (IX).

#### *Morbidity*

Within the normal-birth-weight trial, we conducted a subgroup study of the effect on rotavirus infection and diarrhoea of vitamin A supplementation with BCG at birth. Receiving vitamin A at birth was associated with increased risk of rotavirus infection and rotavirus diarrhoea below 6 months of age in both sexes, (68). At the same time vitamin A at birth was associated with decreased risk of non-rotavirus diarrhoea in boys below 6 months of age, but an increased risk in girls 6 months or older (68). Unexpectedly we also experienced a measles epidemic, which provided an opportunity to study the effect on measles incidence. Significant sex-differential effects, with a tendency for a negative effect of vitamin A in girls, were also seen for measles infection (115). The two findings could not be explained by a chance allocation of a group of “weak” girls and/or “strong” boys to vitamin A supplementation, since they were independent of each other. Hence, the morbidity findings supported the existence of sex-differential effects of vitamin A supplementation at birth. Furthermore, the diarrhoea subgroup study was conducted in 1-8 months children who almost exclusively had DTP vaccine as their last vaccine during the study period (68), and the negative effect of vitamin A supplementation on measles infection in girls was seen among girls who had received DTP vaccine (unpublished data). Hence, the findings were compatible with the hypothesis that the negative effect in girls could be due to negative interaction between vitamin A supplementation and subsequent DTP vaccine in girls.

#### *Vitamin A status*

As a part of the normal-birth-weight trial, we also studied the effect of vitamin A supplementation on vitamin A status at 6 weeks of age and 4 months of age. Overall, vitamin A status improved during this period. However, there was a significant inverse relationship between increase in vitamin A status and number of DTP vaccines received in girls, which was particularly evident among vitamin A recipients (61). The finding supported the possibility of a negative interaction between vitamin A supplementation and subsequent DTP vaccinations in girls.

#### *Discussion*

While we conducted our trials, two other trials of neonatal vitamin A supplementation had been initiated. One of the trials was conducted in Zimbabwe as a two-by-two factorial trial, randomising mothers and their newborn infants to vitamin A or placebo. Children were followed to 12 months of life. The trial was reported separately for HIV-negative (101) and HIV-negative mothers (102). Among HIV-negative mothers there was no overall effect of neonatal vitamin A supplementation on mortality, but the survival curves indicated that the placebo-placebo group had higher mortality in the first months of life, but later the mortality was higher among vitamin A recipients (101, **Figure 5**). Unfortunately data were not reported by sex, and our request for this information has not been met yet (XI). Among HIV-positive mothers, neonatal vitamin A supplementation had divergent effects depending on whether the child did or did not become HIV-positive. Among the large majority of children, who remain-

ed HIV-negative at 6 weeks of age, vitamin A supplementation was associated with significantly negative effects on mortality (102). The other trial was conducted in Bangladesh (103). It showed that neonatal vitamin A supplementation was associated with a significant decrease in overall mortality within the first 6 months of life in boys as well as girls. In addition to these two trials, which were designed specifically to test the effect of neonatal vitamin A supplementation, a trial from Nepal among 0-6 month-old infants also included a group of children below 1 month of age, i.e. neonates. In this subgroup, vitamin A was not associated with beneficial effects (24). Hence, a total of seven trials of neonatal vitamin A supplementation have now been published. Three of four trials from South Asia showed beneficial effects on mortality of neonatal vitamin A supplementation (55, 56, 103). Three trials from Africa found no overall beneficial effect, all estimates going in the other direction (VII, IX, 101, 102). The trials are summarised in **Table 3**.

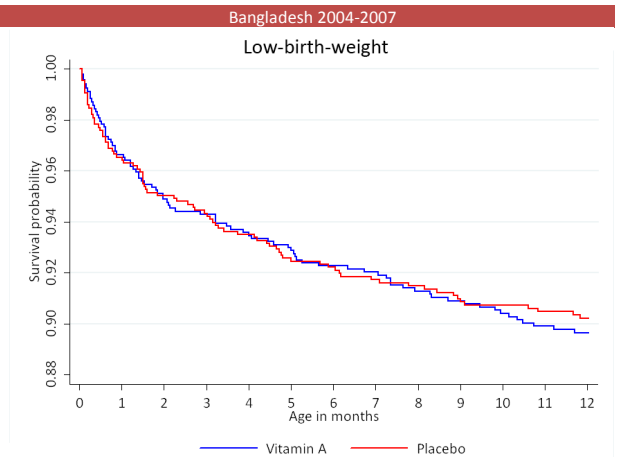
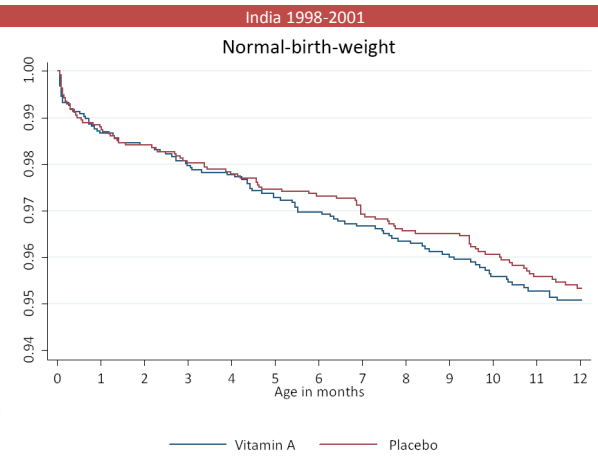
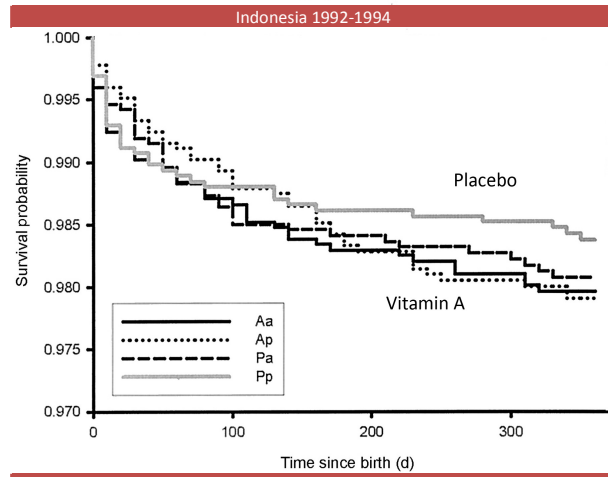
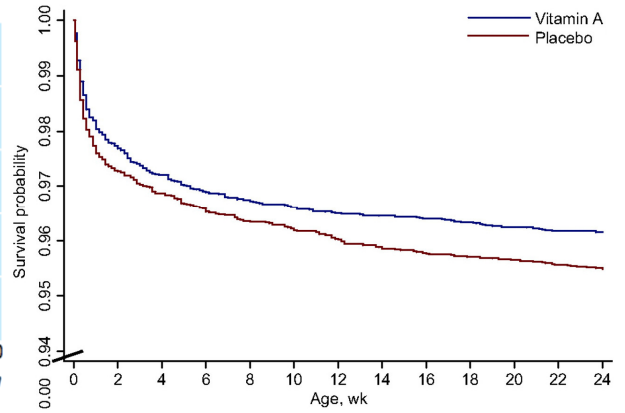
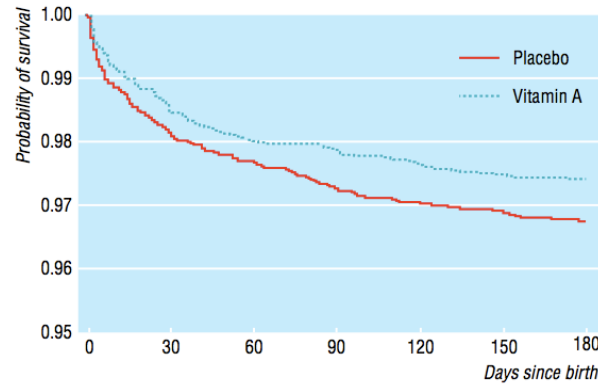
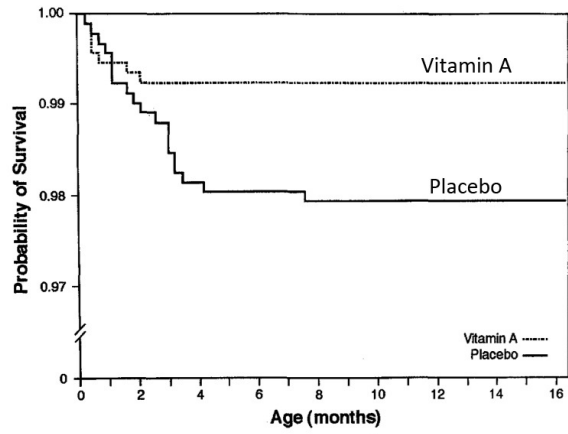
Differences in vitamin A status do not seem to explain the divergent results. It is not easy to assess the vitamin A status since the trials have provided very different measurements of vitamin A deficiency (**Table 3**). The Indian and Bangladeshi trials probably had the highest degree of vitamin A deficiency, and found a good effect, whereas none of the women in the African trials suffered from night blindness. However, it should also be noted that the Indonesian trial had a good vitamin A status for the mothers and a very good effect of vitamin A supplementation. Also, the 4,495 HIV-positive women in the Zimbabwe trial presumably suffered from vitamin A deficiency, and vitamin A had no beneficial effect in that group; it even had a significantly negative effect in the large majority of the children who remained HIV-negative (**Table 3**). The mortality level, which is considered a marker of vitamin A deficiency (1), is easier to assess than vitamin A status (**Table 3**). A plot of the effect of neonatal vitamin A supplementation as a function of baseline mortality in the placebo groups reveals no association (**Figure 6**), and supports that differences in vitamin A status are not the only explanation for the divergent results.

Divergent results would be expected if vitamin A had a particularly good (or bad) effect on certain pathogens and there were regional differences in the prevalence of such pathogens. The relative risk estimates of vitamin A versus placebo for specific causes of death varied considerably in the trials, e.g. for diarrhoea deaths they varied from 0.4 in Indonesia (55) to 2.4 in Zimbabwe (101). Hence, there was no indication that the effect of vitamin A was limited to certain disease categories, though it does not exclude that vitamin A could have differential effect on different pathogens (See also Chapter 6).

There was considerable variation in the causes of death over the trials, for instance almost two thirds of the deaths were ascribed to pneumonia in the Zimbabwe trial (101, 102) compared with less than a quarter in Guinea-Bissau (VII, IX), and septicaemia was by far the major cause of death in Indonesia (55) whereas it was a less common cause of death in India and Zimbabwe (101, 102). These differences are most likely to reflect major variation in the way that causes of deaths are assigned (by verbal autopsy) and they do not correlate with vitamin A effects. Hence, variation in causes of death over sites does not seem to explain the divergent results.

We have proposed that the divergent results may be explained by differences in vaccination intensity (VIII, 63, 65).

Figure 5. Survival curves from neonatal vitamin A supplementation trials



Zimbabwe 1997-2001  
References (in order of appearance): 55, 56, 103, 101, VII, IX

Guinea-Bissau 2002-2008

In our normal-birth-weight trial from Guinea-Bissau, all children received BCG at the same time as VAS or placebo (VII). Having received VAS tended to be beneficial as long as BCG was the last vaccine to be received. However, once children received DTP vaccine, mortality in girls who had received VAS at birth was significantly 2-fold higher compared with girls who had received placebo at birth (VIII). Though vaccination coverage was lower and the schedule more varied, and though we collected vaccination data with larger intervals among low-birth-weight infants, the negative effect of vitamin A was also most evident among older girls, who had received DTP in the low-birth-weight trial (**Table 2**). Hence, in our experience neonatal vitamin A supplementation could have a beneficial effect as long as BCG is the last vaccine, but may have a negative effect for girls once they receive DTP.

Such vitamin A-vaccine interactions could help explain the variation in trial results. Vaccination intensity was high in Guinea-Bissau (**Table 3**) and probably also in Zimbabwe as judged by the national coverage data (116). If there is negative interaction between vitamin A and DTP and the coverage for DTP is high, then one would expect the survival curves of vitamin A and placebo recipients to cross once the children start receiving DTP around two months of age. This pattern is seen both in Guinea-Bissau and among the children of HIV-negative mothers in Zimbabwe (**Figure 5**). The Zimbabwe trial did not provide survival curves for children of HIV-positive mothers, but we calculated that the relative risk of mortality was 1.24 (0.63-2.43) from enrolment to 6 weeks of age, but 1.41 (0.89-2.23) from 6 weeks to 12 months of age (based on Table 3 in the paper, maternal placebo groups only).

Vaccine coverage was low, and vaccines were given with great delays in the trials from India and Bangladesh (**Table 3**). The trial from Indonesia was conducted 15 years ago, when vaccination coverage may have been lower. Hence, one would not expect to see the same negative interaction in these trials. Importantly, as seen from the curves from Bangladesh and India (**Figure 5**), the trials only followed children to 6 months of age, so a negative interaction between vitamin A and DTP vaccine would have had little time to become apparent. Furthermore, for reasons we do not know, all the Asia studies were characterised by a high neonatal mortality, but low mortality in the subsequent months, during which a negative interaction between vitamin A at birth and DTP vaccine would matter. Hence, the existing data are compatible with the hypothesis that neonatal vitamin A may interact negatively with subsequent DTP vaccine in girls.

#### A REANALYSIS OF ONE OF THE ORIGINAL VITAMIN A SUPPLEMENTATION TRIALS

The latest opportunity to test our hypothesis came when we were allowed to reanalyse data from one of the original vitamin A trials. The trial, conducted in rural Ghana from 1989 to 1991 (16), enrolled 6-90-month-old children, and randomised them to vitamin A or placebo every 4 months for a period of 2 years. In the beginning of the trial, vaccination status was assessed. The trial was undertaken in the period, when coverage with routine vaccinations was low, and many children had no vaccination card. The original trial had not analysed data by vaccination status; it reported a 19% significant mortality reduction after vitamin A supplementation (16). However, when we reanalysed the data, we found that vitamin A supplementation only had a beneficial effect in children without a vaccination card. The mortality reduction

was 36% (12-53%) in these children, but only 5% (-26%-28%) in children with a health card (X). This differential effect was particularly pronounced in girls. Among children with a card the effect of vitamin A supplementation differed significantly in boys and girls ( $p=0.046$ ). This was entirely due to a negative effect of vitamin A supplementation in girls who had received 0 to 2 doses of DTP at enrolment and were likely to receive DTP during follow-up (2.60 (1.41-4.80)) (X).

#### Discussion

The reanalysis supports the hypothesis that vitamin A supplementation interacts with vaccines, and that the effect differs between the two sexes, in particular that the combination of vitamin A supplementation and subsequent DTP vaccine may have negative effects in girls. This finding corroborates the findings from our neonatal vitamin A trials. It could be speculated that the observed negative effect of vitamin A supplementation among girls in the neonatal vitamin A trials was due to a negative effect of neonatal vitamin A in infant girls *per se*, rather than a negative interaction between vitamin A and DTP. However, the reanalysis encompassed much older children, and the finding of a negative interaction between vitamin A and DTP vaccine also in older girls supports that this is a true interaction, and not just a result of an unknown age and sex-differential effect of vitamin A. None of the other original vitamin A trials reported data by vaccination status, but it should be noted that they were all carried out during the roll-out of the vaccination programme, and they may likewise have had differential effects in vaccinated and unvaccinated children.

#### DISCUSSION

The initial formulation of the *Vitamin A-vaccine-interaction* hypothesis led to a series of studies of different designs. We conducted an observational study during a vitamin A campaign (VI), a randomised trial testing the effect of two different doses of vitamin A during another campaign (V), two randomised trials of vitamin A given with BCG at birth (VII, IX), and we reanalysed data from an old randomised trial of vitamin A from the perspective of vaccination status (X).

The results do not support the existing interpretation that vitamin A acts by preventing vitamin A deficiency, since a smaller dose seemed more beneficial than a larger dose in girls and the effect of vitamin A supplementation was harmful in certain subgroups of participants.

The results, on the other hand, supported the hypothesis that vitamin A and vaccines interact. *First*, the effect of vitamin A given with DTP was significantly different from the effect of vitamin A given with measles vaccine, and children who received vitamin A with DTP had higher mortality than children, who had received vitamin A alone or who did not receive anything. *Second*, neonatal vitamin A interacted negatively with subsequent DTP vaccines in girls. *Third*, the effect of vitamin A to older children depended on vaccination status, being beneficial in boys, but harmful in girls who received DTP during follow-up.

Each of our studies has its weaknesses and sources of bias. However, it is quite remarkable that though they did not always produce the expected result, e.g. we did not find a beneficial effect of vitamin A given with BCG vaccine, they provided support for the hypothesis that vitamin A and vaccines interact, e.g. vitamin A given with BCG vaccine interacted negatively with subsequent DTP vaccine.

**Table 3. Overview of the neonatal vitamin A supplementation (VAS) trials**

	Participants	Maternal supplement	Age at follow-up	Mortality rate /1000 pyrs in control group	% deaths during the first mo in control group	Level of vitamin A deficiency	Vaccine coverage information	Effect of VAS on mortality ALL	Effect of VAS on mortality BOYS	Effect of VAS on mortality GIRLS
<b>Asian trials</b>										
Nepal (24)	1,621	No	4 mo	132	N/A	No information	No information	1.07 (0.66-1.72)	N/A	N/A
Indonesia (55)	2,067	No	12 mo	20 (12 mo)	21 (1 mo) <sup>1</sup> 48 (2 mo) <sup>2</sup>	MMSR: 1.77 umol/L	No information	0.36 (0.16-0.87)	0.15 (0.03-0.68)	0.84 (0.26-2.77)
India (56)	11,619	No	6 mo	69 <sup>3</sup> (6 mo)	61 (1 mo) <sup>2</sup> 70 (2 mo) <sup>2</sup>	5-6% NB in pregnancy	BCG: 59% DTP1: 66% DTP3: 17% (6 mo)	0.78 (0.63-0.97)	0.70 (0.52-0.94)	0.87 (0.65-1.17)
Bangladesh (103)	15,937	Yes (3*2 factorial)	5½ mo	45 (5½ mo) <sup>3</sup>	69 (1 mo) <sup>1</sup> 82 (2 mo) <sup>2</sup>	MMSR in first trimester: 1.15 umol/L. 10% NB in last pregnancy	BCG: 72% DTP1: 65% (5½ mo) <sup>4</sup>	0.87 (0.67-1.12) <sup>5</sup>	0.89 (0.72-1.10)	0.81 (0.65-1.00)
<b>African trials</b>										
Zimbabwe HIV negative mothers (101)	9,208	Yes (2*2 factorial)	12 mo	17 (12 mo)	53 (1 mo) <sup>2</sup> 65 (2 mo) <sup>2</sup>	MSR: 6%<1.05 umol/L (6 wks) No NB	No information	1.18 (0.76-1.83) <sup>5</sup>	N/A	N/A
Zimbabwe HIV positive mothers (102)	4,495	Yes (2*2 factorial)	24 mo	155 (2 yrs) (all children)	28 (6 wks) <sup>6</sup>	MMSR: ~1.00 umol/L after delivery, No NB		1.21 (0.99-1.46) <sup>5</sup> HIV positive: 0.88 (0.58-1.32) <sup>5</sup> HIV negative: 1.89 (1.05-3.40) <sup>5</sup> 1.16 (0.98-1.38) <sup>5</sup>	N/A	N/A
Zimbabwe combined (102)	14,110	Yes (2*2 factorial)	N/A	N/A	N/A	No NB			N/A	N/A
Guinea-Bissau, Normal-birth-weight (VII)	4,345	No	12 mo	47 (12 mo) 53 (6 mo)	23 (1 mo) 30 (2 mo)	No NB MMSR at 4 mo: 1.66 umol/L Mean child SR: 0.95 umol/L (6 wks) 1.07 umol/L (4 mo)	BCG: 100% DTP1: 98% DTP3: 85% (6 mo)	1.07 (0.79-1.44)	0.84 (0.55-1.27)	1.39 (0.90-2.14)
Guinea-Bissau, Low-birth-weight (IX)	1,717	No (but 2*2 factorial with BCG vaccine)	12 mo	102 (12 mo)	31 (1 mo) 48 (2 mo)	No NB	BCG: 85% DTP1: 83% DTP3: 55% (6 mo)	1.08 (0.79-1.47)	0.74 (0.45-1.22)	1.42 (0.94-2.15)

MSR= Maternal serum retinol. MMSR= Mean maternal serum retinol. NB=night blindness

<sup>1</sup> Presented in paper

<sup>2</sup> Extrapolated from mortality curves in papers

<sup>3</sup> Note, mortality per 1000 live births

<sup>4</sup> Estimates presented in the paper without indication of when collected - presumably at age 5½ months.

<sup>5</sup> Effect in maternal placebo groups

<sup>6</sup> Calculated from table 3 in paper

Besides the papers presented above, we have recently conducted two trials in which vitamin A supplementation came to play an unexpected role. In a trial testing the effect of providing BCG revaccination, children were randomised to BCG revaccination or no BCG revaccination at age 19 months. An unexpected cluster of deaths occurred in the BCG revaccination group in connection with a vitamin A supplementation campaign. In a *post hoc* analysis, the BCG versus no BCG hazard ratio was 4.14 (1.17-14.7) among children who received vitamin A, but 1.29 (0.29-5.75) among children, who had not received vitamin A ( $p=0.042$  for interaction between vitamin A and BCG revaccination) (67). Very recently, we have analysed data from a two-dose measles vaccination trial. Children who had received all three doses of DTP were invited to participate. They were randomised to receive early measles vaccine or no early measles vaccine at 4.5 months of age. All children were given the usual measles vaccine at 9 months of age. Overall, early measles vaccine was associated with decreased mortality. Some of the children had taken part in neonatal vitamin A trials. In a *post hoc* analysis we found evidence for interaction between neonatal vitamin A and early measles vaccine ( $p=0.01$ ). The beneficial effect of early measles vaccine was only seen in children, who had not received neonatal vitamin A; neonatal vitamin A seemed to negate the beneficial effect of the vaccine (117). These findings of negative interactions between vitamin A and BCG revaccination and early measles vaccine, respectively, were clearly unexpected; we were surprised that the interventions seemed to interact when given so many months apart – and if anything we would then have expected the interactions between vitamin A and the live vaccines to be beneficial. Nonetheless, the findings support that vitamin A is an important immuno-modulator, which interacts with vaccines.

The possible interactions between different health interventions are virtually never explored. Only one other group has studied the interaction between vitamin A and vaccines. In the Indian neonatal vitamin A trial, vaccine information was collected every 14 days, and the authors published a separate paper on the potential non-specific effects of vaccines (118). In that paper, the authors censored the first week after enrolment and vitamin A supplementation, and followed the children from 1 week after enrolment to 6 months of age. They found a negative effect of receiving BCG and DTP vaccine among girls in the placebo group. However, they also reported that this negative effect was mitigated by vitamin A (118). Curiously, there were 40 versus 48 deaths in the vitamin A and the placebo group in males, corresponding to the overall estimate for vitamin A versus placebo in males of 0.70 presented in the main paper, but there were 56 versus 54 deaths in females – not corresponding to the overall estimate of 0.87 in the main paper. Hence, the censoring of the first week after enrolment censored fewer deaths among female vitamin A recipients than among female placebo recipients. In other words, it seems as if the observed tendency for a beneficial effect of neonatal vitamin A in females in the Indian trial was entirely due to a beneficial during the first week of life. The estimates for vitamin A versus placebo by vaccination status cannot be deducted from the paper – but it may look as if the combination of vitamin A and DTP vaccine was worse for girls (HR= 0.57 for vitamin A plus DTP compared with vitamin A in unvaccinated children) than placebo and DTP vaccine (HR=0.11 for placebo plus DTP compared with placebo in unvaccinated children), whereas the opposite was the case for boys (HR=0.00 vs. 0.90) (118). This would corroborate our hypothesis of vitamin A amplifying the non-specific negative effect of DTP vaccine in girls. Based on the

way the data was reported, no conclusions can be made. We have asked the authors whether they would be willing to provide vitamin A-placebo estimates stratified by vaccination status in both sexes to further explore the compatibility of their findings with our hypothesis, but unfortunately received no answer.

Even if most data seem compatible with the *Vitamin A-vaccine-interaction* hypothesis, some riddles remain to be solved. The findings of a sex-differential effect of the interactions have not been consistent. Also, the timing of vitamin A supplementation and vaccines is unclear. The campaign study was the only study, in which different vaccines were given at the same time as vitamin A (VI). In the neonatal vitamin A trials, vitamin A seemed to interact with DTP given several months later (VIII). In the Ghana reanalysis, it was also vaccines given during follow-up, rather than status at inclusion, which determined the response (X). Lastly, the two-dose measles trial showed that neonatal vitamin A may interact with a vaccine given 4.5 months after. It would seem important to study the window during which vitamin A and vaccines may interact. In particular, we wonder whether neonatal vitamin A may have priming effects on the immune system that may have long-lasting consequences.

So far, our data on the vitamin A-vaccine interactions on mortality are strongest for a negative interaction between vitamin A and DTP (VI-X). There is less evidence for a positive interaction between vitamin A and measles vaccine (VI). The final proof of vitamin A-vaccine interactions having both beneficial and detrimental effects will have to come from randomised trials. Since it is unethical to withhold recommended vaccines, such a trial would have to be designed in special ways, for instance testing the effect of vitamin A or placebo with DTP versus the effect of vitamin A or placebo with measles vaccine.

## 5. SEX-DIFFERENTIAL EFFECTS OF VITAMIN A

Since the first finding of a sex-differential effect of vitamin A supplementation on the immune response to measles vaccine, stratification by sex has been compulsory in our analyses of vitamin A effects.

Our studies have produced the following observations of sex-differential effects:

1. The effect of vitamin A given with measles vaccine at age 9 months on the antibody response to measles vaccine was most pronounced and only significant in boys. The effect was significantly different in boys and girls ( $p=0.009$ ) (II).
2. Vitamin A supplementation with BCG vaccine temporarily diminished the proportion of boys, who had a positive PPD reaction at 2 months of age. The effect was significantly different in boys and girls ( $p=0.048$ ) (62).
3. A low dose of vitamin A compared with the recommended dose of vitamin A was associated with significantly reduced mortality in girls, but not in boys. The effect was significantly different in boys and girls ( $p=0.004$ ) (V).
4. The effect on mortality of vitamin A given with BCG vaccine to normal-birth-weight infants tended to be beneficial in boys, but not in girls. The same was seen among low-birth-weight infants. In a combined analysis, there was statistically significant interaction ( $p=0.01$ ) between vitamin A and sex,

and the effect of vitamin A was significantly negative in girls (Hazard ratio=1.41 (1.04 to 1.90)) (VII, IX).

5. Vitamin A given with BCG to normal-birth-weight infants was associated with significantly higher mortality in girls after subsequent DTP vaccination, but not in boys. The effect was borderline significantly different in boys and girls ( $p=0.05$ ). The same tendency was seen in low-birth-weight infants. In a combined analysis, vitamin A at birth was associated with 75 % (9-183%) increased mortality among girls who subsequently received DTP vaccine, significantly different from the effect in boys ( $p=0.04$ ) (IX)
6. The effect of vitamin A given with BCG vaccine on non-rotavirus diarrhoea was beneficial in boys, but not in girls, and differed significantly between boys and girls ( $p=0.03$ ) (68).
7. The effect of vitamin A given with BCG vaccine on the incidence of measles infection was beneficial in boys, but not in girls, and differed significantly between boys and girls ( $p=0.03$ ) (115).
8. There was a significant inverse relationship between increase in vitamin A status and number of DTP vaccines received in girls, which was particularly evident among vitamin A recipients ( $p=0.01$  for interaction between sex and DTP and vitamin A and DTP) (61).
9. The effect of vitamin A supplementation in Ghana differed between boys and girls who had a health card ( $p=0.046$ ) (X).
10. The effect of vitamin A supplementation in Ghana differed between boys and girls who had a health card and had measles vaccine as their last vaccine ( $p=0.009$ ). This was particularly pronounced among children, who had received 0-2 doses of DTP and were likely to receive more doses of DTP during follow-up ( $p=0.0004$ ) (X).

However, it should be noted that not all studies found sex-differential effects:

11. The negative effect of receiving vitamin A with DTP during a campaign compared with receiving vitamin A plus measles vaccine was seen in both boys and girls (VI).
12. The negative effect of vitamin A given with BCG vaccine on rotavirus infection and rotavirus diarrhoea was seen in both boys and girls (68).

Nonetheless, it should be quite clear that we found more sex-differences in the vitamin A response than could be expected by chance.

**Table 4** summarises all trials that have reported the effect on mortality of vitamin A supplementation by sex. Surprisingly few trials have done that.

There is an overweight of trials, which have found a more beneficial effect in boys than in girls in the youngest age group (**Table 4**). Also, several studies have found sex-differences in the immune response after simultaneous administration of vitamin A and vaccines (II, 62, 97). Sex-differences in the mortality response to other micronutrient interventions have also been seen, in particular in the youngest age groups (66).

There are at least three possible explanations why such sex-differences should occur.

*First*, they may be a product of initial differences in micronutrient status. Boys have been observed to be born with lower levels of vitamin A and to be more iron-deficient during infancy (114, 119). They grow more rapidly and their requirements may be larger than girls' and as such they may benefit more from supplementation (fits the *Prevention-of-deficiency* hypothesis). *Second*, there may be underlying immunological differences between boys and girls, which determine a differential response to vitamin A (this would imply that vitamin A is an immuno-modulator). *Third*, the sex-differences may be the result of interaction with vaccines, as we have proposed. This third possibility can co-exist with the two other explanations. It builds on the observation that vaccines have non-specific sex-differential effects on mortality. The live vaccines BCG and measles vaccine have been shown to reduce overall mortality much more than can be ascribed to the prevention of tuberculosis and measles, respectively, and these effects are strongest for girls. On the other hand the inactivated DTP has been shown to increase mortality from other diseases in girls (70-75). We have proposed that the non-specific effects of vaccines are amplified by high doses of vitamin A (IV). Our studies provide support for the latter notion because most of the observations of a more beneficial effect in boys and a negative effect in girls have been done in connection with DTP exposure. Also, the mere finding of a negative effect indicates that other mechanisms than prevention of deficiency are in play – it might be possible that girls are less deficient and experience less beneficial effects of vitamin A supplementation, but it is difficult to imagine why a high dose of vitamin A should cause increased mortality many months after supplementation, unless it does more than merely prevent deficiency.

The potential biological mechanisms behind the observed sex-differences are discussed further in Chapter 6. Irrespective of the underlying mechanism, all future studies of vitamin A should be analysed stratified by sex. If such sex-differences exist, it could have profound implications for the vitamin A supplementation policy. The present results suggest that while neonatal vitamin A does not seem to benefit girls, it should perhaps be given to boys. The acknowledgement of the existence of sex-differences in the response to vitamin A would also encourage that all other health interventions be analysed by sex. To take it to its logical conclusion, we might have to treat boys and girls differently to treat them equally.

## 6. BIOLOGICAL MECHANISMS

Though the immunological effects of vitamin A supplementation are not the main theme of the present thesis, I have reviewed the potential biological mechanisms behind the interactions between vitamin A and the specific and non-specific vaccine effects in boys and girls. Since our current hypotheses are based on observations of the effect of vitamin A on T-cell subsets, and on underlying differences in the immune systems of boys and girls, I have summarised the effect of vitamin A and sex, respectively, on T-cell subsets. I have focused on the balance between T helper (Th) 1 and Th2 cytokines, and the balance between anti-inflammatory and pro-inflammatory cytokines, though it should be noted that this is very simplistic (120). The effect of vitamin A supplementation on different pathogens may also shed light on the differential effects of vitamin A when given with different vaccines; it has also been briefly reviewed.



**Table 4. Estimates of the effect of vitamin A supplementation on mortality by age group and sex\***

Author, country and year of publication	Overall effect	Boys	Girls	Comments
<b>At-birth supplementation</b>				
Humphrey, Indonesia 1996 (55)	0.36 (0.16-0.87)	<b>0.15 (0.03-0.68)</b>	<b>0.84 (0.26-2.77)</b>	Hospital deliveries. No vaccination information
Rahmatullah, India 2003 (56)	0.78 (0.63-0.96)	<b>0.70 (0.52-0.94)</b>	<b>0.87 (0.65-1.17)</b>	Low vaccination coverage
Benn, Guinea-Bissau 2008 (VII)	1.07 (0.79-1.44)	<b>0.84 (0.55-1.27)</b>	<b>1.39 (0.90-2.14)</b>	Negative effect of vitamin A in girls only seen after DTP (VIII)
Klemm, Bangladesh 2008 (103)	0.85 (0.73-1.00)	0.89 (0.72-1.10)	0.81 (0.65-1.00)	Low vaccination coverage
Benn, Guinea-Bissau 2010 (IX)	1.08 (0.79-1.47)	<b>0.74 (0.55-1.22)</b>	<b>1.42 (0.94-2.15)</b>	Combined analysis of the two studies from Guinea-Bissau: significant interaction between vitamin A and sex
<b>0-11 months</b>				
Pathwardhan, Jordan 1966 (23)	0.50 (0.13-1.94)	<b>0</b> <i>(one placebo death)</i>	<b>0.59 (0.15-2.30)</b>	1-5-month old. Pre-vaccination era
Sommer, Indonesia 1986 (10)	0.83 (0.51-1.37)	<b>0.59 (0.33-1.06)</b>	<b>1.06 (0.62-1.81)</b>	0-11-month old. No vaccination information
West, Nepal 1995 (24)	1.11 (0.86-1.42)	1.24 (0.86-1.78)	0.98 (0.68-1.42)	0-5-month old. No vaccination information
Mahalanabis, Bangladesh 1997 (28)	1.06 (0.52-2.18)	<b>0.70 (0.21-2.36)</b>	<b>1.40 (0.59-3.34)</b>	6-17 weeks. Vitamin A given with each of the three doses of DTP
<b>12 months+</b>				
Sommer, Indonesia, 1986 (10)	0.66 (0.44-0.97)	<b>0.59 (0.37-0.95)</b>	<b>0.80 (0.46-1.40)</b>	12-71-month old. No vaccination information
West, Nepal, 1991 (13)	0.70 (0.57-0.87)	0.77 (0.55-1.09)	0.65 (0.48-0.89)	6-60-month old (~90% of the children were between 12-60 months). No vaccination information
Daulaire, Nepal, 1992 (14)	0.74 (0.55-0.99)	<b>0.72 (0.48-1.08)</b>	<b>0.76 (0.48-1.19)</b>	1-59-month old (~85% of the children were between 12-59 months). No vaccination information
Herrera, Sudan, 1992 (15)	1.06 (0.82-1.37)	1.25 (0.85-1.83)	0.93 (0.66-1.31)	9-72-month old. No vaccination information
Ghana Vast Study Team, Ghana, 1993 (16)	0.81 (0.68-0.98)	<b>0.73 (0.59-0.92)</b>	<b>0.90 (0.71-1.15)</b>	6-90-month old. Subsequently reanalysed by vaccination status (X)

Note: All major studies that have addressed the effect of vitamin A supplementation by sex. Some of the studies of children aged 12 months and older have also included younger children, but apart from Dr. Sommer's study it is not possible to extract the sex-specific mortality ratios for separate age groups. All trials finding a better effect of vitamin A in boys than in girls are in bold.

#### VITAMIN A AND T-CELLS

In immunological studies, vitamin A deficiency has been associated with Th1 deviation, and vitamin A supplements have mostly been shown to enhance Th2 type responses (37, 76, 77). It was the background for studying the effect of vitamin A on the development of atopy; if vitamin A supplementation led to Th2-deviation when given with measles vaccine at 9 months of age, it could perhaps lead to increased incidence of atopy. In our small study we found no indication that vitamin A enhanced the risk of being skin prick-positive 6-7 years later, but a recent study actually reported that excessive intake of vitamin A modulated the Th1/Th2 development with a shift towards Th2, and increased the risk or severity of asthma in mice (121). However, vitamin A may not always induce Th2 deviation. One *in vitro* study suggested that the effect of vitamin A may depend on the environment; vitamin A enhanced Th1 responses under Th1 conditions, but enhanced Th2 responses under Th2 conditions (122). This could be interpreted as if vitamin A amplifies whatever is ongoing in the immune system. Very recently it has also been shown that vitamin A promotes the generation of immune-suppressive regulatory T cells, while they suppress the T cell differentiation into inflammatory Th17 cells (123). Hence, based on the current literature vitamin A seems mostly to induce Th2 and anti-inflammatory responses.

Most of the above work has been done in rodents and in adults, and results have not always been consistent. Furthermore, the results may be of limited relevance to infants, because the Th1/Th2 dichotomy may be less distinct in humans than in rodents (124), and because infants differ immunologically from adults (125). There are very few studies on the immunological effects of vitamin A status and vitamin A supplementation in children. In South Africa, among children with severe diarrhoea, there was no association between vitamin A status and neopterin concentrations (neopterin being a marker of Th1 activity) (126). In Indonesia, on the other hand, vitamin A deficiency was associated with increased neopterin concentrations, but significantly reduced *ex vivo* IFN- $\gamma$  production, indicating that vitamin A deficiency was associated with Th1 dominance in the steady state, but impairment of Th1 response after stimulation (127). In Venezuela, vitamin A deficient children had significantly diminished serum IL-10 levels (128). There was no measurable effect on cytokine levels of providing a single large dose of 200,000 IU vitamin A to preschool children (129). Within the normal-birth-weight neonatal vitamin A trial in Guinea-Bissau, we conducted an immunological subgroup study. Vitamin A status was positively correlated with *in vitro* IL-5 and IL 13 production to PPD in 6-week-old infants, supporting a positive association between vitamin A and Th2-responses. However, we found no effect of vitamin A supplementation on the Th1/Th2 balance, but vitamin A supplementation was associated with significantly decreased spontaneous TNF- $\alpha$  production supporting an anti-inflammatory effect of vitamin A supplementation (130).

#### SEX AND T-CELLS

There are indications that males may have a more pronounced Th1 profile than females, since they have stronger delayed type hypersensitivity (DTH) responses (62, 131). Conversely, females may have a stronger Th2 profile, as they seem to mount a higher antibody response than males (II, 132-133). To our knowledge, very few studies have addressed the Th1/Th2 balance in boys and girls. In a cohort of children at high risk of developing allergic disease, IFN- $\gamma$  responses to PHA were higher in boys than in

girls at 1 year and 3 years of age, but IL-5 responses were also higher at 3 years of age (134). There were no differences in PHA-induced IL-10 production between the sexes. We have found no sex-differences in the *in vitro* cytokine production within the placebo group in our trial, except for lower TNF- $\alpha$  response to LPS among girls (130).

A possible explanation for the sex-differences in the Th1/Th2 balance could be oestrogen levels. Very few studies have been done on oestrogen levels in prepubertal children, but they generally seem to report that girls have higher levels than boys (135-136). Oestrogen is positively associated with Th2-cytokine concentrations, whereas it seems to dampen Th1-cytokine production (137). Hence, the effects of oestrogen on the generation of Th2-derived cytokines in infant girls might inhibit secretion of Th1-derived cytokines and skew the immune response towards Th2-type responses compared with boys.

Another possible explanation could be differences in vitamin A levels; if boys were more vitamin A deficient and vitamin A deficiency was associated with Th1-deviation, it could be speculated that boys were more Th1-deviated for that reason. Indeed, there are indications that boys are born with lower vitamin A levels (114). However, in Guinea-Bissau boys were no more vitamin A-deficient than girls at 6 weeks of age, and at 4 months of age they had higher RBP levels than girls (61).

#### VITAMIN A AND PATHOGENS

There is accumulating evidence for interaction between vitamin A supplementation and specific infectious pathogens. Given the protective effects of vitamin A supplementation on mortality, and the observation that vitamin A deficiency increases morbidity from diarrhoea and respiratory infections, it was presumed that vitamin A supplementation would have beneficial effects on morbidity. However, the findings from the existing studies, almost all supplementing children above 6 months of age, are not consistent. The effects of vitamin A supplementation on the prevention of diarrhoea and respiratory infections have recently been summarised (77, 138-139). With regard to diarrhoea, a meta-analysis estimate yielded no overall effect of vitamin A on the incidence (1.00 (0.94-1.07)) (138). However, our study of diarrhoea (68), along with studies from Mexico (140-143), has indicated that the effect of vitamin A supplementation on diarrhoea is pathogen-specific. For instance, vitamin A reduced the prevalence of enteropathogenic *E. coli* (EPEC) infections, but increased the duration of *Giardia* infections in Mexico (140), and we found a negative effect of vitamin A on rotavirus infection in both sexes, but age and sex-specific effects of vitamin A on non-rotavirus diarrhoea, the effect being beneficial in the youngest boys (68). With regard to respiratory infections, two recent meta-analysis yielded estimates of vitamin A effects of 1.08 (1.05-1.11) (138) and 1.13 (0.88-1.43) (139). Hence, paradoxically vitamin A supplementation may increase the incidence of respiratory infections. Used as treatment against specific diseases, vitamin A has been beneficial in the treatment of measles infection (144), but potentially harmful in the treatment of respiratory infections (145). Hence, the data on the effect of vitamin A in the prevention and treatment of infectious diseases suggest that the effect of vitamin A may differ, depending on which pathogens the immune system encounters. Unfortunately, very few studies have presented data stratified by sex.

We speculated that the divergent effect of vitamin A supplementation on different disease categories could be the underlying

ing explanation for a strong interaction between vitamin A and season observed in our normal-birth-weight neonatal vitamin A trial (VII). However, we were not able to confirm such an interaction among low-birth-weight infants (IX). Nonetheless, we recommend that season should always be considered when evaluating vitamin A effects.

Different pathogens induce different immune responses and this may be the explanation for the divergent effects of vitamin A supplementation on disease incidence and outcome. For instance, compared with placebo recipients, 6-15-month-old Mexican children who received vitamin A supplementation every 2 months had reduced IL-4, IL-6 and IFN- $\gamma$  levels in faecal samples when infected with EPEC. In contrast, they had increased IL-4 levels when infected with *A. lumbricoides* (141).

The pathogen-differential effect of vitamin A on infectious disease incidence, disease outcome and cytokine production during disease, supports the notion that vitamin A effects on mortality may depend on vaccine type, and the effects can be beneficial, but also at times harmful.

#### VITAMIN A AND SPECIFIC EFFECTS OF VACCINES

As described in Chapter 3, there is some evidence for interaction between vitamin A supplementation and vaccines in terms of specific immune response to the vaccine. Vitamin A has been shown to enhance the antibody response to measles vaccine, oral polio vaccine and hepatitis B vaccine in some, but not in all studies. The increased antibody response seems biologically plausible based on vitamin A's stimulating effect on Th2 responses. Though very few studies have analysed the data stratified by sex, the effect of vitamin A supplementation might be most pronounced in boys, i.e. vitamin A may enhance the humoral immune response in terms of the antibody titres more in boys than in girls (II, 97). In contrast, vitamin A may decrease the cell-mediated delayed type hypersensitivity response in boys (62). These two observations could be the result of two factors. First, as described above boys seem to be more Th1 prone than girls; boys tend to have stronger cell-mediated responses than girls, but lower antibody titres. Second, as also described above, vitamin A supplementation may bias the immune response in a Th2 direction. If boys are more Th1 prone, they may benefit more from the Th2-deviating antibody-enhancing effects of vitamin A, but vitamin A supplementation could also lead to loss of the Th-1 driven stronger cell-mediated responses. Based on our studies, vitamin A supplementation made boys reach the same measles antibodies levels and the same prevalence of positive PPD responses as girls; in other words, vitamin A supplementation made boys respond more like girls. If vitamin A has anti-inflammatory capacities, this would be expected to lead to decreased humoral as well as cellular responses in both sexes, this, however, does not fit with the observations, perhaps because that anti-inflammatory effect would be less pronounced if the children were marginally vitamin A deficient.

#### VITAMIN A AND NON-SPECIFIC EFFECTS OF VACCINES

With regard to the interactions between vitamin A and the non-specific effects of vaccines, we clearly need to understand the biological mechanisms behind the non-specific effects on mortality of vaccines before we can come to fully understand how vitamin A may amplify these non-specific effects. Biologically there is no obvious immunological mechanism that can explain non-specific effects of vaccines. However, only biological proc-

esses already known are plausible. In fact, several animal experiments have shown that infection with one pathogen strongly affects the way the immune system reacts towards other unrelated pathogens – so-called heterologous immunity (146). These effects can be beneficial, leading to a more sufficient response, or harmful, leading to impaired response. From that perspective it would not be implausible that vaccines could do the same, i.e. influence the response to subsequent unrelated pathogens with potential beneficial, but also at times harmful, consequences.

Immunological studies have mainly reported that live vaccines were associated with Th1 deviation (47-51), and inactivated vaccines with Th2 deviation, also to unrelated pathogens (49-54), though the results have by no means been consistent, in particular the whole cell DTP vaccine used in low-income countries seems to induce a more Th1-deviated response than the acellular DTP vaccine used in high-income countries (147-148). However, a general picture seems to be that live and inactivated preparations of the same antigen elicit Th1 and Th2-deviated responses, respectively (49-51). A recent study aiming to develop a live pertussis vaccine have found that a live pertussis vaccine induces a more Th1 deviated response than the inactivated pertussis vaccine (149). Aluminium based adjuvants used in DTP vaccine induce a Th2 response (150-151).

Since infants are born with a Th2-deviated immune system, we have speculated that the Th1 inducing effect of the live vaccines may be part of the explanation for their non-specific beneficial effects on overall mortality, whereas the Th2-deviation induced by inactivated vaccines and the aluminium-based adjuvants may be detrimental. If vitamin A amplifies these immunological effects as proposed, it could influence the overall effect of vitamin A supplementation on mortality, and the effect could possibly be more important than the beneficial effect of vitamin A supplementation in vitamin A-deficient children. A negative effect would be particularly evident in settings without widespread vitamin A deficiency. Hence, it might be speculated that the potentially negative effect of vitamin A given with DTP in girls is due to a too strong unnecessary or excessive Th2 deviation, which would be particularly problematic in girls, who were more Th2-prone than boys.

## 7. CONCLUSIONS AND FURTHER RESEARCH QUESTIONS

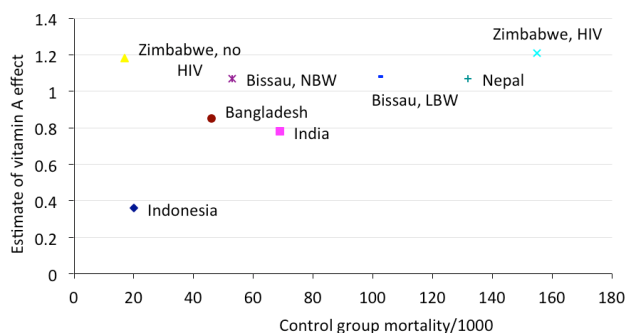
When I started working within the field of vitamin A, it was a common perception that vitamin A worked by preventing and treating vitamin A deficiency. I interpreted the findings of increased measles antibody titres after supplementation as the result of a well functioning, vitamin A replete, immune system. However, my subsequent research has clearly shown that this interpretation is not sufficient to explain all the effects of vitamin A. Vitamin A is also an immuno-modulator, and its effect is modified by other factors, which influence the immune system. The effects are different for boys and girls.

#### REFUTING THE PREVENTION OF-DEFICIENCY HYPOTHESIS

There is no doubt that vitamin A does prevent deficiency and thereby protects against vitamin A deficiency-related diseases and mortality. However, that assumption fails to explain all the existing evidence. Four major contradictions are mentioned below:

First, as shown in the first meta-analysis of the original trials there is no association between the degree of pre-existing vitamin A deficiency and baseline mortality on one hand, and the effect of vitamin A supplementation on the other hand (19). This observation has been confirmed in the recent neonatal vitamin A supplementation trials (Figure 6). If vitamin A merely acted by preventing deficiency one would expect a clear association with a better effect in the more deficient populations. Especially low-birth-weight infants are known to suffer from vitamin A deficiency (114), and the lack of a beneficial effect in our normal-birth-weight trial was by some researchers explained by the fact that we had not studied low-birth-weight children (152). The lack of a beneficial effect among low-birth-weight infants in our trial clearly disproved such speculations (IX).

**Figure 6. Baseline mortality and estimated effect of neonatal vitamin A supplementation**



Note: Bangladesh only provided mortality per 1000 live births, not per 1000 person years at risk as the rest of the trials.

Second, the *Prevention-of-deficiency* hypothesis does not explain the heterogeneity in effects over age groups. Though the picture is less consistent now, vitamin A still seems to be better for older children than for infants, and vitamin A has no beneficial effects in 1-5-month-old infants (Figure 3), though several of the studies found that a substantial proportion of the children had low serum retinol levels (25-26). This should be seen in the light that all infants are born with limited vitamin A stores. During the first 6 months of life children increase their liver stores by drinking breast milk or formula. However, studies from Bangladesh, Brazil and Indonesia reported that a quarter to over 90% of 6-month-old infants had inadequate liver stores (153-155). A necropsy study of American infants reported deficient liver vitamin A concentrations in two-thirds of the infants under 3 months of age and in a quarter of 4-6-month-old infants, but none among 6-12-month-old infants (156). If this is the general age-related biological trend, it does not make sense that vitamin A is not beneficial among the 1-5-month-old infants in low-income countries.

Third, almost all the trials, which studied the effect of different doses, found a better effect of the smaller dose (V, 26, 108-112). It is generally acknowledged that high-dose vitamin A supplementation can have transient toxic effects in the form of bulging fontanels or vomiting, but otherwise it is considered safe (5). Hence, it is not logical that a smaller dose should have a more beneficial effect than a large dose several weeks to months after supplementation.

Fourth, importantly, if vitamin A supplements merely treated vitamin A deficiency, it would not explain our consistent observa-

tions of a differential effect of vitamin A according to vaccine status. We hypothesised that it would be so, we tested it using several different designs – and we found the same again and again (V-X) as well as in different age groups (infants in the two neonatal vitamin A trials (VII-IX), older children in the reanalysis of the Ghana trial (X)): vitamin A supplementation was significantly associated with negative effects on mortality for girls when given in relation to DTP vaccine. These findings were supported by specific morbidity studies (68,115) and studies of vitamin A status (61). There is no way that this effect can be explained by specific deficiency-treating effects of vitamin A.

#### VITAMIN A IS AN IMMUNO-MODULATOR AND ITS EFFECTS ARE MODIFIED BY THE ACTIVITIES IN THE IMMUNE SYSTEM

Our research has emphasised that vitamin A is an immuno-modulator, and its effects depend on what is going on in the immune system at the time of supplementation. These interactions may lead to a more beneficial effect of vitamin A supplementation than can be explained merely from prevention of deficiency, for example the strong beneficial effects after 6 months of age may also relate to an amplification of the beneficial effects of measles vaccine, which is usually given in this age group. These interactions may, however, also lead to negative effects, which may help explain the otherwise incomprehensible findings of negative effects of vitamin A in some subgroups of recipients.

Vitamin A beyond doubt has profound effects on the immune system. Several studies have shown that vitamin A and vaccines cannot be seen as two independent interventions, since vitamin A enhanced the antibody response to the vaccine. The morbidity studies have shown quite clearly that the effect of vitamin A supplementation on disease outcome is modified by pathogens. Though they are yet not well understood, vaccines have profound effects on the immune system, influencing its capacity to respond to subsequent infections. The effects can be beneficial, as seen for BCG and measles vaccine, but also harmful, as seen for DTP vaccine. Seen from that perspective, it is not so surprising that we have found that the mortality effect of vitamin A supplementation may depend on the type of vaccine with which it is given. In particular, we consistently found that vitamin A may be negative when provided close to DTP vaccine. This would explain the lack of effect when vitamin A was provided in the age group 1-5 months, when DTP vaccine is the predominant vaccine, as well as the non-significant but worrying tendencies for increased mortality in vitamin A recipients, when vitamin A was given directly with DTP vaccine (See Chapter 8). It may also explain that a smaller dose of vitamin A is more beneficial than a large dose, especially if DTP is the most recent vaccine. Lastly, it may explain why vitamin A supplementation even to older children no longer seems to confer the same benefits as it did in the original vitamin A trials, which were carried out before the vaccine programme was rolled out (See Chapter 8). So far, we have more limited evidence for a synergistic beneficial effect on overall mortality of vitamin A and measles vaccine; more trials are needed.

#### VITAMIN A HAS SEX-DIFFERENTIAL EFFECTS

In all our studies we have looked for sex-differential effects in the response to vitamin A supplementation, and we have found them. Vaccines also have sex-differential effects on overall mortality. Seen from my perspective it should be compulsory to conduct all analyses stratified by sex. It is incomprehensible how much money is being invested in conducting genetic analyses

with the aim to provide individualised treatment – when the potentially most important genetic variation is easy and free to test and virtually unexplored.

The *Vitamin A-vaccine-interaction* hypothesis offers an explanation for the sex-differential effects of vitamin A supplementation, since many studies have shown that the negative non-specific effects of DTP vaccine are particularly marked for girls. However, as also pointed out previously, there are several other potential explanations for the sex-differences, and we still see hypothesis that vitamin A has sex-differential effects as independent of the *Vitamin A-vaccine-interaction* hypothesis.

#### FURTHER RESEARCH QUESTIONS

Our research raised many questions, which need to be studied further. Among them I find the following most intriguing:

##### *Timing of vitamin A and vaccines*

Our hypothesis raises the question of the optimal window of supplementation. According to our results, vitamin A can interact negatively with DTP vaccine in girls, even if the two interventions are given several months apart (VII-X). Further studies would be needed to determine a safety time window between the two interventions.

##### *Combined vaccinations*

We have observed that the combination of BCG vaccine and DTP vaccine may carry survival benefit for girls (72), an observation which has been confirmed in several recent analyses of datasets from Asia (unpublished). According to the WHO-recommended vaccination schedule, children should receive BCG vaccine at birth, and subsequently DTP vaccine at 6 weeks of age (Figure 1). Our studies have been done in an urban setting, where most children follow this schedule. However, in many rural areas children get their vaccinations with delay, and many children have reached the age of DTP vaccination when they come for BCG vaccination, and receive both vaccines at the same time. If vitamin A is provided at birth, it may be speculated that it would be beneficial for girls, if it was followed by combined BCG and DTP vaccination instead of a DTP vaccine only. I would anticipate that such a beneficial effect of neonatal vitamin A supplementation would be seen until the girls received their second DTP vaccine, after which the effect would be negative.

According to the WHO schedule, DTP should be given before measles vaccine. However, many children receive DTP delayed, together with measles vaccine. The effect of providing vitamin A with combined DTP and measles vaccine remains to be studied.

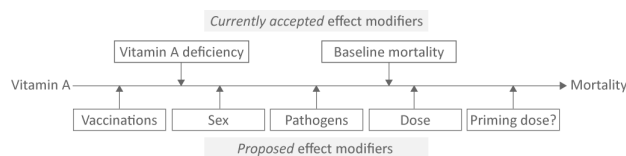
##### *Other modifiers of vitamin A effects*

Apart from vaccines and sex, other factors may modify the effect of vitamin A. They are depicted in Figure 7. Among them are pathogens. It is well recognised that vitamin A has differential effects on diarrhoea and respiratory infections. Curiously this has not led to openness to the proposition that the effect may also differ by vaccination status.

We have also shown that the dose of vitamin A may be important. This observation is supported by all other studies, which have looked at dose. From my perspective it seems plausible that more frequent dosing with smaller doses is more beneficial than the more non-physiological larger doses, in particular when vitamin A is provided in the same time window as a DTP vaccine

to girls, or at the time of a specific pathogen infection, for which the immuno-modifying effects of high-dose vitamin A may be detrimental.

**Figure 7. Potential modifiers of the effect of vitamin A supplementation on mortality**



Priming with early vitamin A supplementation may also be important. Recent results have indicated that early vitamin A supplementation may prime a beneficial response to subsequent doses of vitamin A. A follow-up study of the participants in the neonatal vitamin A trial in normal-birth-weight infants showed that after a vitamin A supplement at 12 months of age (at the end of follow-up), mortality was significantly lower among girls, who had received vitamin A at birth compared with girls, who had received placebo at birth. Among children who were not at home to get vitamin A at 12 months of age, mortality continued to be higher among girls who had received vitamin A compared with placebo at birth (157). Furthermore, another trial of different doses during a vitamin A campaign revealed that the benefit of a small dose versus a high dose was confined to children, who had not received vitamin A at birth; children who had received vitamin A at birth benefitted from a high dose (158).

To me there is no doubt that there will be other modifiers of vitamin A supplementation. The proposition that vitamin A as an immuno-modulator interacts with other factors, which affects the immune system, opens up for a large range of possible interactions. The important issue in the future will be to be open to such interactions, continuously exploring their existence, keeping in mind that while such interactions would not necessarily need to be biologically plausible in the first place, they would have to be repeatable.

##### *Other micronutrients*

Several recent large-scale randomised trials providing iron and zinc supplementation to infants have shown disappointing results (159-162). In one trial, the iron arm was closed prematurely due to a significantly increased incidence of serious adverse events including deaths (159). We made the observation that the negative effect seemed limited to girls below one year of age, and proposed that similar negative interactions with DTP exist for iron and zinc (66). If DTP has negative non-specific effects on the immune system leading to increased mortality, girls who have DTP as their most recent vaccine may be better off if their immune system is not functioning optimally due to marginal micronutrient deficiency.

#### IMPLICATIONS OF SPECIFIC AND NON-SPECIFIC VITAMIN A-VACCINE INTERACTIONS

The work in the present thesis has shown both *specific* and *non-specific* vitamin A-vaccine interactions of relevance for the current WHO policy of providing vitamin A with vaccines to children above 6 months of age. The policy has never been evaluated in

randomised trials. Our results imply that the policy needs to be evaluated – and not merely for its effect on vitamin A status or on the immune response to the vaccines. Vitamin A may have *specific* beneficial effects in terms of improving vitamin A status or the immune response to the vaccine(s) with which it is given. However, our work has shown that even though vitamin A supplementation improves vitamin A status and the immune response to vaccines, it can also have important *non-specific* effects on survival. The overall benefit of providing vitamin A supplementation can be seen as the sum of vitamin A's specific and non-specific effects. If there are strong negative non-specific effects on overall survival of providing vitamin A with certain vaccines, this could overrule the beneficial specific effects. Research should be done to provide evidence for policy – and the research needs to focus on the important outcomes in order to produce the relevant answers. Our work has emphasised that beneficial specific effects such as improved vitamin A status and increased immune responses cannot be interpreted as equivalent to an overall beneficial effect - overall mortality is the most important outcome.

#### A NEW FRAMEWORK: INTERVENTIONS INTERACT AND HAVE SEX-DIFFERENTIAL EFFECTS

It is a common assumption within public health in low-income countries that interventions can be combined without unexpected consequences. According to the existing scientific paradigm, health interventions are specific. Health interventions are also seen as independent of each other. Furthermore, health interventions are assumed to have the same effect in both sexes. However, the work presented in this thesis confronts this paradigm. My work suggests a more complex, yet hopefully more sensible framework: vaccines and vitamin A are immuno-modulatory interventions that programme the immune system. These non-specific effects may interact with unexpected effects on survival. Combining interventions can be convenient and lead to synergistic health benefits, but we documented several examples where it also leads to unexpectedly increased mortality.

Thus, to optimise the child health intervention policy in low-income countries a shift in paradigm is needed. Health interventions should no longer be seen as merely specific and independent, and the policy should probably not be the same for boys and girls. Though more complex, it is necessary to evaluate all health interventions in terms of their effect on overall mortality - and their potential interactions with other health interventions and potential sex-differential effects should always be investigated. Only in this way can we assure that the children in the poorest countries get the best possible treatment and avoid using large amounts of money and resources on interventions, which may, in worst case, kill them.

To sum up, the consequences of public health importance are

- 1) Vitamin A supplementation is not always beneficial, and we need to seek the optimal policy, taking advantage of the beneficial effects of vitamin A supplementation, but avoid using it when it puts children at risk
- 2) Child health interactions may interact with consequences for mortality
- 3) There is a need for continuing evaluation of interventions, which are already recommended, since their effect may change in the context of new interventions being launched.
- 4) Sex of the child should always be considered when evaluating the effect of health interventions.

In the last chapter, I will provide examples of the consequences of my work for public health.

## 8. PUBLIC HEALTH CONSEQUENCES

Our findings have contributed to major public health issues. The findings have been commented upon in a number of editorials (152, 163-167) and influenced policy decisions on several occasions:

### VITAMIN A WITH MEASLES VACCINE

While we conducted our trial of the effect on measles-specific antibody titres a paper was published reporting that vitamin A supplementation had negative effect on the antibody response to measles vaccine administered at 6 months of age (84). The paper raised a lot of concern, which was debated in *The Lancet* (168-170). The benefits of measles vaccine and vitamin A supplementation were weighed against each other, in the case that simultaneous administration of the two interventions should be abandoned and one should be prioritised over the other (168-170). Our paper was published with an accompanying editorial stating that there was no need for concern if vitamin A was given with measles vaccine at the recommended age of 9 months (163). It closed the debate, also at the WHO (171).

### VITAMIN A WITH DIPHTHERIA-TETANUS-PERTUSSIS VACCINE

Since the beginning of the nineties there has been interest in extending the beneficial effects of vitamin A in older children to younger children. In 1998, the WHO multicenter trial was published, showing no effect of providing 25,000 IU vitamin A with each of the three DTP vaccines, even though the majority of the participants were vitamin A-deficient (26). The natural response would have been to explore the reasons for this lack of effect – and perhaps to start questioning whether the underlying assumption, that vitamin A worked merely by preventing vitamin A deficiency, could be true. However, the reaction was rather the opposite – it was argued that the dose of vitamin A had been too small – and in 2002 the International Vitamin A Consultative Group (IVACG) came out with a strong recommendation that young children should have 50,000 IU vitamin A with each of the three DTP vaccines (32).

Since the formulation of our hypothesis we have argued that it would potentially be a very bad idea to provide vitamin A with DTP. We have repeatedly emphasised the observation that vitamin A has never proven beneficial when given in the age group of DTP vaccine - and our subsequent trials have supported that.

All existing data of providing 50,000 IU with DTP (alone or as a part of pentavalent vaccine) suggest that this could have been detrimental, if the IVACG recommendations had been followed. A small trial from Ghana, which provided 50,000 IU vitamin A with the three pentavalent vaccines and compared it with placebo, had five deaths in the vitamin A group and 1 death in the placebo group, resulting in a relative risk of 4.65 (95% CI 0.55–39.5),  $p = 0.12$  (29). Another small trial from Bangladesh gave 50,000 IU or placebo with the first DTP vaccine to children hospitalised with diarrhoea, and followed up with vitamin A to the vitamin A recipients when they came for the second and third DTP. The dose of 50,000 IU vitamin A tended to be beneficial when given to the children with diarrhoea, but it tended to be negative when given with the second and the third dose of DTP

(3.54 (0.76–16.5)), the effect of vitamin A given with the second and third dose of DTP being significantly different from the effect of vitamin A given with the first dose of DTP (28, 57). Another study from The Gambia, testing the effect of 50,000 IU versus 25,000 IU vitamin A with the three DTP vaccines, found that those who received the high dose had two deaths versus none in the low dose group, and they had significantly more clinic attendances during the first 6 months of life ( $p=0.018$ ) (110-111).

The recommendation to provide any dose of vitamin A with DTP has now been abandoned. To our regret there has been little interest in pursuing why vitamin A was not beneficial.

#### NEONATAL VITAMIN A SUPPLEMENTATION

While writing the present thesis a heated debate regarding a global or regional recommendation of neonatal vitamin A supplementation is ongoing. As outlined in Chapter 4, three of four studies from Asia found a beneficial effect whereas three African trials found no effect and even negative effects in some subgroups (girls, in particular after DTP, in our trials; children of HIV-positive mothers, who remained HIV-negative themselves, in Zimbabwe).

When some trials found a positive effect of neonatal vitamin A supplementation, and others no effect, a subgroup vitamin A supplementation policy is only acceptable if there is convincing evidence that the subgroup encompasses the children who benefit, and not those who might be harmed. Apparently, many seem to accept the geographical area “South Asia” as a reasonable definition of such a subgroup; several voices have been raised in favour of recommending blanket neonatal vitamin A supplementation in South Asia (152, 172-173). We think it might be premature to implement neonatal vitamin A supplementation in South Asia before we understand the background for the contradictory effects in existing trials (65).

According to the advocates for “South Asia”, differences in vitamin A status and baseline mortality in the neonatal vitamin A supplementation trials is the best way of reconciling the contradiction between the trials. However, as described in Chapter 4, there is limited data on vitamin A status, and differences in baseline mortality do not explain the divergent results.

As argued in Chapter 4, the divergent results may be explained by differences in vaccination intensity in the trials (VIII, 63, 65). If our hypothesis is correct and neonatal vitamin A supplementation is made a general policy in South Asia, the intervention may cease to be beneficial or even become detrimental, as the DTP coverage increases and more children are vaccinated early in life - especially in populations in which mortality is not limited to the first months of life. However, there will be no way of knowing, because it is considered unethical to conduct further trials once an intervention has become policy.

WHO has now decided to carry out three new trials of neonatal vitamin A supplementation. It was initially proposed that these studies should only follow children to 6 months of age. It was not suggested to collect data on vaccination status and the trials were not designed with the *a priori* hypothesis that the effect could be different in boys and girls. However, based on our findings from Guinea-Bissau, it has now been decided to prolong follow-up to 12 months of age, and vaccination data will be collected (Magnus Tingstrøm, personal communication).

An important question for further research is what happens when BCG and DTP are administered simultaneously, as often happens in rural areas. We suspect that vitamin A supplementation in this situation may be more beneficial for girls, since we have previously experienced that combined BCG and DTP vaccinations are better for girls than for boys (72). The previous African neonatal vitamin trials were carried out in urban areas, and most children received their vaccinations in the recommended schedule, first BCG and then DTP (VIII, Jean Humphrey, personal communication). If the two new African trials are carried out in rural settings where BCG and DTP are often given together, then the results may be different, with a beneficial effect for girls, at least until the second dose of DTP.

#### IS VITAMIN A SUPPLEMENTATION BENEFICIAL IN THE VACCINATION ERA?

Among the eight original vitamin A trials leading to the policy of providing vitamin A to all children between 6 months and 5 years of age, two used fortification or small weekly doses of vitamin A and did in fact not test the effect of high-dose supplementation (11, 17). Two of the other trials did not find a beneficial effect (12, 15). Recently, a large Indian trial was conducted, showing no effect of vitamin A supplements in 1-5-year-old children (22). The trial has not yet been published, but it is hard to believe that such a result can be a chance finding or due to bias. The original trials were carried out while the vaccination programme was in its youth. Our re-analysis of the Ghana trial showed that almost half of the children had not received any vaccines (X). It also showed that the beneficial effect of vitamin A was limited to children, who had no vaccination card. The prevalence of vitamin A deficiency is declining. Hence, the beneficial effect of vitamin A on the prevention of deficiency, and thereby on mortality, may be less than it used to be. Furthermore, overall performance of immunisation programmes is often tracked with the coverage of three doses of DTP vaccine (DTP3). As a result of the drive to increase DTP3 coverage, many children now receive DTP vaccine together with or after measles vaccine, and the coverage of DTP3 is now higher than the measles vaccine coverage in many countries. Furthermore, many countries provide DTP booster vaccine around 18 months of age. Hence, also older children may now receive vitamin A supplements close to DTP vaccine. It could be speculated that the effect of vitamin A supplementation is only beneficial in deficient children and/or in unvaccinated children – and as the prevalence of such children declines, the effect ceases to be beneficial. If vitamin A interacts negatively with certain pathogens or certain vaccines, it may even become detrimental. For a long time it has been considered completely unethical to conduct randomised trials of vitamin A supplementation in children aged 6 months to 5 years. The time may have come for it to be done, since it is also unethical to continue using large amounts of money and resources on interventions that may, in worst case, kill some children.

## SUMMARY

The present thesis is based on 11 papers from 1995-2010. The studies have mainly taken place at the Bandim Health Project in Guinea-Bissau, West Africa, but a reanalysis of a randomised trial from Ghana is also included.

My research has explored the consequences of combining high-dose vitamin A supplementation and childhood vaccines. Vitamin A deficiency is associated with increased mortality. To protect against the consequences of vitamin A deficiency the World Health Organization recommends that high-dose vitamin A supplements be given together with routine vaccines to children between 6 months and 5 years of age in more than 100 low-income countries. The recommendation is based on logistical considerations. The consequences of combining vitamin A and vaccines were not investigated in randomised trials prior to the implementation of this policy – it was assumed that the interventions were independent.

My first project aimed to study the effect on the immune response to measles of providing vitamin A together with measles vaccine. We found that the two interventions were not independent. Vitamin A enhanced the antibody response to measles vaccine given at 9 months of age significantly, especially in boys. The effects were sustained over time; the children who had received vitamin A with their measles vaccine were more protected against measles at 6-8 years of age.

Though vitamin A supplementation had a beneficial effect on the immune response to measles vaccine, it intrigued me that the effect of vitamin A supplementation on overall mortality was not always beneficial. While vitamin A was beneficial when given after 6 months of age, and two studies had shown a beneficial effect when given at birth, all studies testing the effect between 1-5 months of age had found no effect. These time windows are dominated by three different childhood vaccines: BCG vaccine given at birth, diphtheria-tetanus-pertussis (DTP) vaccine given between 1-5 months of age, and measles vaccine given at 9 months of age. These vaccines have been shown to have strong effects on mortality from infectious diseases in general, so-called non-specific effects. The live BCG and measles vaccine protects against more mortality than can be ascribed to the prevention of tuberculosis and measles, respectively. The inactivated DTP vaccine worryingly has been associated with increased mortality from other infectious diseases. Both positive and negative effects are strongest for girls. I proposed the hypothesis that vitamin A amplifies not only the specific vaccine effects, as we saw for measles vaccine, but also the non-specific effects of vaccines on mortality from other infectious diseases. According to my hypothesis, vitamin A would enhance the non-specific beneficial effects on mortality of BCG and measles vaccine, but also the negative effects of DTP vaccine. Hence, the hypothesis offered an explanation for the mortality-age pattern after vitamin A supplementation.

Since it was formulated, I have aimed to test this hypothesis. Since it is associated with ethical problems to randomise children above 6 months of age to vitamin A supplementation, and to randomise children in general to recommended vaccines, we have had to be pragmatic when designing the trials. Hence, our studies have taken many different forms. We conducted an observational study during a vitamin A campaign in which missing vaccines were also provided, and a randomised trial testing the effect of two different doses of vitamin A during another

campaign; we tested the effect of providing vitamin A with BCG at birth in two randomised trials, and we reanalysed data from one of the original randomised trials of vitamin A supplementation from the perspective of vaccination status. In all studies the main outcome was mortality.

The results document that vitamin A supplements do more than protect against vitamin A deficiency. They support the hypothesis that vitamin A supplements interact with vaccines with important consequences for mortality. First, a smaller dose of vitamin A was more beneficial than a larger dose for girls. Second, the effect of vitamin A given with DTP vaccine was significantly different from the effect of vitamin A given with measles vaccine, and children, who received vitamin A with DTP vaccine, had higher mortality than children, who had received vitamin A alone, or who did not receive anything. Third, vitamin A given with BCG at birth interacted negatively with subsequent DTP vaccines in girls. Fourth, the effect of vitamin A to older children in Ghana depended on vaccination status, being beneficial in boys, but harmful in girls who received DTP vaccine during follow-up. The results also show that boys and girls respond differently to vitamin A and vaccines.

It is a common assumption within public health in low-income countries that interventions can be combined without producing unexpected consequences. The work presented in this thesis confronts this assumption; the results show that vitamin A and vaccines should be seen not only as specific interventions with specific and independent effects, but as immuno-modulators, which can interact with important consequences for overall mortality. Combining interventions can be convenient and lead to synergistic health benefits, but we documented several examples, where it also leads to unexpectedly increased mortality.

Thus, to optimise the child health intervention policy in low-income countries a shift in paradigm is needed. Health interventions should no longer be seen as merely specific and independent, and the policy should probably not be the same for boys and girls. Though more complex, it is necessary to evaluate all health interventions in terms of their effect on overall mortality - and their potential interactions with other health interventions and potential sex-differential effects should always be investigated. Only in this way can we assure that the children in the poorest countries get the best possible treatment and avoid using large amounts of money and resources on interventions which may, in worst case, kill them.

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