Vitamin A Supplementation Fails to Reduce Incidence of Acute Respiratory Illness and Diarrhea in Preschool-Age Indonesian Children[1,2]

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ABSTRACT Vitamin A supplementation of populations of vitamin A–deficient preschool-age children has been shown to reduce childhood mortality, but the primary preventive effects of such supplements on childhood infectious diseases have not been carefully evaluated. We conducted an individually randomized, placebo-controlled, double-masked trial among 1,407 Indonesian preschool-age children, to measure the effects of high dose vitamin A on acute respiratory and diarrheal illnesses. Signs and symptoms of morbidity were monitored using every other day home surveillance by trained interviewers. High dose vitamin A supplements increased the incidence of acute respiratory illnesses (ARI) by 8%, and acute lower respiratory illnesses (ALRI) by 39%. These detrimental effects on acute lower respiratory illnesses were most marked in children with adequate nutritional status (rate ratio 1.83, 95% confidence interval 1.257–2.669). In contrast, vitamin A tended to be protective of ALRI in chronically malnourished children (rate ratio 0.71, 95% confidence interval 0.375–1.331). There was no overall effect of high-dose vitamin A supplements on the incidence of diarrheal disease (rate ratio 1.06, 95% confidence interval 0.920–1.225). However, we found a significant interaction between supplementation and age: vitamin A increased the incidence of diarrhea in children <30 mo of age, but tended to reduce the incidence in older children. The finding of a significant adverse effect of vitamin A supplements in adequately nourished children highlights the need to review the criteria for selecting populations of preschool-age children for vitamin A supplementation. J. Nutr. 126: 434–442, 1996.

INDEXING KEY WORDS:

- *vitamin A deficiency*
- *vitamin A supplementation*
- *preschool children*
- *acute respiratory illness*
- *diarrheal diseases*

Vitamin A deficiency is a major nutritional deficiency among preschool-age children from the developing world with ~ 14 million having had eye damage from vitamin A deficiency and over 190 million being at risk of vitamin A deficiency (ACC/SCN 1993). Prospective, controlled community trials of vitamin A supplementation in deficient populations have demonstrated an average reduction in childhood mortality of 23 (Beaton et al. 1993) to 30% (Fawiz et al. 1993, Glasziou & Mackerras 1993). However, the mechanisms by which vitamin A supplementation reduces childhood mortality are less clear.

It has been hypothesized that vitamin A supplements decrease all-cause childhood mortality by a reduction in infectious diseases, the intermediary steps to most childhood deaths in developing countries. This hypothesis originated from three sets of observations: *1)* laboratory studies that documented a decreased resistance to infection in vitamin A–deficient animals (Nauss 1986); 2) a series of longitudinal observational studies (Bloem et al. 1990, Milton et al. 1987, Sommer

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et al. 1984) that reported an increased risk of acute respiratory illness (ARI) and possibly of diarrhea in vitamin A–deficient children; and 3) an intervention trial in Aceh, Indonesia (Sommer et al. 1986) that demonstrated a 34% reduction in preschool child mortality following supplementation with high dose of vitamin A.

Most of the prospective trials designed to test the above hypothesis failed to demonstrate a primary preventive effect on the incidence of childhood infectious illnesses (Abdeljaber et al. 1991, Barreto et al. 1994, Bhandari et al. 1994, Ghana VAST Study Team 1993, Rahmathullah et al. 1991, Stansfield et al. 1993, Vijayaraghavan et al. 1990). The supplementation trial conducted in Ghana (Ghana VAST Study Team 1993), although unable to demonstrate any impact on the prevalence of diarrhea or acute respiratory illness, did report reduced clinic visits and hospitalizations of vitamin A–supplemented children. The trial conducted in Brazil (Barreto et al. 1994) reported a beneficial effect of supplementation on episodes of severe diarrhea. The trial from India (Bhandari et al. 1994) reported a reduced incidence of measles in children <23 mo but no effect on the incidence of diarrhea and acute respiratory illness.

In a population of rural Indonesian preschool children with a high prevalence of subclinical vitamin A deficiency, we conducted a study which aimed to assess the role of high dose vitamin A supplementation in the prevention of common childhood illnesses. The primary study hypothesis was that the incidence and severity of acute respiratory illness and diarrheal disease would be less in children supplemented with vitamin A every 4 mo.

SUBJECTS AND METHODS

Study population. The study was carried out in 34 rural villages located on the southern coast of Central Java, Indonesia. A census conducted in 25 of these villages in September, 1989, identified a total population of 14,350 with 762 children aged 6–47 mo. In December, 1990, an additional nine villages were added resulting in a total of 1036 children who were age-eligible for the trial.

Written informed consent was obtained from the guardians of all study subjects. Meetings with village leaders and a program of community education about the study supplemented the informed consent process. The protocol was reviewed and approved by the Committee on Human Research, The Johns Hopkins University, School of Hygiene and Public Health, the Indonesian National Vitamin A Research Steering Committee and the Committee on Ethics in Human Biomedical Research of the Faculty of Medicine, Gadjah Mada University.

Randomization and supplementation. Children aged 6–47 mo were randomly allocated to receive either vitamin A or placebo on their entry into the study and the same treatment every 4 mo thereafter. At the start of each cycle, the children were examined and those with evidence of chronic illnesses (cerebral palsy, flaccid paralysis, mental retardation, epilepsy, congenital or rheumatic heart disease) were permanently excluded; those with weight-for-height more than 3.00 SD below the WHO growth reference mean (Dibley et al. 1987) or acute xerophthalmia were excluded for one cycle and treated with high dose vitamin A.

Randomization of the treatments was done with a 1:1 allocation ratio in blocks of eight, based on a table of random permutations of integers (Cochrane and Cox 1950). The treatments were administered in a fixed geographic pattern with the blocking designed to ensure balance of groups by village and distance from health services. All investigators, field and laboratory staff, and participants were masked to the treatment code.

The dose of vitamin A administered to children < 12 mo of age was 107 μmol (103,000 IU) retinyl ester plus 40 μmol (17 IU) vitamin E (*all-rac-α*-tocopherol) in oil, while older participants received 214 μmol (206,000 IU) vitamin A ester plus 84 μmol (37 IU) vitamin E in oil. The placebo capsules contained the same oily base and either 40 or 84 μmol vitamin E depending on the age of the subject. The capsules were packaged in opaque blister packs with a unique treatment code. The oily contents of the vitamin A and placebo capsules were of similar taste and color. A quantitative laboratory analysis of a random sample of capsules revealed 99.7% potency of the vitamin A after 2 y of field storage.

Data collection procedures. Data were collected during six treatment cycles. Before the start of these cycles, the census was repeated to identify new, age-eligible children for the trial. The date of birth data were gathered by checking birth certificates and village birth registries. Detailed socioeconomic information was collected at the start of each child's 1st cycle.

Special village clinics were conducted at which children enrolling or active in the study had a physical examination, anthropometric measurements collected, immunization status assessed, blood samples collected and trial treatments administered. The field pediatricians used standardized diagnostic algorithms based on history and physical examination to identify children with chronic illnesses. The ophthalmologists used the standardized diagnostic criteria of the World Health Organization (WHO 1982) to identify children with xerophthalmia. Trained anthropometrists measured the child's weight to the nearest 0.1 kg with a hanging Salter scale (Salter Industrial Measurement, West Midlands, UK) and the child's recumbent length (or stature for children > 24 mo) to the nearest 0.1 cm using a portable calibrated board. Anthropometric

procedures were standardized (Habicht 1973) at the start of each treatment cycle. The child's immunization status was ascertained by questionnaire and examination of local health center records. Serum retinol was measured before treatment at the start of the 1st cycle, and again during the 6th cycle.

Morbidity data were collected by trained interviewers who visited the children at home every other day to record symptoms of diarrhea and acute respiratory illnesses using standardized precoded forms. The longest recall period allowed was 4 d. An anthropologist had conducted focus group discussions and in-depth interviews with village women to identify local Javanese terms for symptoms and childhood illnesses. The interviewers were retrained regularly and their work was monitored independently by a supervisor. During each treatment cycle a sample of ~ 1% of trial subjects was selected for independent same-day reinterview by a supervisor.

Children with cough had minute respiratory rates counted twice using an electronic audible timer (Micronta, Tandy Corporation, Hong Kong). At the start of each cycle, the respiratory rate measurements were standardized with a method similar to that used for the anthropometric measurements. Children reported to have cough or fever had their axillary body temperature measured to the nearest 0.1°C using a digital thermometer with an audible alarm (Becton Dickinson, Rutherford, NJ). Children with diarrhea had a stool sample collected.

Case definitions. *Episodes of diarrhea* were defined as adjoining days for which the child was reported to have three or more loose stools per 24-h period. They were ended by two or more symptom-free days or missing data days. *Episodes of acute respiratory illness (ARI)* were defined as two or more adjoining days for which the child was reported to have cough. They were ended by three or more symptom-free days or missing data days. This definition of ARI includes most upper respiratory tract infections except for nasal discharge or sore throat alone. It also includes lower respiratory tract infections and children with prolonged chronic cough. *Episodes of acute lower respiratory illness (ALRI)* were defined as periods of two or more adjoining days for which the child was reported to have cough and during which there was at least one elevated respiratory rate measurement. As in earlier epidemiological studies of acute respiratory tract infections in children (Selwyn 1990), 50 respirations per minute defined an elevated respiratory rate for all age groups. The episodes were ended by three or more cough-free days or missing data days. This definition of an episode of ALRI was similar to the diagnostic algorithm described in the World Health Organization case management guidelines for pneumonia (WHO 1990).

Laboratory procedures. Blood samples were collected by ante-cubital venipuncture into a capped colored glass tube, then centrifuged at 1000 × *g* for 10 minutes and the serum pipetted before transportation on ice on the same day to the laboratory. The retinol levels were assayed by HPLC using standard methods (Arroyave et al. 1982). The within-assay coefficient of variation for the retinol analyses was < 5%.

Data monitoring. After 12 mo of data collection, an effects-monitoring committee reviewed the data for evidence of acute side effects following treatment and the effect of treatment on cough and diarrheal disease episode rates. Although small differences (not significant) were found between the treatment groups for some of these outcomes, the committee recommended that the study be continued.

Data management and statistical analysis. Field and laboratory data were collected on precoded forms that were subject to a system of editing before data entry. The dSURVEY (Corner 1989) software was used for data entry and data cleaning. A computer program that employed user-defined diagnostic criteria to count episodes of diarrhea, ARI, ALRI and their associated person-time denominators from daily symptom data for each child. The person-time denominators for the incidence rates were counts of the observed days at risk between episodes and excluded the days the child had symptoms of that illness. Thus, these counts of person-time denominators were calculated separately for each of the major trial outcomes. Episodes beginning before the start of a treatment cycle were excluded from the count of events for that cycle.

Data analyses were carried out on microcomputers using SYSTAT (Wilkinson 1990) and SAS (SAS 1991) statistical software. Anthropometric indicators were calculated using the World Health Organization international growth reference (Dibley et al. 1987) with computer subroutines provided by the U.S. Centers for Disease Control. Base-line differences between treatment groups were tested by χ^2 test for general association and for trend.

Parametric Weibull survival models, with each observation being one time-to-event (or censoring) for each child in each cycle, were fitted to estimate the effects of vitamin A on morbidity while allowing for adjustment of covariables that change within a cycle or from episode to episode. The generalized estimating equations method (Zeger and Liang 1986) was modified (Moulton 1993) to adjust the variance and confidence interval estimates from the parametric survival model to take account of the correlated longitudinal data collected in the trial. Interaction terms in the models were assessed by the Wald test. Differences between treatment groups were judged to be significant at $P < 0.05$.

Incidence curves by age were adjusted for the effects of the treatment cycle (season) and the number of cycles the child had participated in the trial. The rate levels were determined using the child's 1st cycle in the trial and the average effect over all treatment cycles. Finally, the data were smoothed using four-knot restricted cubic splines (Harrell et al. 1988).

RESULTS

Participation and compliance. A total of 1405 children were allocated a treatment, and on average 782 participated in each cycle with 386 in the placebo and 396 in the vitamin A group. The number of children age-eligible for the trial, the number treated, and losses before and after treatment are detailed for each cycle in **Table 1.** An average of 9% of the families declined to participate. Compliance was equally high in both treatment groups, and on average 89% of the age-eligible children received a treatment. On average 4.6% of the children migrated and 3.5% withdrew before completion of the treatment cycle. Morbidity surveillance was completed by 96% of the placebo and 97% of the vitamin A group. Less than 2% of the daily morbidity records were missing, mainly due to temporary absences.

Characteristics of the treatment groups. Demographic, clinical and nutritional characteristics at the start of the 1st cycle were similar for both groups (**Table 2**). Furthermore, the groups remained balanced by these characteristics at the start of each of the other five treatment cycles (data are not presented).

Based on commonly used criteria (WHO 1982), the study population can be characterized as having a high prevalence of subclinical vitamin A deficiency but a low prevalence of clinical vitamin A deficiency. Serum retinol measurements taken from 97% (n = 682) of the children enrolled in the 1st cycle revealed that 6% had very low serum retinol (≤0.35 μmol/L) and 52% had moderately low serum retinol (0.35–0.69 μmol/L). Serum retinol measurements taken from 74% (n = 629) of the children who were active during the 6th cycle (31% of these children were in the 1st cycle) demonstrated a response to treatment. The mean serum retinol of the vitamin A group (0.89 ± 0.255 μmol/L, mean ± SD) was 24% higher than in the placebo group (0.71 ± 0.228 μmol/L). Furthermore, 56% of the placebo group had a serum retinol ≤0.70 μmol/L compared with 32% from the vitamin A group. The prevalence of low serum retinol at base line and in the placebo group exceeds the World Health Organizations's criteria to define vitamin A deficiency as a public health problem (WHO 1982). Eye disease was largely absent with only one case of Bitot's spots and no cases of corneal ulceration in trial subjects.

Based on standard anthropometric definitions (Waterlow et al. 1977), a similar proportion of children was malnourished in each of the treatment groups. Stunting affected a far larger proportion of trial subjects than did wasting (Table 2). Immunization coverage was balanced by treatment group in cycles 2–6 (Table 2). Seventy percent of the children were reported to have been vaccinated against measles.

There were no significant differences between the treatment groups in the proportion of days the children were ill with cough or cough and elevated respiratory rate during the 2 wk prior to the start of trial. However, the children in the placebo group reported a

TABLE 1

Enrollment and retention of study participants

	Treatment cycle					
	1	2	3	4[1]	5	6
Age-eligible for study, n	762	739	732	1036	1116	1031
Agreeing to study, %	98	97	90	91	85	85
Losses before treatment						
Migrated, n	13	2	3	8	5	7
Withdrawal of consent, n	13	8	8	26	8	4
Excluded from study, n	13	19	9	14	15	17
Children treated with placebo, n	350	335	312	441	456	423
Children treated with vitamin A, n	357	350	328	452	466	423
Losses after treatment[2]						
Migrated—placebo, n	8	16	11	7	8	0
Migrated—vitamin A, n	12	10	12	5	7	0
Withdrawals—placebo, n	5	10	5	1	4	0
Withdrawals—vitamin A, n	12	10	12	5	7	0
Completed surveillance						
Placebo treated,[3] %	96	92	95	98	97	100
Vitamin A treated,[4] %	94	95	96	99	98	100
Study eligibles,[5] %	92	89	85	87	82	84

[1] Nine additional villages recruited into study starting this cycle.
[2] There was one death in placebo group in 5th cycle.
[3] Percentage treated with placebo who completed surveillance for that cycle.
[4] Percentage treated with vitamin A who completed surveillance for that cycle.
[5] Study eligibles are age-eligible minus migrations and exclusions before treatment.

TABLE 2

Characteristics of study participants on entry to 1st treatment cycle in the placebo and vitamin A treatment groups

Characteristic	Placebo *n* (%)	Vitamin A *n* (%)
Sex*		
Male	181 (51.7)	179 (50.1)
Female	169 (48.3)	178 (49.9)
Age,** *mo*		
6–11	45 (12.9)	54 (15.1)
12–23	89 (25.4)	98 (27.5)
24–35	95 (27.1)	101 (28.3)
36–47	111 (31.7)	99 (27.7)
48–53	10 (2.9)	5 (1.4)
Serum retinol,** *μmol/L*		
≤0.350	26 (7.4)	18 (5.0)
0.351–0.700	173 (49.4)	173 (48.5)
0.701–1.050	123 (35.2)	128 (35.9)
>1.050	10 (2.9)	15 (4.2)
Missing	18 (5.1)	23 (6.4)
Anthropometric status**[1]		
Stunted	126 (36.0)	137 (38.4)
Stunted and wasted	13 (3.7)	19 (5.3)
Wasted	12 (3.4)	12 (3.4)
Normal	194 (55.5)	185 (51.8)
Missing	5 (1.4)	4 (1.1)
Immunization status[2]		
Ever vaccinated for polio	212 (65.2)	202 (59.9)
Ever vaccinated for measles	236 (72.6)	225 (66.8)
Base-line morbidity prevalence[3]		
Diarrhea*	0.85	0.44
Cough	10.65	11.05
Cough and elevated respiratory rate	0.09	0.00

[1] Stunted defined as height-for-age < reference mean minus 2 SD and weight-for-height ≥ mean minus 2 SD; wasted defined as height-for-age ≥ mean minus 2 SD and weight-for-height < mean minus 2 SD; stunted and wasted defined as height-for-age < mean minus 2 SD and weight-for-height < mean minus 2 SD; and normal defined as height-for-age ≥ mean minus 2 SD and weight-for-height ≥ mean minus 2 SD.

[2] Data for children at start of 2nd treatment cycle (placebo group n = 325 vitamin A group n = 337).

[3] Proportion of days the children in the vitamin or placebo group were ill with diarrhea, cough or cough and elevated respiratory rate during the 2 wk prior to the start of the first treatment cycle.

* $P > 0.60$ from χ^2 test; ** $P > 0.10$ from Cochran-Mantel-Haenzel trend test.

higher proportion of days ill with diarrhea during this base-line period (Table 2). Although the difference was significant, the magnitude of the difference was small and may have arisen because of the limited duration of base-line morbidity monitoring.

Acute respiratory illness. A total of 10,735 episodes of ARI were observed with 88% of the children experiencing two or more such episodes. The incidence of ARI was 8% (95% confidence interval: 0.7%, 19%), higher in the vitamin A group than in the placebo group (**Table 3**), and this pattern was consistently detected in all six cycles. The age, anthropometric status and gender of the child did not affect the impact of treatment with vitamin A on the incidence of ARI (data are not presented).

Treatment with vitamin A was not associated with the mean duration of ARI episodes (vitamin A 4.6 d, placebo 4.5 d, $P = 0.62$) or with the proportion of episodes longer than 14 d (vitamin A 4.9%, placebo 5.1%).

Acute lower respiratory illness. A total of 177 episodes of ALRI were observed with less than 2% of the children experiencing two or more such episodes. The incidence of ALRI was 39% (95% confidence interval: 0.3%, 93%), higher in the vitamin A than in the placebo group (Table 3). This increased incidence of ALRI with vitamin A treatment was detected in five of the six treatment cycles: rate ratios for cycles 1–6 were 1.74, 1.35, 2.38, 1.37, 0.85 and 1.53, respectively.

There was no significant interaction ($P = 0.44$) between vitamin A and the age of the child on the incidence of ALRI as illustrated in **Figure 1.** At 18 mo the rate ratio (the ratio of the heights of the two curves in Figure 1) was 1.35, whereas at 36 mo it was 1.46, indicating a similarly increased risk of ALRI at both ages.

The marked interaction between stunting and the effect of vitamin A on the incidence of ALRI was significant using the Wald test (**Table 4**). Vitamin A treatment significantly increased the risk of ALRI in children who were not stunted at entry into the study (rate ratio 1.83) but did not increase the risk in children who were initially categorized as stunted (rate ratio 0.71). The survival models used to detect this interaction controlled for the effects of age, gender, treatment cycle (season) and the number of cycles the child had participated in the trial. There was no interaction of wasting with the effect of vitamin A treatment on ALRI (data are not presented). Gender also tended ($P = 0.09$) to affect the impact of treatment with vitamin A on ALRI, but the Wald test for this interaction was not significant (Table 4).

Diarrheal disease. A total of 1311 episodes of diarrhea were observed with 23% of the children experiencing two or more episodes. The incidence of diarrhea for all age groups over the six treatment cycles was < one episode of diarrhea per child-year. The highest incidence was in the 1st treatment cycle (1.4 episodes per child-year) and with children < 24 mo of age (see **Figure 2**). There was no overall effect of the treatment with vitamin A on the incidence of diarrhea (Table 3), and this finding was consistently observed in all cycles.

There was a significant interaction between vitamin A and the age of the child on the incidence of diarrhea as illustrated in Figure 2. For children < 30 mo of age, the average rate ratio for diarrhea associated with vitamin A treatment was 1.19 (95% confidence interval:

TABLE 3

Cause-specific morbidity incidence of children aged 6–54 mo and the incidence rate ratios of vitamin A vs. placebo treatment groups

	Placebo			Vitamin A			
	Observed child-days	Episodes *n*	IR[1]	Observed child-days	Episodes *n*	IR	Rate ratio[2] (95% CI)[3]
Acute respiratory illness	239 611	5126	7.81	243 633	5609	8.41	1.08 (1.007–1.190)
Acute lower respiratory illness	273 630	73	0.97[4]	280 186	104	1.36[4]	1.39 (1.003–1.931)
Diarrhea	273 585	628	0.84	280 182	683	0.89	1.06 (0.920–1.225)

[1] IR = incidence rate per child-year.
[2] Adjusted for within-child correlation.
[3] CI = confidence interval.
[4] Incidence rate per 10 child-years.

1.02, 1.40). The corresponding rate ratio for children over 30 mo of age was 0.82% (95% confidence interval: 0.56, 1.20). The *P*-value for the test of a difference of these two rate ratios (for the interaction term) was 0.01. At 18 mo the rate ratio (the ratio of the heights of the two curves in Figure 2) was 1.29, indicating that treatment with vitamin A increased the risk of diarrhea. However, at 36 mo of age, vitamin A tended to be protective with a rate ratio of 0.79. There was no interaction of stunting, wasting, or gender with the effect of vitamin A on the incidence of diarrhea (data are not presented).

The mean duration of diarrhea episodes was 1.6 ± 1.11 d, and only 1.8% of episodes lasted longer than 5 d. Treatment with vitamin A did not reduce the mean duration of diarrhea episodes (vitamin A 1.7 d, placebo 1.6 d, $P = 0.57$).

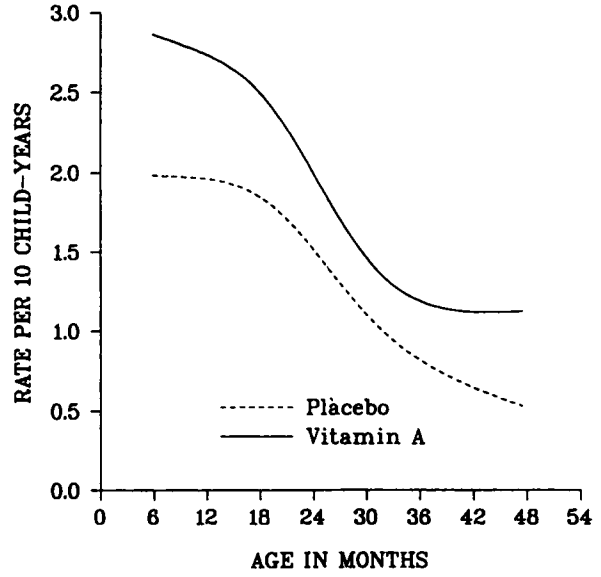

FIGURE 1 Incidence of Acute Lower Respiratory Infection (ALRI) by Age at Time of Episode by Treatment Group. Smoothed incidence curves adjusted for effects of the treatment cycle and number of cycles in the trial. The rate levels were determined using the child's 1st cycle in the trial and the average effect over all treatment cycles. The rate ratio for vitamin A vs. placebo did not vary significantly by age with $P = 0.44$ using the Wald test.

DISCUSSION

The most striking finding from the trial was the effect of high dose vitamin A on respiratory infections.

TABLE 4

Incidence of acute lower respiratory infections (ALRI) by nutritional status and gender, and the incidence rate ratios of vitamin A vs. placebo treatment groups

Incidence of ALRI	Placebo IR[2]	Vitamin A IR	Rate ratio[1] (95% CI)[3]	Significance level for interaction[4]
Nutritional status				
Stunted	4.412	3.118	0.71 (0.375–1.331)	0.01
Not stunted	1.307	2.394	1.83 (1.257–2.669)	
Gender				
Male	2.295	2.548	1.11 (0.709–1.739)	0.09
Female	1.306	2.573	1.97 (1.225–3.169)	

[1] Adjusted for within child correlation.
[2] IR = incidence rate per 10 child-years.
[3] CI = confidence interval.
[4] From Wald test.

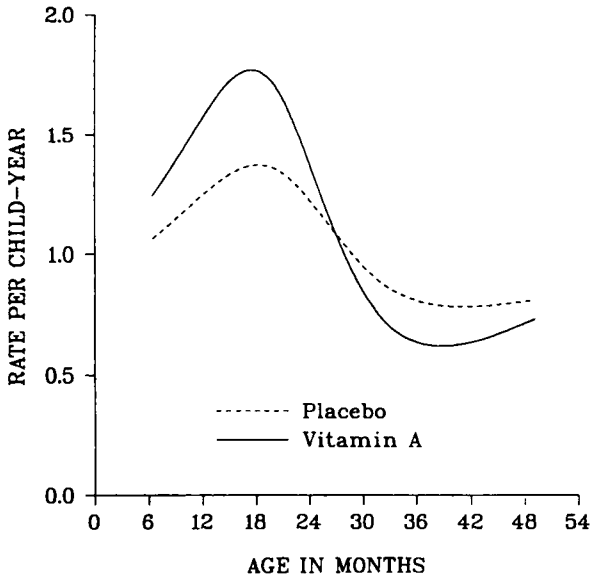

FIGURE 2 Incidence of Diarrhea by Age at Time of Episode by Treatment Group. Smoothed incidence curves adjusted for effects of the treatment cycle and number of cycles in the trial. The rate levels were determined using the child's 1st cycle in the trial and the average effect over all treatment cycles. The rate ratio for vitamin A vs. placebo did vary significantly by age with $P = 0.01$ using the Wald test.

Overall, vitamin A slightly increased (8%) the incidence rate of ARI, but substantially increased (39%) the incidence rate of ALRI. These deleterious effects of high dose vitamin A on ALRI were found mainly in children who had adequate nutritional status. In contrast, vitamin A did not increase the risk of ALRI in children who were stunted.

The physiological response to a high dose of vitamin A depends on the underlying vitamin A status of the child. In vitamin A deficiency, retinyl ester is rapidly absorbed in the liver and bound to the accumulated apo-retinol binding protein (Loerch et al. 1979). However, in normal adults, higher concentrations of plasma retinyl ester have been found following a high dose of vitamin A than are found in adults with clinical conditions involving fat malabsorption and vitamin A deficiency (Johnson et al. 1992). Thus high doses of vitamin A given to children with adequate stores might result in vitamin A being presented to the tissues, including the immune system, in a different way than would occur in a child with vitamin A deficiency.

Humoral and cell-mediated immune responses appear to be very sensitive to vitamin A. Both chronic excess or deficient vitamin A intake in animals depresses immune responses (Friedman et al. 1991, Friedman and Sklan 1989, Nauss 1986). It is plausible that high dose vitamin A administered to children with adequate vitamin A stores might cause a temporary immune suppression and lead to increased rates of infectious diseases. This would explain our findings of increased incidence of ALRI following treatment with vitamin A in subgroups of children who are more likely to have adequate vitamin A stores.

No consistent pattern of findings has been reported from six prospective randomized trials (Abdeljaber et al. 1991, Barreto et al. 1994, Bhandari et al. 1994, Ghana VAST Study Team 1993, Stansfield et al. 1993, Vijayaraghavan et al. 1990) that aimed to assess the role of high dose vitamin A in the primary prevention of childhood infectious diseases including acute respiratory tract infections. It is difficult to compare our findings on ALRI with these morbidity trials because most of them used less specific and less sensitive case definitions of respiratory illness.

Three of these studies (Abdeljaber et al. 1991, Ghana VAST Study Team 1993, Vijayaraghavan et al. 1990) reported no effect of vitamin A on the prevalence of respiratory symptoms including cough, reported difficulty in breathing and rapid breathing. In contrast, a trial in Haiti (Stansfield et al. 1993) found that vitamin A significantly increased the prevalence of cough and reported rapid breathing. The variability in the findings from these studies might be related to the lack of specificity of the case definitions based on symptoms alone. However, these studies also differed in other important design aspects including the duration of the recall period for morbidity symptoms and the time between treatment and the measurement of morbidity.

Using methods similar to our own, a morbidity trial from Brazil (Barreto et al. 1994) found no effect of vitamin A on the incidence of ALRI. However, this trial, using 50 respirations per minute to define elevated respiratory rates, reported incidence rates of ALRI that were 2–3 times higher than those we observed. Variations in the precision of respiratory rate measurements between studies could result in apparent differences in rates of ALRI. Studies with less precise respiratory rate measurements would tend to misclassify more children as having ALRI. The recent trial conducted in India (Bhandari et al. 1994) also measured respiratory rates to define cases of ALRI but used 40 respirations per minute to define elevated respiratory rates. As would be expected with this case definition, the reported incidence of ALRI was very high. No difference was observed in the incidence of ALRI between treatment groups; however, the mean daily prevalence of ALRI was 42% higher in the vitamin A–supplemented children < 23 mo of age. Finally, none of the six trials reported the effect of high dose vitamin A in nonstunted children, the group in which we found the adverse effect of supplementation.

Meta-analyses of community-based vitamin A mortality trials have reported either no effect (Beaton et al. 1993) or a slight increase (Glasziou and Mackerras 1993) in deaths from respiratory disease following treatment with vitamin A. Our findings would suggest that vitamin A might have reduced respiratory deaths only in the subpopulations with stunting. It

may have been a risk factor for respiratory disease deaths in the subpopulations with adequate nutritional status. This possibility would explain why treatment with high dose vitamin A does not appear to consistently protect against pneumonia deaths in mortality studies (Daulaire et al. 1992, West et al. 1991).

Vitamin A supplementation appeared to have no overall effect on the incidence or duration of diarrhea, but we found a significant interaction between treatment with vitamin A and age on the incidence of diarrhea. Observational epidemiological studies from Thailand (Bloem et al. 1990) and India (Milton et al. 1987) suggest that vitamin A deficiency is only a weak risk factor for diarrhea, although these studies did not examine the relationship with severe diarrhea. Our findings of little overall effect of vitamin A on the incidence of diarrhea but a small reduction in children > 30 mo of age are consistent with these observational studies. Only two prospective morbidity trials have reported age-stratified analyses of the effects of vitamin A on diarrhea. The Thailand trial (Bloem et al. 1990) found a tendency for protection in older children and a tendency for increased diarrhea rates in younger children, whereas the Haiti trial (Stansfield et al. 1993) found no interaction between vitamin A and age on diarrhea rates.

A limitation of our trial was the low incidence of diarrhea observed in the study population, making it more difficult to detect differences between the treatment groups. A review of five longitudinal studies in developing countries that used frequent home-based surveillance to monitor diarrhea in preschool-age children reported a median of three episodes per child-year (Snyder and Merson 1982). It is possible that the protective effect of vitamin A supplements we noted in older children might be more prominent in populations with higher rates of more severe diarrhea.

The lack of impact of high dose vitamin A supplementation on diarrhea incidence contrasts with the reported 30–40% reduction in diarrheal disease deaths in vitamin A mortality studies (Beaton et al. 1993, Fawiz et al. 1993, Glasziou and Mackerras 1993). It seems unlikely that vitamin A causes this reduction in diarrheal disease deaths through a lower overall incidence of diarrhea. More likely explanations are that vitamin A protects against severe episodes of diarrhea as was reported in the Brazil trial (Barreto et al. 1994) or that it reduces the severity of measles-associated diarrhea (Latham 1993). We were unable to test either of these hypotheses because of the mild nature of diarrhea and the lack of measles in our study population.

In summary, our findings indicate that the distribution of high dose vitamin A to preschool children has little role to play in the primary prevention of diarrheal diseases or acute respiratory infections. It is possible that the increased incidence of ALRI we observed in children with adequate nutritional status following supplementation might have occurred by chance. However, such an outcome is biologically plausible given the immunological activity of vitamin A and the different physiological response to high dose supplements in subjects with adequate nutritional status. Furthermore, most of the other trials that assessed the impact of vitamin A supplements on respiratory illness used less specific case definitions than were used in this trial. The apparent identification of a significant adverse effect of high dose vitamin A supplementation on adequately nourished children should cause nutrition program managers to review carefully which populations of preschool-age children should receive high dose vitamin A supplements.

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LITERATURE CITED

Abdeljaber, M. H., Monto, A. S., Tilden, R. L., Schork, A. & Tarwotjo, I. (1991) The impact of vitamin A supplementation on morbidity: a randomized community intervention trial. Am. J. Public Health 81: 1654–1656.

ACC/SCN, Administrative Committee on Coordination/Subcommittee on Nutrition (1987) Second Report on the World Nutrition Situation, p. 29. United Nations, Geneva, Switzerland.

Arroyave, G., Chichester, C. O., Flores, H., Glover J., Mejia, L. A., Olson, J. A., Simpson, K. L. & Underwood, B. A. (1982) Biochemical Methodology for the Assessment of Vitamin A Status. The Nutrition Foundation, Inc., Washington, DC.

Barreto, M. L., Santos, L.M.P., Assis, A.M.O., Araújo, M.P.N., Farenzena, G. G., Santos, P.A.B. & Fiaccone, R. L. (1994) Effect of vitamin A supplementation on diarrhea and acute lower-re-

spiratory tract infections in young children in Brazil. Lancet 344: 228–231.

Beaton, G. H., Martorell, R., Aronson, K. J., Edmonston, B., McCabe, G., Ross, A. C. & Harvey, B. (1993) Effectiveness of Vitamin A Supplementation in the Control of Young Child Morbidity and Mortality in Developing Countries. ACC/SCN State-of-the-Art Series, Nutrition Policy Discussion Paper No. 13. United Nations, Geneva, Switzerland.

Bhandari, N., Bhan, M. K. & Sazawal, S. (1994) Impact of massive dose of vitamin A given to preschool children with acute diarrhea on subsequent respiratory and diarrheal morbidity. Br. Med. J. 309: 1404–1407.

Bloem, M. W., Wedel, M., Egger, R. J., Speek, A. J., Schrijver, J., Saowakontha, S. & Schreurs, W.H.P. (1990) Mild vitamin A deficiency and risk of respiratory tract disease and diarrhea in preschool children and school children in northeastern Thailand. Am. J. Epidemiol. 131: 332–339.

Cochrane, W. G. & Cox, G. M. (1950) Experimental Designs. John Wiley Co., New York, N.Y.

Corner, G. (1989) dSURVEY User Manual. Helkorn Pty. Ltd., Pearce, ACT 2607, Australia.

Daulaire, N.M.P., Starbuck, E. S., Houston, R. M., Church, M. S., Stukel, T. A. & Pandey, M. R. (1992) Childhood mortality after a high dose of vitamin A in a high risk population. Br. Med. J. 304: 207–210.

Dibley, M. J., Goldsby, J. B., Staehling, N. W. & Trowbridge, F. L. (1987) Development of normalized curves for the international growth reference: historical and technical considerations. Am. J. Clin. Nutr. 46: 736–48.

Fawiz, W. W., Chalmers, T. C., Herrera, M. G. & Mosteller, F. (1993) Vitamin A supplementation and child mortality: a meta-analysis. J. Am. Med. Assoc. 269: 898–903.

Friedman, A., Meidovsky, A., Leitner, G. & Sklan, D. (1991) Decreased resistance and immune response to *Escherichia coli* infection in chicks with low or high intakes of vitamin A. J. Nutr. 121: 395–400.

Friedman, A. & Sklan, D. (1989) Antigen-specific immune response impairment in the chick as influenced by dietary vitamin A. J. Nutr. 119: 790–795.

Ghana VAST Study Team (1993) Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions, and child mortality. Lancet 342: 7–12.

Glasziou, P. P. & Mackerras, D.E.M. (1993) Vitamin A supplementation in infectious diseases: a meta-analysis. Br. Med. J. 306: 366–370.

Habicht, J-P. (1973) Standardization procedures for quantitative epidemiologic field methods. In: Manual of Internationally Comparable Growth Studies in Latin America and the Caribbean. Pan American Health Organization, Washington, DC.

Harrell, F. E. Jr., Lee, K. L. & Pollock, B. G. (1988) Regression models in clinical studies: determining relationships between predictors and response. J. Natl. Cancer Inst. 80: 1198–1202.

Johnson, E. J., Krasinski, S. D., Howard, L. J. & Alger, S. A. (1992) Evaluation of vitamin A absorption by using oil-soluble and water-miscible vitamin A preparations in normal adults and in patients with gastrointestinal disease. Am. J. Clin. Nutr. 55: 857–864.

Latham, M. (1993) Vitamin A and child mortality. Lancet 342: 549.

Loerch, J. D., Underwood, B. A. & Lewis, K. C. (1979) Response of plasma levels of vitamin A to a dose of vitamin A as an indicator of hepatic vitamin A reserves in rats. J. Nutr. 109: 778–786.

Milton, R. C., Reddy, V. & Naidu, A. N. (1987) Mild vitamin A deficiency and childhood morbidity: an Indian experience. Am. J. Clin. Nutr. 46: 827–829.

Moulton, L. H. (1993) The Weibull/GEE Model for Multivariate Survival Analysis. The Biometric Society ENAR Spring Meeting, Philadelphia, PA.

Nauss, K. M. (1986) Influence of vitamin A status on the immune system. In: Vitamin A Deficiency and Its Control (Bauernfeind, J. C., ed.), pp. 207–43. Academic Press, Orlando, FL.

Rahmathullah, L., Underwood, B. A., Thulasiraj, R. D. & Milton, R. C. (1991) Diarrhea, respiratory infections and growth are not affected by a weekly low-dose vitamin A supplement: a masked, controlled field trial in children in southern India. Am. J. Clin. Nutr. 54: 568–77.

SAS Institute Inc. (1991) SAS Language and Procedures: Usage 2, version 6, 1st ed. SAS Institute Inc., Cary, NC.

Selwyn, B. J. (1990) The epidemiology of acute respiratory tract infection in young children: comparison of findings from several developing countries. Rev. Infect. Dis. 12(Suppl. 8): S870–S888.

Snyder, J. D. & Merson, M. M. (1982) The magnitude of the global problem of acute diarrheal disease: a review of active surveillance. Bull. WHO 60: 605–613.

Sommer, A., Katz, J. & Tarwotjo, I. (1984) Increased risk of respiratory disease and diarrhea in children with pre-existing mild vitamin A deficiency. Am. J. Clin. Nutr. 40: 1090–1095.

Sommer, A., Tarwotjo, I., Djunaedi, E., West, K. P., Jr., Loeden, A. A., Tilden, R., Mele, L. & The Aceh Study Group (1986) Impact of vitamin A supplementation on childhood mortality: a randomized, controlled community trial. Lancet 1: 1169–1173.

Stansfield, S. K., Pierre-Louis, M., Lerebours, G. & Augustin, A. (1993) Vitamin A supplementation and increased prevalence of childhood diarrhoea and acute respiratory infections. Lancet 342: 578–582.

Vijayaraghavan, K., Radhaih, G., Prakasam, B. S., Sarma, K.V.R. & Reddy, V. (1990) Effect of massive dose vitamin A on morbidity and mortality in Indian children. Lancet 336: 1342–1345.

Waterlow, J. C., Buzina, R., Keller, W., Lane, J. M., Nichaman, M. Z. & Tanner, J. M. (1977) The presentation and use of height and weight data for comparing nutritional status groups of children under the age of 10 years. Bull. WHO 55: 489–98.

West, K. P., Jr., Pokhrel, R. P., Katz, J., LeClerq, S. C., Khatry, S. K., Shrestha, S. C., Pradhan, E. K., Tielsch, J. M., Pandey, M. R. & Sommer, A. (1991) Efficacy of vitamin A in reducing preschool child mortality in Nepal. Lancet 338: 67–71.

World Health Organization (1982) Control of Vitamin A Deficiency and Xerophthalmia. Tech. Rept. Ser. 672. WHO, Geneva, Switzerland.

World Health Organization Programme for the Control of Acute Respiratory Infections (1990) Acute respiratory infections in children: case management in small hospitals in developing countries. A manual for doctors and other senior health workers. Unpublished document WHO/ARI/90.5.

Wilkinson, L. (1990) SYSTAT version 5.02. SYSTAT Inc., Evanston, IL.

Zeger, S. L. & Liang, K.-Y. (1986) Longitudinal data analysis for discrete and continuous outcomes. Biometrika 42: 121–130.