The Beneficial Effects of Weekly Low-dose Vitamin A Supplementation on Acute Lower Respiratory Infections and Diarrhea in Ecuadorian Children

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ABSTRACT. *Background.* **Previous studies of large-dose vitamin A supplementation on respiratory morbidity have produced conflicting results in a variety of populations. The influence of malnutrition has not been examined in the majority of these trials. We hypothesized that weekly low-dose vitamin A supplementation would prevent respiratory and diarrheal disease morbidity and that malnutrition might influence the efficacy of vitamin A supplementation.**

Methods. **In a randomized, double-blind, placebo-controlled field trial of 400 children, 6 to 36 months of age in a high Andean urban slum, half of the children received 10 000 IU of vitamin A weekly and half received placebo for 40 weeks. Children were visited weekly at home by physicians and assessed for acute diarrheal disease and acute respiratory infections.**

Results. **Acute diarrheal disease and acute respiratory infection did not differ globally or by severity between supplement-treated and placebo groups. However, the incidence of acute lower respiratory infection (ALRI) was significantly lower in underweight (weight-for-age z score [WAZ] <−2 SD) supplement-treated children than in underweight children on placebo (8.5 vs 22.3 per 10^3 child-weeks; rate ratio: 0.38 [95% CI: 0.17–0.85]). ALRI incidence was significantly higher in normal-weight (WAZ >−2 SD) supplement-treated children than in normal-weight children on placebo (9.8 vs 4.4 per 10^3 child-weeks; rate ratio: 2.21 [95% CI: 1.24–3.93]). By logistic regression analysis the risk of ALRI was lower in underweight supplement-treated children than in underweight children on placebo (point estimate 0.148 [95% CI: 0.034–0.634]). In contrast, risk of ALRI was higher in normal-weight supplement-treated children (WAZ >−1 SD to mean) than in normal-weight children on placebo in the same WAZ stratum (point estimate: 2.51 [95% CI: 1.24–5.05]). The risk of severe diarrhea was lower in supplement-treated children 18 to 23 months of age than in children on placebo in this age group (point estimate: 0.26 [95% CI: 0.06–1.00]).**

Conclusions. **Weekly low-dose (10 000 IU) vitamin A supplementation in a region of subclinical deficiency protected underweight children from ALRI and paradoxically increased ALRI in normal children with body weight over −1 SD. Protection from severe diarrhea was consistent with previous trials. Additional research is warranted to delineate potential beneficial and detrimental interactions between nutritional status and vitamin A supplementation regarding ALRI.** *Pediatrics* **1999;104(1). URL: http://www.pediatrics.org/cgi/content/full/104/1/e1; *vitamin A, lower respiratory infection, underweight children, diarrhea.***

ABBREVIATIONS. ARI, acute respiratory infections; ADD, acute diarrheal disease; AURI, acute upper respiratory infection; ALRI, acute lower respiratory infection; WAZ, weight-for-age Z score; HAZ, height-for-age z score; NCHS, National Center for Health Statistics; TMP/SMX, trimethoprim/sulfamethoxazole; RR, relative risk rates.

Vitamin A deficiency has been associated with increased rates of mortality among children.[1] Children with even subclinical vitamin A deficiency are at increased risk of developing acute respiratory infections (ARI).[2–4] Controlled trials to assess the impact of vitamin A supplementation on mortality risk have shown variable results with rate ratios of 0.5 to 1.04.[5,6] A recent meta-analysis based on these trials concluded that vitamin A supplementation in young children reduces the overall mortality rate ~22%.[7] In contrast, five recent community-based studies of vitamin A supplementation, when examined in another meta-analysis, did not find a protective or detrimental effect on pneumonia-specific mortality in young children 6 months to 5 years of age.[8] Moreover, the overall incidence of pneumonia was not affected, although some previous trials have suggested a possible increase in the risk of ARI symptoms.[9,10] Subsequent reports from Nepal, Indonesia, Tanzania, and Peru have suggested that vitamin A supplementation may be linked to adverse ARI effects.[11–14]

These findings are not consistent with the demonstrated positive impact vitamin A has on reducing global mortality,[6–8,15–17] because pneumonia contributes substantially to the burden of deaths in young children. This inconsistency mandates new studies to disclose the critical variables that could be associated with a protective role. Because previous trials admin-

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istering large-dose episodic vitamin A supplementation, including those in Latin America, failed to be protective against respiratory morbidity, and because a national survey in a sample of an impoverished children population showed 18% prevalence of biochemical deficiency of serum retinol (<20 μg/dL),[18] we hypothesized that weekly low-dose vitamin A supplementation would prevent respiratory and diarrheal disease morbidity. Because most pneumonia deaths occur in malnourished children and based on the clearly demonstrated benefit of vitamin A supplementation to reduce respiratory morbidity and mortality by measles in these children,[19,20] we also hypothesized that subpopulation analysis based on nutritional status might explain those previous paradoxical findings.

To test these hypothesis we conducted a placebo-controlled, randomized, double-blind trial of weekly low-dose vitamin A supplementation in a high Andean urban slum. Children 6 to 36 months of age were followed intensively on a weekly basis for ARI and acute diarrheal disease (ADD) during 40 weeks. Nutritional status was assessed in all children participating at the start and at the end of the supplementation period as well as serum retinol in a subset of children.

METHODS

Study Area and Population

The study was conducted at Comité del Pueblo (People's Committee) neighborhood between July 1996 and April 1997, at the northwestern region of the city of Quito, Ecuador (2800 meters above sea level). Electricity is present, but no municipal source of potable water or sewerage is in place. Most dwellings have two rooms of cement block construction with metal sheet or cardboard roofs. Only one street is paved. The neighborhood is inhabited by poor immigrants from small cities and rural areas. We chose this community, because it is representative of most Ecuadorian high Andean slums with substantial rates of malnutrition and subclinical vitamin A deficiency. Overt vitamin A deficiency is uncommon in Ecuador.[18]

All study participants were children 6 to 36 months of age living in the neighborhood.

Enrollment

The study was conducted under a clinical protocol that was approved by the ethical committee of the Ecuadorian Biotechnology Corporation and by the National Research Institute of Health (IIDES) which is part of the Ministry of Public Health. After a census performed by members of the study team, all children (N = 613) between 6 to 36 months of age were considered eligible. Age was verified through birth certificates. To decrease the dropout rate, we selected those children who reliably stayed at home or at day care centers during weekdays (n = 525), and then we excluded children whose families had lived in the neighborhood for <1 year (n = 60). Children who had been given multivitamins in the last 3 months also were excluded (n = 6). A total of 459 children were available for entry into the study. These children were examined by an ophthalmologist for signs of xerophthalmia. No cases of xerophthalmia were found. Finally, 400 children completing the basal anthropometric test were included.

During the screening period, detailed information about the trial was delivered to the parents and community leaders through meetings and home visits. Information on vitamin A supplementation aims, objectives, risks, and potential benefits was provided. Formal written signed consent was obtained freely from the parents of all participating children.

Design

We conducted a randomized, placebo-controlled, double-blind trial to assess the impact of a weekly low dose of vitamin A on the incidence and severity of ARI and ADD in children 6 to 36 months of age without clinical vitamin A deficiency.

A total of 400 children were included in this study. The majority of the children stayed at home and some of them stayed at day care centers located in the same neighborhood. Children were assigned randomly to the active supplementation group (n = 200) or to the nonsupplemented placebo group (n = 200). For random allocation of each child to treatment or placebo group the following procedure was performed. Identical flasks containing vitamin A or placebo were numbered from 1 to 400 by members of the study team in Boston, Massachusetts. The local Ethical Committee of the Ecuadorian Biotechnology Corporation in Quito did not know the identity of the active or placebo flasks, because they did not have the code. Then, this committee assigned each flask to a specific child from a random list by using a table of random numbers. After randomization, the ethical committee received the confidential code from Boston and kept it for the remainder of the study, when it was revealed.

Children in the supplement-treated group received a weekly dose of 10 000 IU of vitamin A (3000 μg of retinol) for 40 weeks, and children in the nonsupplement group received a weekly placebo for the same period.

During the study period, each child was visited weekly at home or at their day care center by physicians who administered vitamin A or placebo. Respiratory and diarrheal disease symptoms and signs were monitored carefully at the same time. On days 0 and 280, anthropometric tests were conducted in each child. In a random subsample of 100 children, we assessed serum retinol concentrations on days 0 and 280. Vitamin A dietary intake was evaluated in the last 2 months of the study.

Information on potable water source (bought from a private tanker, piped water, or well), excrement disposal (use of latrine or field), and the ratio of persons per room was collected from each household during the census.

Sample Size

In a previous study conducted by our team, the incidence of diarrheal disease in children living in a poor neighborhood of Quito, Ecuador was 46 episodes per 1000 child-weeks.[21] To detect a 25% reduction in the incidence of diarrhea with 80% power and two-tailed significance $P < .05$ ($\alpha = 0.05$), we would have required 120 children in each group for an observational period of 40 weeks (4774 child-weeks per treatment arm). Based on our previous experience, we estimated a 30% annual dropout rate (36 children). Thus, we estimated that each arm of the study would require at least 156 children. To decrease loss, we included the study children whose families had lived in the neighborhood for at least 1 year. Calculations for sample size were performed as indicated by Smith and Morrow.[22]

The reported yearly incidence rate of ARI in Ecuador is similar to that of ADD (1460/100 000 vs 1542/100 000 population, respectively).[23] In the absence of information to the contrary, we inferred that group sizes of 200 children in each arm were likely to be sufficient.

Supplementation

The vitamin A syrup was manufactured and kindly donated by Astra Pharmaceuticals (Astra, Westborough, MA). The placebo syrup was manufactured by the Pharmacy Department of St Elizabeth's Medical Center of Boston, Massachusetts. Both the vitamin A and placebo syrups were in identical amber glass containers with calibrated eyedroppers and were not distinguishable (yellow with anise flavoring). The syrups were administered at home and at day care centers by study researchers who were blinded to the presence or absence of active drug. The neighborhood was divided into 16 areas, and each weekday (Monday, Tuesday, Thursday, or Friday) the field investigators visited 4 of the areas according to a schedule previously agreed on with the mothers. Syrups were transported in closed bags to avoid sunlight. Between visits, syrup bottles were stored in a dry, well ventilated room. Supplement-treated children received 10 000 IU of vitamin A (3000 μg of retinol) in 0.2 mL of syrup once weekly, and the nonsupplemented group received an identical volume of placebo weekly.

Follow-up During and Between Weekly Visits

Each child in the study was visited weekly at home or at his or her day care center throughout the study by a physician investi-

gator. Each physician examined 25 children daily for active case detection of diarrheal or respiratory disease. All children were examined weekly, and the mothers or guardians were questioned about the presence or absence of illness. Mothers were instructed to contact the physician researcher in the neighborhood during the week if their child became ill between scheduled visits. If this occurred, the child was visited at home for a complete clinical examination. All cases of ARI and ADD were treated by the researchers during these visits.

Definitions

Diarrhea

Diarrhea was defined as the presence of three or more liquid or semiliquid stools in a period of 24 hours or less. Severe diarrhea was defined as the presence of visible blood in the stool and/or the presence of dehydration. Nonsevere diarrhea was defined as diarrhea without blood or dehydration. If 3 or more days had passed since the resolution of a previous episode, it was considered a new case of diarrhea.

ARI

Mild acute upper respiratory infection (AURI) was defined as the presence of cough, nasal and/or postnasal secretions, and diminished activity. Moderate AURI was defined as mild AURI plus fever >38°C (rectal temperature). Severe AURI included otitis (fever, local pain, or aural pus), and pharyngitis (fever, local inflammation, and/or anterior cervical lymphadenitis). All cases of acute lower respiratory infection (ALRI) were considered severe and were defined as tachypnea (respiratory rate >40/min) and/or lower respiratory tract secretions (alveolar or bronchoalveolar) assessed by thoracic auscultation with one or more of the following symptoms: cough, fever, and chest retractions. If 7 or more days had passed since the last symptoms of AURI, a case was judged to be a new episode. The interval was required to be 14 days to be considered a new episode of ALRI.[8]

Nutritional Status

All children had their weight-for-age z score (WAZ) and height-for-age z score (HAZ) determined using the National Center for Health Statistics (NCHS) standards from the United States.[24, p63–101] We defined underweight children as having a WAZ ≤ -2 SD, stunted children as having an HAZ ≤ -2 SD, and all other children as normal.

Treatment

Children were treated using Ministry of Public Health guidelines. Children with mild or moderate AURI were treated symptomatically. Children with otitis received 10 days of trimethoprim/sulfamethoxazole (TMP/SMX), children with pharyngitis received penicillin (penicillin G plus procaine plus benzathine, 1 dose, imam) if petechial hemorrhages were present, and children with ALRI received TMP/SMX for 7 days. All children with ADD were treated with oral rehydration. When there was macroscopic blood in the stool, ampicillin or TMP/SMX was administered for 5 days. ALRI and severe ADD cases were attended daily at home, and complicated cases were referred to the Baca Ortiz Hospital for Children.

Procedures

Respiratory rates were counted for 1 minute when the child was not crying, and the mean of three counts was recorded.

Information on potable water source, excrement disposal, and the ratio of number of persons per room was based on two random direct observations in each household.

On days 0 and 280, height (or length in children <24 months of age) and weight were measured by standard procedures. Weight was measured with a DETECTO balance (DETECTO, Webb City, MO) and recorded to the nearest 0.1 kg. Height (or length) was obtained with a foot-board or with a calibrated scale and recorded in centimeters. All instruments were calibrated by the Ecuadorian Institute for Normalization.

During the last 2 months of the study, vitamin A dietary intake was evaluated by the 24-hour recall method in a random home visit. The estimated daily vitamin A intake per child was based on the Ecuadorian Food Table.[25] An experienced nutritionist performed this evaluation.

Serum retinol concentrations were assessed in a random subsample of 100 children on days 0 and 280. We obtained 3 mL of peripheral venous blood after the child had fasted for 12 hours by using a 21 × 1 needle and a vacutainer tube without anticoagulant. Vacutainers were covered with aluminum foil to prevent exposure to sunlight. The sample was centrifuged in a darkened room, and the serum was aliquoted into two amber plastic tubes and frozen at −20°C until analysis. To decrease potential interassay variation, basal and final serum samples were analyzed simultaneously. We performed the high performance liquid chromatography technique described by Bieri et al.[26] Serum was treated with methanol to denaturate serum proteins, and vitamin A was extracted with hexane and was redissolved in methanol/ether after evaporation. An aliquot was then examined using a Perkin-Elmer high performance liquid chromatography (Norwalk, CT) with a C18 3.6 × 80.3 mm column. Retinyl palmitate oil solution and retinyl acetate were used as vitamin A standard and internal standard, respectively. Methanol/water was used as eluent. All samples were assayed in duplicate and the mean value was used for analysis. The interassay variation coefficient was <4% and the correlation coefficient was 0.995. Serum retinol assays were performed in the Biochemistry Laboratory of the Medical School of the Central University, Quito, Ecuador.

Data Management

Field investigators had two field precoded forms, one to register supplementation and the other to register signs and symptoms of ADD and ARI. Consistency of registration was audited every 2 weeks by supervisors from the Ecuadorian Biotechnology Corporation and then entered into an EPI INFO 6.0 (Centers for Disease Control and Prevention, Atlanta, GA) database on a personal computer. Consistency was checked by an independent clerk who resolved any differences by using the original field record form.

Standardization

Investigators made repetitive assays of clinical signs of ADD and ARI in series of children. Sensitivity (95%) and specificity (95%) were evaluated comparing data from the reference observer who was the most experienced with those from each researcher. Sensitivity and specificity were monitored continuously by the reference observer during the study period. Height and weight measurements were standardized according to World Health Organization guidelines.[24, p41–45]

Statistical Analysis

Incidence rates of ADD and ARI, the primary outcome variables were evaluated globally and by severity in supplement-treated and nonsupplemented groups. Similar analysis was performed for both groups by age and nutritional status. The global or subgroup incidence rates were estimated per thousand child-weeks of exposure, based on the number of weeks each child had lived in the study area during the study period. Relative risk rates (RR) (incidence rate in the supplement-treated group/incidence rate in nonsupplemented group), 95% CI, and significance tests (χ^2) were also calculated.

The mean ± SD of HAZ, and the mean ± SD of WAZ were calculated. In addition, we calculated the mean ± SD of serum retinol concentrations. The differences between supplement-treated and placebo groups were examined using the Student's *t* test. Intragroup variation was examined by ANOVA. A significance level of <.05 was accepted.

We performed a multiple logistic regression analysis to strengthen the efficiency of comparisons between supplement-treated and nonsupplemented groups. To assess the adequacy of the model, a goodness of fit test was applied. The logistic model included the following variables: vitamin A supplementation, WAZ, HAZ, age, potable water source, excrement disposal, and persons per room ratio. CI for the point estimates (≈RR: vitamin A/placebo) was calculated with a 95% level. All calculations were conducted by using EPI INFO 6.0 and SPSS 3.1 software with an IBM-compatible computer.

RESULTS

A total of 400 children were enrolled in the study. Observations were made over a cumulative total of

5719 child-weeks in the supplement-treated group and 5707 child-weeks in the nonsupplemented group. A total of 306 children finished the study, because 50 children from the supplement-treated group and 44 from the nonsupplemented group were lost to follow-up when their families moved to other neighborhoods. Of all children, 70%, including those lost to follow-up, accumulated >30 weeks of observation. On day 0, there were no significant differences between the vitamin A and placebo groups in age, sex distribution, weight, height, weight/height, persons per room ratio, potable water source, access to latrine, and retinol serum concentrations (Table 1). Children with incomplete follow-up were distributed evenly in relation to the baseline variables (Table 2).

There were 82 underweight children (40 in the supplement-treated group and 42 in the nonsupplemented group; 20% of total studied population); 111 children were stunted (56 in the supplement-treated group and 55 in the nonsupplemented group; 27.8% of total studied population); and 247 were normal children (123 in the supplement-treated group and 124 in the nonsupplemented group; 61.8% of total studied population).

The mean ± SE of the estimated vitamin A dietary intake based on recall method conducted in the last 2 months and expressed as retinol equivalents was similar in both supplement-treated and placebo groups (386.6 ± 66.7 vs 425.9 ± 74.5, respectively). There were no significant differences of retinol equivalents between supplement-treated and placebo groups by nutritional status (data not shown).

In the supplement-treated group, 301 cases of diarrhea were diagnosed (incidence rate: 52.6/1000 child-weeks) and in the nonsupplemented group 277 cases were diagnosed (incidence rate: 48.5/1000 child-weeks). A total of 305 cases of ARI (AURI + ALRI) were diagnosed in the supplement-treated group (incidence rate: 53.3/1000 child-weeks), and 287 cases were diagnosed in the nonsupplemented group (incidence rate: 50.3/1000 child-weeks). There were no significant differences related to severity of ADD and ARI between the supplement-treated and placebo groups (Table 3).

TABLE 1. Characteristics of Vitamin A and Placebo Groups (Day 0)

	Vitamin A $n = 200$	Placebo $n = 200$	P
Age (mo)			
(Mean ± SD)	19.5 ± 9.1	20.7 ± 9.1	NS
Sex (females) (%)	48.2	51.8	NS
WAZ (mean ± SD)	−1.15 ± 1.09	−1.22 ± 1.03	NS
HAZ (mean ± SD)	−1.42 ± 1.24	−1.49 ± 1.21	NS
WHZ (mean ± SD)	−0.32 ± 0.97	−0.33 ± 0.87	NS
Ratio persons/room			
(Mean ± SD)	3.97 ± 3.6	3.79 ± 3.42	NS
Potable water source (%)			
Bought from private tankers	71.5	65.2	NS
Piped water	18.6	24.4	NS
Well	9.8	9.8	NS
Access to latrine (%)	79.8	87.0	NS
Serum retinol (ug/dl)			
(Mean ± SD)	40.8 ± 11.2	41.9 ± 10.9	NS

TABLE 2. Baseline Characteristics of Children From Vitamin A and Placebo Groups Who Withdrew From the Study

	Vitamin A $n = 50$	Placebo $n = 44$	P
Age (mo)			
(Mean ± SD)	19.2 ± 9.0	20.4 ± 9.2	NS
Sex (females) (%)	50	48.4	NS
WAZ (mean ± SD)	−1.18 ± 1.37	−1.39 ± 1.15	NS
HAZ (mean ± SD)	−1.73 ± 1.45	−1.82 ± 1.42	NS
WHZ (mean ± SD)	−0.15 ± 1.18	−0.28 ± 0.92	NS
Ratio persons/room			
(Mean ± SD)	4.77 ± 3.99	5.06 ± 4.04	NS
Potable water source (%)			
Bought from private tankers	77.5	76.4	NS
Piped water	12.5	11.7	NS
Well	10	11.7	NS
Access to latrine (%)	82.5	73.5	NS
Serum retinol (ug/dl)			
(Mean ± SD)	36.1 ± 14.5	37.9 ± 9.7	NS

TABLE 3. Crude Analysis of Respiratory and Diarrhea Infections in Children Who Took Vitamin A or Placebo

	Vitamin A IR	Placebo IR	RR	95% CI	P
Add	52.6	48.5	1.08	0.92–1.27	.32
Severe ADD	6.6	7.7	0.86	0.56–1.33	.49
Nonsevere ADD	45.9	40.8	1.13	0.95–1.34	.17
ARI	53.3	50.3	1.06	0.91–1.24	.46
Mild AURI	10.1	11.0	0.92	0.64–1.31	.63
Moderate AURI	9.3	11.6	0.80	0.56–1.15	.22
Severe AURI	25.2	20.3	1.24	0.97–1.58	.08
ALRI	8.9	7.4	1.21	0.81–1.82	.35

Abbreviations. IR, number of cases in each group/1000 child-weeks. RR, incidence rate in supplemented group/incidence rate in non supplemented group.

There were no significant differences between supplement-treated and placebo groups regarding AURI, globally or by severity, when either nutritional or age-stratified analysis was performed (data not shown). A total of 91 cases of ALRI were diagnosed during the study period (incidence rate: 8.1/1000 child-weeks). This total number was distributed evenly between supplement-treated and placebo groups (49 vs 42). Age-stratified analysis did not show differences (data not shown). However, analysis by nutritional status did show significant differences between supplement-treated and nonsupplemented groups in underweight (RR = 0.38; $P = .01$) and normal (RR = 2.21; $P = .005$) children (Table 4).

Analysis of ALRI based on mutually exclusive nutritional subgroups revealed differences of marginal significance between supplement-treated and placebo groups in wasted-not stunted (RR = 0.37; 95% CI: 0.12–1.08; $P = .104$), and stunted-and-wasted (RR = 0.29; 95% CI: 0.08–1.00; $P = .063$) subgroups. There was no difference in the stunted-not wasted subgroup (RR = 3.54; 95% CI: 0.41–3.02; $P = .40$).

Logistic regression analysis of ALRI showed differences between the supplemented and nonsupplemented groups based on WAZ. Three WAZ strata were defined for this analysis: ≤−2 SD ($n = 82$), >−2 SD to −1 SD ($n = 169$), and >−1 SD to mean ($n = 89$). These cutoff points were established to disaggregate the impact of supplementation among

TABLE 4. Analysis of ALRI in Children Who Took Vitamin A or Placebo by Baseline Nutritional Status

Nutritional Status	Vitamin A			Placebo			RR	95% CI	*P*
	No. Cases	CW	IR	No. Cases	CW	IR			
Underweight	8	937	8.5	24	1075	22.3	0.38	0.17–0.85	.01
Stunted	8	1703	4.7	16	1635	9.8	0.48	0.21–1.12	.08
Normal	36	3655	9.8	17	3835	4.4	2.21	1.24–3.93	.005

Abbreviations. IR, number of cases in each group/1000 child-weeks. RR, incidence rate in supplemented group/incidence rate in non supplemented group. CW, number of child-weeks.

normal weight children. The point estimate (≈RR: vitamin A/placebo) was significant for the lowest (≈RR = 0.148; 95% CI: 0.034–0.634) and the highest (≈RR = 2.51; 95% CI: 1.24–5.05) strata, but it was not significant for the middle stratum (≈RR = 1.66; 95% CI: 0.699–3.937). The remaining children (n = 60) with body weight over the mean were not included in the stratified analysis, because only 2 cases of ALRI occurred in this group during the follow-up.

There were no differences regarding ADD between supplemented and placebo groups globally or by severity when either nutritional or age-stratified analysis was performed (data not shown). Logistic regression analysis showed differences in severe ADD between supplement-treated and nonsupplemented groups based on age. The point estimate (≈RR: vitamin A/placebo) was significant only in the subgroup from 18 to 23 months of age (≈RR = 0.26; P = .05) (Table 5).

A total of 100 children were selected randomly on day 0 for serum retinol determinations. Four samples were lost because of a transportation accident. Baseline concentrations (μg/dL) were similar in both supplement-treated (n = 54) and nonsupplemented (n = 42) groups (40.8 ± 11.2 vs 41.9 ± 10.9, respectively). On day 280, we reassessed 89 of the 100 children's serum retinol levels, because 11 children had been lost to follow-up. The overall mean value was significantly higher in the supplement-treated group (n = 46) than in the placebo group (n = 43; 48.5 ± 12.6 vs 42.9 ± 10.5, respectively; P = .02). On day 280, differences between vitamin A and placebo groups were significant in the lowest (P = .04) and highest (P = .04) strata based on WAZ (Table 6) .

Final serum retinol concentration increased over the baseline in each stratum based on WAZ in the supplement-treated group. However, the intragroup variation between basal and final concentrations was significant in the middle (delta = 7.4; P = .04) and in the highest (delta = 13.3; P = .03) strata but not in the lowest (delta = 5.9; P = .23) stratum.

There were no significant differences in weight and height between supplement-treated and placebo groups in underweight and in stunted children on days 0 and 280. WAZ and HAZ scores improved in all groups during the course of the study (data not shown).

DISCUSSION

The most remarkable finding in our study was the strongly protective effect vitamin A exerted against ALRI in underweight children, and the paradoxical increase seen in children whose body weight was nearer the NCHS mean value. These differences would have been masked in a global analysis that did not allow stratification by nutritional status. Our results may explain why vitamin A supplementation has been found to decrease mortality from all causes but has not been found consistently to decrease ARI. As others have found, vitamin A supplementation did not have a detectable effect on the incidence of ARI in this study, either globally or by severity. The risk of ALRI that we detected was similar to that found in other reports, indicative of global nonprotection.[16,27,28] In other reports and meta-analysis, stratification based on age has shown some slight protection for supplemented children older than 11 months.[8] We note that in many societies, nutritional status worsens after weaning, which occurs at approximately this age. Although some trials from Africa, Asia, and Latin America have reported underweight children in the population studied, ranging from 12% in Brazil[27] to 43% in Ghana.[16] No stratified analysis by nutritional status was published.

Our population can be characterized as having only subclinical vitamin A deficiency, similar with what others have reported from Brazil.[27] One trial conducted in India did not find a protective effect of low-dose weekly (8333 IU) vitamin A supplementation in malnourished children.[6,29] However, the children in that study suffered from prominent clinical deficiency, because 37.5% of the children had serum retinol levels <20 μg/dL, and 11% had clinical xerophthalmia.[6,29] It is possible that low-dose vitamin A is protective in malnourished children with subclinical deficiency, such as is found commonly in South America, but not in children with more severe deficiencies.

TABLE 5. Severe Diarrhea in Children Who Took Vitamin A or Placebo by Age

Age Groups	Vitamin A			Placebo			RR	≈RR	95% CI	*P*
	No. Cases	CW	IR	No. Cases	CW	IR				
<18 mo	21	2629	7.9	23	2230	10.3	0.76	0.78	0.38–1.59	.52
18–23 mo	7	952	7.3	11	1021	10.7	0.68	0.26	0.06–1.0	.05
≥24 mo	10	2138	4.6	10	2456	4.0	1.15	1.06	0.38–3.0	.89

Abbreviations. IR, number of cases in each group/1000 child-weeks; RR, incidence rate in supplemented group/incidence rate in non supplemented group; ≈RR, point estimate; CW, number of child-weeks.

TABLE 6. Serum Retinol Concentration (ug/dL) in Children Who Took Vitamin A or Placebo by WAZ Strata

WAZ Strata	Day 0			Day 280		
	Vitamin A (*n*)	Placebo (*n*)	*P*	Vitamin A (*n*)	Placebo (*n*)	*P*
≤−2 SD	40.8 ± 9.9 (12)	37.7 ± 10.7 (8)	NS	46.9 ± 10.2 (11)	36.6 ± 11.4 (7)	.04
>−2 SD to −1 SD	40.2 ± 12.4 (27)	43.8 ± 12.1 (23)	NS	47.6 ± 12.4 (23)	43.0 ± 10.5 (20)	.18
>−1 SD to mean	44.9 ± 11.3 (7)	40.6 ± 9.8 (10)	NS	58.2 ± 6.5 (5)	45.3 ± 10.0 (5)	.04

We found that, given the same supplemental dose of vitamin A, the increment in serum retinol concentrations was lowest in underweight children and greatest in the children that were best nourished. Moreover, the lowest individual values that we recorded in our study were in the group that was the most malnourished (≥2 SD below the mean for weight) with values near biochemical deficiency (<20 μg/dL). It is possible that a rapid utilization of the vitamin is linked to malnutrition. For example, measles mortality has been related to an exhaustion of vitamin A stores.[20,30] It should be emphasized that most of the mortality from measles occurs in malnourished, and not only vitamin A-deficient, children.

Normal weight supplement-treated children (WAZ within 1 SD of the NCHS mean) had a significantly higher incidence of ALRI than did normal, nonsupplemented children. This difference was also masked in the global analysis. Our study suggests that this population is not helped, and may be placed at risk by low-dose weekly supplementation.

Other aspects of our study suggest the internal and external validity of our results. For example, the global incidence of ADD in this study was very similar to the incidence we found in a previous study conducted in a poor setting in Quito based on two home visits per week.[21] Although ADD in this high Andean population is less frequent than has been seen in other populations of children, a protective effect of vitamin A against severe diarrhea in children 18 to 23 months of age was found. This could be related to the transitional pattern of pathogens from viruses to bacteria in this age group, as has been described in malnourished children.[31] The degree of protection against severe diarrhea that we found is consistent with previous trials.[27,32] In addition, we reassuringly found consistent improvements in WAZ and HAZ scores in all groups during this study. Rahmathullah and colleagues[29] have suggested that this is a predictable effect of close medical monitoring, which shortens the detrimental impact of episodes of illness.

CONCLUSION

In this study, we present evidence that weekly low-dose vitamin A supplementation in regions of subclinical deficiency protects underweight children from ALRI but may place better nourished children at risk of ALRI. We believe this phenomenon may explain the conflicting results others have obtained regarding the efficacy of vitamin A supplementation in protecting against ARI. These results suggest that additional prospective studies in underweight and normal children are warranted. A reanalysis of previous studies to see whether nutritional stratification plays a role in the incidence of ARI may prove enlightening. If our results are proved valid by confirmatory studies, targeted but not population-wide vitamin A supplementation in areas of subclinical deficiency may prove the wisest choice.

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