

VITAMIN A SUPPLEMENTATION FOR EXTREMELY-LOW-BIRTH-WEIGHT INFANTS

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ABSTRACT

Background Vitamin A supplementation may reduce the risk of chronic lung disease and sepsis in extremely-low-birth-weight infants. The results of our pilot study suggested that a dose of 5000 IU administered intramuscularly three times per week for four weeks was more effective than the lower doses given in past trials.

Methods We performed a multicenter, blinded, randomized trial to assess the effectiveness and safety of this regimen as compared with sham treatment in 807 infants in need of respiratory support 24 hours after birth. The mean birth weight was 770 g in the vitamin A group and 769 g in the control group, and the respective gestational ages were 26.8 and 26.7 weeks.

Results By 36 weeks' postmenstrual age, 59 of the 405 infants (15 percent) in the vitamin A group and 55 of the 402 infants (14 percent) in the control group had died. The primary outcome — death or chronic lung disease at 36 weeks' postmenstrual age — occurred in significantly fewer infants in the vitamin A group than in the control group (55 percent vs. 62 percent; relative risk, 0.89; 95 percent confidence interval, 0.80 to 0.99). Overall, 1 additional infant survived without chronic lung disease for every 14 to 15 infants who received vitamin A supplements. The proportions of infants in the vitamin A group and the control group who had signs of potential vitamin A toxicity were similar. The proportion of infants with serum retinol values below 20 μg per deciliter (0.70 μmol per liter) was lower in the vitamin A group than in the control group (25 percent vs. 54 percent, $P < 0.001$).

Conclusions Intramuscular administration of 5000 IU of vitamin A three times per week for four weeks reduced biochemical evidence of vitamin A deficiency and slightly decreased the risk of chronic lung disease in extremely-low-birth-weight infants. (N Engl J Med 1999;340:1962-8.)

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INFANTS with extremely low birth weights (≤ 1000 g) have low plasma and tissue concentrations of vitamin A,^{1,3} and vitamin A deficiency may predispose these infants to chronic lung disease.⁴ A meta-analysis⁵ of clinical trials of vitamin A supplementation for preterm infants⁶⁻⁹ revealed a 17 percent increase in the rate of survival without chronic lung disease, which approached statistical significance. The current relevance of these trials is lim-

ited by changes in the vitamin A concentration in infant formulas and by the use of antenatal glucocorticoid and postnatal surfactant therapy. Moreover, in a pilot study⁵ we found that many infants given the dose of vitamin A administered in these trials had low serum retinol concentrations.^{3,7,10}

We conducted a multicenter, randomized trial to determine the effectiveness and safety of a higher dose of vitamin A than used in previous trials in extremely-low-birth-weight infants. We hypothesized that vitamin A supplementation would increase the rate of survival without chronic lung disease and, on the basis of prior studies,^{6,9,11,12} reduce the risk of hospital-acquired sepsis.

METHODS

Study Subjects and Design

We studied 807 infants with birth weights ranging from 401 to 1000 g who received mechanical ventilation or supplemental oxygen at 24 hours of age in one of the participating centers (see the Appendix) between January 1996 and July 1997. To reduce the likelihood of early death that was unrelated to vitamin A status and to facilitate enrollment, we enrolled infants 24 to 96 hours after birth. We excluded infants with major congenital anomalies, those with congenital nonbacterial infection, those thought to have a terminal illness (as indicated by a pH below 6.80 or by the presence of hypoxia with bradycardia for more than two hours), and those who were to receive vitamin A in a parenteral fat emulsion or in doses exceeding recommendations for multivitamin preparations. The study was approved by the institutional review board at each center, and informed consent was obtained for each infant.

The infants were stratified according to center and birth weight (401 to 750 g or 751 to 1000 g) and assigned to the vitamin A or control group by a hospital pharmacist using a randomization list

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(or, at four centers, by a research nurse using sealed envelopes containing the treatment assignments). Vitamin A was given intramuscularly because of its poor enteral absorption^{5,13} and unreliable delivery in crystalloid solutions¹⁴: a dose of 5000 IU (0.1 ml) was given on Mondays, Wednesdays, and Fridays for four weeks (study days 0 to 25 or 26) in a 0.3-ml insulin syringe (Becton Dickinson, Cockeysville, Md.) with a 29-gauge needle. The same dose was used regardless of birth weight, because the smallest infants have the highest incidence of chronic lung disease, the lowest vitamin A stores at birth,² and the lowest enteral intakes.^{15,16} The vitamin A preparation (Aquasol A, Astra USA, Westborough, Mass.) was refrigerated and shielded at all times from direct light. The concentration (50,000 IU per milliliter) of 11 vials was independently verified. To avoid pain and potential side effects and to facilitate enrollment, control infants received a sham procedure rather than placebo injections. For each treatment, a screen was placed around the bed, a pacifier was used to minimize crying after injections, and the injection site was covered with an adhesive bandage (or gauze in areas where the skin was fragile). The same covering was placed on control infants. The research nurse removed the covering at the next treatment. With the small needle, the injection site was either not visible or visible only on close inspection.

To assess vitamin A supplementation under usual clinical circumstances, we made sure that the staff of the neonatal intensive care unit retained responsibility for decisions regarding the use of therapies other than vitamin A, including parenteral fluids, mechanical ventilation, and glucocorticoids.

Evaluation

All vitamins and enteral feedings provided on the 12 treatment days were recorded for the first 247 infants. Experienced personnel (usually a neonatologist or fellow) who were not involved in the infants' care assessed the infants for signs of potential vitamin A toxicity¹⁷ each week for four weeks (on the days of the 3rd, 6th, 9th, and 12th treatment procedures). The examiners assessed the fontanelle, fronto-occipital circumference, and liver size and checked each infant for edema, cutaneous abnormalities, bony tenderness, lethargy, and irritability. They also assessed whether any abnormal findings could be explained by factors other than vitamin A toxicity. If vitamin A toxicity was suspected, the attending neonatologist decided whether to continue treatment on the basis of clinical findings without knowledge of the infant's treatment assignment.

Study Outcomes

Chronic lung disease was defined as the need for oxygen at 36 weeks' postmenstrual age (calculated at birth according to the original method of Ballard et al.¹⁸). Radiologic findings were not assessed, because the need for oxygen at 36 weeks' postmenstrual age is predictive of later pulmonary complications^{19,20} and because we wished to avoid unnecessary exposure to radiation, expense, variability in interpretations, and the logistic problems of central interpretations. Sepsis was defined on the basis of a positive blood culture and treatment with antibiotics for at least five days (unless the infant died within five days). Research nurses recorded and transmitted all data electronically to the biostatistical coordinating center. To verify that chronic lung disease was reliably recorded, the biostatistical coordinating center independently assessed the hospital records of 210 randomly selected infants. Chronic lung disease was erroneously reported to be present in two infants in the vitamin A group and two infants in the control group.

Studies of Vitamin A Metabolism

Serum vitamin A was measured in a central laboratory (University of Illinois at Chicago, Chicago) at base line and at 28 days in the first 300 infants. On study day 28 (two to three days after the last treatment and immediately after a blood sample was collected), the relative dose-response was evaluated. A high relative dose-response is considered a sensitive indicator of vitamin A deficiency

in premature infants^{21,22} and other subjects.^{23,24} As recommended by Jayant Shenai (personal communication), a dose of 2000 IU per kilogram of body weight was administered, and a second blood sample was obtained three hours later. The relative dose-response was calculated as the change in the serum retinol concentration divided by the preinjection concentration. On the basis of previous work²¹ and data for our control infants, we designated a relative dose-response of more than 10 percent as high. When study infants died and an autopsy was performed within 24 hours, liver and lung samples were obtained and frozen at -20°C for subsequent analysis.

We did not measure serum retinol frequently or discontinue supplementation with vitamin A when high concentrations were identified, because of uncertainty about the serum retinol concentration that is desirable in extremely-low-birth-weight infants, difficulty in interpreting the results after the administration of glucocorticoids (which appear to raise serum retinol concentrations²⁵ by reducing tissue concentrations²⁶), the low serum concentrations and absence of toxicity in our pilot study,⁵ and the expense of weekly determinations for all infants.

Serum retinol and retinyl esters (palmitate and stearate) were measured by high-performance liquid chromatography,²⁷ and serum retinol-binding protein was measured by radial immunodiffusion (Behring Diagnostics, Somerville, N.J.). The interassay coefficients of variation were 2.5 to 3.4 percent for retinol and 8.5 percent for retinol-binding protein. Samples were routinely analyzed in duplicate. Postmortem samples of lung and liver were homogenized in distilled water-methanol (50:50 vol/vol) containing 1 percent pyrogallol and saponified for one hour at 70°C with 10 percent potassium hydroxide (in the final mixture). The samples were extracted with hexane and analyzed by high-performance liquid chromatography. This method, which includes saponification before extraction,²⁸ converts retinyl esters to retinol, with the result that the liver retinol concentrations recorded were substantially higher than in our pilot study⁵ and other neonatal studies.²⁹

Statistical Analysis

Data were analyzed with SAS software (SAS Institute, Cary, N.C.). The two groups were compared according to the intention to treat with the use of Student's *t*-tests, the Wilcoxon rank-sum tests, chi-square analysis, and Fisher's exact tests. The primary outcome was adjusted for center and birth weight by the Mantel-Haenszel procedure. An independent data safety and monitoring committee used the Lan-DeMets procedure with an O'Brien-Fleming spending function to assess the intervention. One interim analysis was performed.

RESULTS

Characteristics of the Patients

Of 1939 extremely-low-birth-weight infants who were screened for enrollment, 689 (36 percent) were ineligible (65 percent of these infants were considered to be terminally ill and 24 percent had not been treated with mechanical ventilation or oxygen at 24 hours). Among the remaining 1250 infants, we enrolled 807. The remaining 443 eligible infants were not enrolled because of parental refusal (68 percent), failure to seek consent or unavailability of the parents (26 percent), or physician refusal (6 percent). The base-line characteristics of the 405 infants in the vitamin A group and the 402 infants in the control group were similar (Table 1).

Effectiveness of Supplemental Vitamin A

The infants in the vitamin A group received 99 percent of the intended doses. The vitamin A intakes

TABLE 1. BASE-LINE CHARACTERISTICS OF THE INFANTS.*

CHARACTERISTIC	VITAMIN A GROUP (N=405)	CONTROL GROUP (N=402)
Birth weight — g	770±135	769±138
Gestational age — wk	26.8±1.9	26.7±1.7
Small for gestational age — no./total no. (%)	86/311 (28)	94/311 (30)
Race or ethnic group — no. (%)†		
Black	198 (49)	189 (47)
White	133 (33)	137 (34)
Hispanic	64 (16)	62 (15)
Other	10 (2)	14 (3)
Male sex — no. (%)	202 (50)	189 (47)
Antenatal glucocorticoid therapy — no. (%)	307 (76)	298 (74)
Apgar score ≤3 — no./total no. (%)		
At 1 min	176/400 (44)	148/398 (37)
At 5 min	41/400 (10)	30/399 (8)
Delivery-room care — no. (%)		
Intubation	364 (90)	363 (90)
Drugs for resuscitation	42 (10)	40 (10)
Surfactant administration — no. (%)	337 (83)	329 (82)
Respiratory status at 24 hr		
Mechanical ventilation — no. (%)	369 (91)	367 (91)
Mean airway pressure — cm of water	6.9±2.6	6.9±2.4
Fraction of inspired oxygen	0.41±0.19	0.41±0.20
Serum retinol — μg/dl‡	16.2±6.2	16.2±6.2
Time from birth to randomization — hr	60.8±21.0	60.5±20.6
Time from birth to first treatment — hr	64.4±20.3	64.1±19.7

*Plus-minus values are means ±SD.

†Because of rounding, percentages may not total 100.

‡To convert values for serum retinol to micromoles per liter, multiply by 0.035.

from other sources were similar in the two groups (Fig. 1).

The primary outcome — chronic lung disease or death by 36 weeks' postmenstrual age — occurred in fewer infants in the vitamin A group than in the control group (55 percent vs. 62 percent; relative risk, 0.89; 95 percent confidence interval, 0.80 to 0.99; $P=0.03$) (Table 2). Overall, 1 additional infant survived without chronic lung disease for every 14 to 15 infants who were treated with vitamin A (calculated as the inverse of the difference in risk, $1 \div (0.62 - 0.55) = 14.3$).³⁰

The relative risk of chronic lung disease or death among infants in the vitamin A group as compared with those in the control group ranged from 0.66 to 1.11 at the various centers. A test of homogeneity indicated that this variation was within that expected by chance. The relative risk of chronic lung disease or death was 0.94 (95 percent confidence interval, 0.82 to 1.06) among infants with birth weights of 401 to 750 g and 0.83 (95 percent confidence interval, 0.68 to 1.02) among infants with birth weights of 751 to 1000 g.

Although the mortality rates were similar in the two groups (Table 2), the percentage of infants with chronic lung disease who were alive at 36 weeks' postmenstrual age was lower in the vitamin A group than in the control group (47 percent vs. 56 percent; relative risk, 0.85; 95 percent confidence interval, 0.73 to 0.98; $P=0.03$). There were no significant differences between groups in the other outcomes (Table 2).

Safety of Vitamin A Supplementation

All physical findings were similar in the two groups (data not shown). A suspected or definite increase in fontanelle tension was recorded in 15 percent of infants treated with vitamin A and 18 percent of infants in the control group ($P=0.26$). Signs of potential vitamin A toxicity that could not be explained by other factors (e.g., posthemorrhagic hydrocephalus causing a full fontanelle) were identified in 1.0 percent of infants in the vitamin A group and 0.8 percent of infants in the control group. Physical findings were also similar in analyses that compared infants with serum retinol, retinyl esters, and total retinol equivalents in the upper quintile with infants with lower values.

The serum concentrations of retinol, retinol-binding protein, and retinyl esters were higher in the vitamin A group than in the control group, irrespective of whether glucocorticoid therapy had been given postnatally ($P<0.001$) (Table 3). Fewer infants in the vitamin A group than in the control group had serum retinol concentrations below 20 μg per deciliter (0.70 μmol per liter) on day 28 (25 percent vs. 54 percent for all infants; 41 percent vs. 73 percent for those who had not received glucocorticoid therapy in the prior two weeks; $P<0.001$). No infant in the vitamin A group had a value below 10 μg per deciliter (0.35 μmol per liter). In contrast, 14 percent of all infants in the control group and 20 percent of those who had not recently been given glucocorticoid therapy had a value below 10 μg per deciliter ($P<0.001$). A relative dose-response of more than 10 percent, suggesting marginal or low vitamin A status, was found in 22 percent of infants in the vitamin A group and 45 percent of infants in the control group ($P<0.001$) (Table 3).

Among infants who were not given glucocorticoid therapy postnatally, the median liver retinol concentration was 141 μg per gram of tissue in six infants in the vitamin A group and 69 μg per gram in six infants in the control group; the corresponding concentrations of retinol in the lung were 0.62 and 0.64 μg per gram. Among infants who were given glucocorticoid therapy postnatally, the median liver retinol concentration was 317 μg per gram in six infants in the vitamin A group and 43.5 μg per gram in one infant in the control group; the corresponding values in the lung were 0.91 and 0.16 μg per gram.

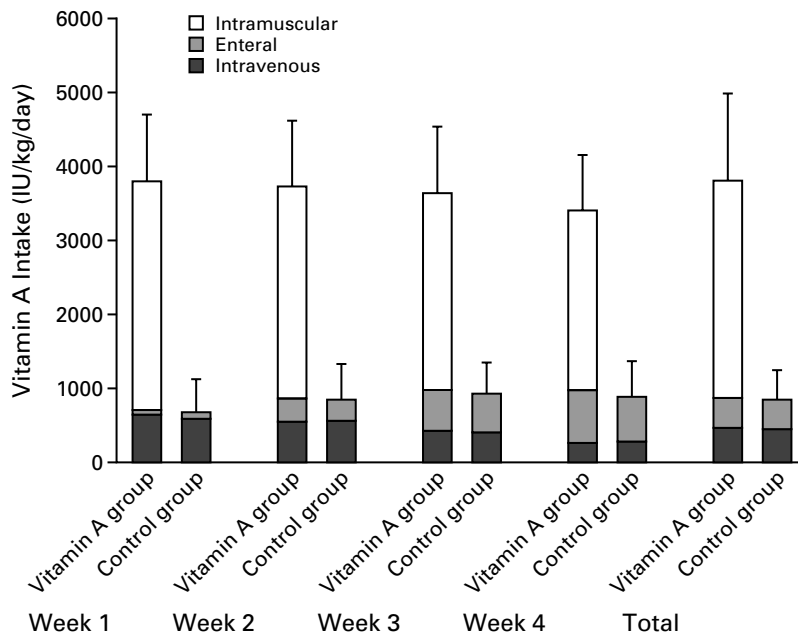


Figure 1. Mean (+SD) Daily Vitamin A Intake in the Two Groups. Vitamin A supplementation was given intramuscularly on Mondays, Wednesdays, and Fridays for four weeks.

TABLE 2. RELATIVE RISKS OF DEATH AND OTHER OUTCOMES.

OUTCOME	VITAMIN A GROUP (N=405)	CONTROL GROUP (N=402)	RELATIVE RISK (95% CI)*	P VALUE
Chronic lung disease or death by 36 wk postmenstrual age — no. (%)	222 (55)	248 (62)	0.89 (0.80–0.99)	0.03†
Death by 36 wk postmenstrual age — no. (%)	59 (15)	55 (14)	1.07 (0.76–1.50)	0.72
Death before discharge — no. (%)	67 (17)	66 (16)	1.01 (0.74–1.38)	0.96
Survival with chronic lung disease at 36 wk postmenstrual age — no./total no. (%)	163/346 (47)	193/347 (56)	0.85 (0.73–0.98)	0.03
Mechanical ventilation — no./total no. (%)	24/346 (7)	22/347 (6)	1.09 (0.63–1.91)	0.76
Median fraction of inspired oxygen (5th, 95th percentiles)	0.21 (0.21, 1.0)	0.25 (0.21, 1.0)		0.12‡
Oxygen at 28 days postnatal age — no./total no. (%)	265/362 (73)	269/356 (76)	0.97 (0.89–1.06)	0.47
Mechanical ventilation — no./total no. (%)	168/362 (46)	171/356 (48)	0.97 (0.83–1.13)	0.66
Median fraction of inspired oxygen (5th, 95th percentiles)	0.30 (0.21, 0.80)	0.30 (0.21, 0.80)		0.88‡
Hospital-acquired sepsis treated with antibiotics ≥5 days — no. (%)	155 (38)	170 (42)	0.91 (0.77–1.07)	0.25
Intracranial hemorrhage — no. (%)	168 (41)	180 (45)	0.93 (0.80–1.09)	0.39
Grade 3 or 4	73 (18)	78 (19)	0.94 (0.70–1.25)	0.65
Periventricular leukomalacia — no./total no. (%)	23/328 (7)	30/318 (9)	0.74 (0.44–1.25)	0.26
Death, grade 3 or 4 intracranial hemorrhage, or periventricular leukomalacia — no. (%)	128 (32)	136 (34)	0.93 (0.77–1.14)	0.50
Necrotizing enterocolitis — no. (%)	47 (12)	51 (13)	0.92 (0.63–1.33)	0.65
Death or necrotizing enterocolitis — no. (%)	95 (23)	89 (22)	1.06 (0.82–1.37)	0.64
Length of hospitalization — median (5th, 95th percentile)				
Infants who survived	94 (58, 174)	92 (54, 173)		0.44‡
Infants who died	19 (5, 211)	19 (4, 140)		0.66‡

*Relative risks are for the occurrence of the outcome in the vitamin A group as compared with the control group. CI denotes confidence interval.

†The primary outcome variable was adjusted for center and birth weight by the Mantel-Haenszel procedure.

‡The P value was calculated with use of the Wilcoxon rank-sum test.

TABLE 3. SERUM ANALYSES ON DAY 28, AFTER THE COMPLETION OF TREATMENT.*

VARIABLE	VITAMIN A GROUP (N=155)	CONTROL GROUP (N=145)	P VALUE†
	median (5th, 95th percentiles)		
Serum retinol — $\mu\text{g}/\text{dl}$	29.8 (12.9, 83.7)	17.8 (7.4, 63.1)	<0.001
No postnatal glucocorticoid therapy within 2 wk before sampling	22.6 (11.6, 57.3)	13.0 (6.4, 28.3)	<0.001‡
Postnatal glucocorticoid therapy within 2 wk before sampling	45.2 (16.5, 87.9)	27.6 (8.6, 76.7)	<0.001‡
Serum retinol-binding protein — mg/dl	2.4 (1.0, 5.8)	1.8 (0.7, 5.6)	<0.001
No postnatal glucocorticoid therapy within 2 wk before sampling	1.8 (0.9, 2.8)	1.5 (0.7, 3.1)	0.01‡
Postnatal glucocorticoid therapy within 2 wk before sampling	3.6 (1.1, 6.5)	2.7 (0.8, 6.8)	0.02‡
Serum retinyl esters — $\mu\text{g}/\text{dl}$	27.2 (5.8, 110.4)	6.6 (0, 45.3)	<0.001
No postnatal glucocorticoid therapy within 2 wk before sampling	22.7 (4.7, 88.8)	7.4 (0, 48.8)	<0.001‡
Postnatal glucocorticoid therapy within 2 wk before sampling	37.7 (8.0, 117.4)	6.2 (0, 35.9)	<0.001‡
Relative dose–response — %§	2.9 (–10.3, 17.6)	7.3 (–6.2, 48.6)	<0.001
Relative dose–response >10% — no. (%)	34 (22)	65 (45)	<0.001

*Measurements were made at base line and at day 28 in the first 300 infants. To convert values for serum retinol, retinol-binding protein, and retinyl esters to micromoles per liter, multiply by 0.035, 0.47, and 0.019, respectively.

†P values were calculated with use of the Wilcoxon rank-sum test.

‡For this subgroup analysis, a P value of less than 0.025 was considered to indicate a significant difference (Bonferroni's adjustment).

§The relative dose–response was calculated as the change in the serum retinol concentration in response to a dose of 2000 IU of vitamin A per kilogram divided by the preinjection concentration. A value of more than 10 percent was considered high.

DISCUSSION

Our findings add to the evidence^{1,2,5,10,14-16,21,22} that vitamin A intake is inadequate in extremely-low-birth-weight infants and that as reported by Shenai and colleagues,⁶ vitamin A supplementation can reduce the risk of chronic lung disease. Vitamin A deficiency may promote chronic lung disease by impairing lung healing, increasing the loss of cilia, increasing squamous-cell metaplasia, increasing susceptibility to infection, and decreasing the number of alveoli.^{6,31} Extremely-low-birth-weight infants are prone to vitamin A deficiency because they have low stores of the vitamin at birth,² minimal intake during feedings for several weeks or longer after birth, and poor enteral absorption of vitamin A^{5,13} and because intravenous delivery in crystalloid solutions is unreliable.¹⁴ In our study, serum retinol concentrations on study day 28 were low (<20 μg per deciliter)^{3,10,14,32} in 54 percent of infants in the control group and in 73 percent of infants in the control group who had not recently been treated with glucocorticoids; 20 percent of the latter infants had a concentration of less than 10 μg per deciliter, a value considered strongly indicative of vitamin A deficiency.³²

Although the dose of vitamin A that we used was larger than that given in previous trials, it can be considered conservative in terms of the resulting serum

retinol concentrations. Because of decreased transport of vitamin A from blood into tissues, less efficient utilization, or increased need for lung repair,^{6,15,16,21} the optimal serum retinol concentration in extremely-low-birth-weight infants might be higher than the mean value for U.S. children who are at least three years of age (37 to 45 μg per deciliter [1.30 to 1.67 μmol per liter]).³ Study of higher doses or alternative approaches to increasing tissue concentrations is warranted.

We found no clinical or biochemical evidence of vitamin A toxicity. Indeed, a questionable or definite increase in fontanelle tension — a sensitive sign of toxicity^{17,33,34} — was identified in somewhat fewer infants in the vitamin A group than in the control group. Increased serum concentrations of retinyl esters are expected because of the absorption of retinyl palmitate after intramuscular administration of vitamin A. Neither the absolute values for liver retinol concentrations nor the ratio of the median value for liver retinol among infants in the vitamin A group to that for infants in the control group³⁵ was in the range indicative of vitamin A toxicity²⁸ with our assay.

In other populations with a deficient vitamin A intake, a single, large enteral dose — 50,000 IU in term newborns or 200,000 IU in older children — safely^{33,34} reduces the risk of infection and death from

all causes.^{11,12} Studies in preterm infants suggest that the initiation of vitamin A supplementation soon after birth reduces the risk of intracranial hemorrhage,^{22,36} chronic lung disease, and infection.^{6,9}

In our trial, vitamin A supplementation significantly reduced the risk of chronic lung disease or death (relative risk, 0.89). It was also associated with nonsignificant trends toward reduced risks of sepsis (relative risk, 0.91), intracranial hemorrhage (relative risk, 0.93), and periventricular leukomalacia (relative risk, 0.74). Nevertheless, these values provide additional reassurance that the regimen is more likely to be beneficial than harmful.

We conclude that the vitamin A regimen assessed in our study should be used in extremely-low-birth-weight infants requiring early respiratory support, because of its biologic plausibility, safety, and reasonable cost, and because a large multicenter trial and a well-performed meta-analysis of multiple small trials found it to be beneficial.^{5,37} Moreover, the number of patients (14 to 15) who needed to be treated in order to prevent 1 adverse outcome (chronic lung disease) was lower than for many widely used therapies. For example, at most hospitals (those with 4 cases or fewer per 1000 births), at least 250 infants would need to be given antibiotics in the intrapartum period to prevent 1 case of early-onset group B streptococcal sepsis, even if prophylaxis were 100 percent effective. Vitamin K would need to be given to at least 2000 healthy newborn infants who are being fed formula in order to prevent hemorrhagic disease in 1 such infant.³⁸

In conclusion, the regimen of vitamin A supplementation that we tested was safe, reduced biochemical evidence of vitamin A deficiency, and resulted in 1 fewer infant with chronic lung disease for every 14 to 15 infants given supplemental vitamin A. Our findings support the use of this regimen for extremely-low-birth-weight infants who require early respiratory support.

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APPENDIX

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