Assessment of vitamin A status with the relative-dose-response test in Peruvian children recovering from pneumonia¹–³

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ABSTRACT

Background: The relative-dose-response (RDR) test is used to identify subjects with marginal liver vitamin A stores, but its use has not been evaluated during episodes of infection.

Objective: The objective was to assess, with the RDR test, the vitamin A status of children recovering from pneumonia.

Design: As part of a double-blind, placebo-controlled clinical trial of high-dose vitamin A supplements among children hospitalized with pneumonia in Lima, Peru, we examined the association of treatment group, nutritional status, severity of disease, and induction of the acute phase response [on the basis of serum C-reactive protein (CRP)] on serum retinol and the RDR test.

Results: Serum retinol was low at admission and increased significantly in both the vitamin A and placebo groups during recovery. Serum CRP had a significant, inverse association with retinol at both admission and discharge. Serum retinol and CRP concentrations never differed significantly between the treatment groups. Among subjects with CRP ≥ 10 mg/L, 21% in the vitamin A group and 20% in the placebo groups (P = 0.83) had a positive RDR test result. Among subjects with CRP < 10 mg/L, 56% in the placebo group but only 6% in the vitamin A group had positive RDR test results (P = 0.002).

Conclusion: The RDR test was useful in assessing the vitamin A status of children recovering from pneumonia when CRP concentrations were < 10 mg/L but not when CRP concentrations were higher. Am J Clin Nutr 2002;76:1351–7.

KEY WORDS Vitamin A, retinol, acute phase response, pneumonia, C-reactive protein, CRP, relative-dose-response test, RDR test, children, Peru

INTRODUCTION

Vitamin A is stored in the liver and is transported to peripheral tissues through the blood by retinol binding protein (RBP). When liver stores fall below a critical level, serum retinol also decreases and can thus be used as an indicator of liver stores. The level at which this occurs varies among persons; therefore, serum retinol is an inaccurate measure for identifying subjects with marginal vitamin A stores. However, the identification of subjects with depleted stores is more reliable. According to generally accepted criteria, when serum retinol is < 1.05 mol/L, stores are likely to be marginal; concentrations < 0.70 μmol/L indicate that stores are probably depleted. Serum retinol concentrations ≤ 0.35 μmol/L are observed in persons with a clinical deficiency (xerophthalmia), when stores are essentially absent (1–3).

The relative-dose-response (RDR) test was developed to more reliably identify individuals with marginal vitamin A stores (1). The RDR test measures serum retinol concentrations before and 5 h after administration of an oral dose of vitamin A. During this 5-h period, the vitamin A dose is absorbed and transported via chylomicrons (as retinyl esters) to the liver. If liver stores are inadequate to maintain serum retinol at a normal concentration, all or part of this oral dose will be released from the liver as retinol-RBP, thereby increasing the serum retinol concentration. Previous work found that an increase of ≥ 20% indicates marginal or depleted liver stores (4).

Serum retinol concentrations may decrease during acute infection but typically rebound to preinfection concentrations within a few days (5). This transient decrease does not reflect a depletion of liver stores and, thus, it can interfere with the use of serum retinol as an indicator of vitamin A status. Many factors act to decrease serum retinol during infection. Infection can decrease retinol absorption from the gut (3), and induction of the acute phase response can decrease the synthesis of RBP in the liver (6). Other factors, including increased metabolic demand and urinary retinol excretion, may also play a role (7). Decreased retinol absorption or diminished RBP synthesis during infection could interfere with the RDR test by blocking the delivery of vitamin A to the liver or impairing the release of retinol-RBP from the liver. This could cause a false-negative RDR test result (ie, a normal result, indicated by an increase in serum retinol of < 20% in a subject with deficient liver stores). On the other hand, the low serum retinol concentrations observed during infection might allow a smaller absolute increase in serum retinol to achieve the 20% threshold, thus potentially causing a false-positive RDR test result.

We examined the utility of the RDR test in a randomized, placebo-controlled, clinical trial of high-dose vitamin A supplements among children hospitalized with pneumonia in Lima, Peru.
C-reactive protein (CRP) measurements varied at each time point. or problems with blood collection, the number of serum retinol or were available for analysis. Because of a limited sample volume 47 subjects in the vitamin A group and 45 in the placebo group was subsequently excluded from the data analysis. Thus, data from 49 to the placebo group. Three subjects in the placebo group had of a purified protein derivative skin test and a medical history. tuberculosis and were excluded from the analysis. One subject 48 h after admission to the study on the basis underlying chronic disease. Informed written consent was solicited www.cdc.gov/nchs/about/major/nhanes/growthcharts/wtstat.txt), a Health Statistics reference standards (available on the Internet: http:// in children hospitalized because of X-ray–confirmed, community-acquired pneumonia in Lima, Peru. We found that this supplementation regimen had a modest, negative effect on recovery from pneumonia, as previously described (8). We assumed during this analysis that children who received the supplements had sufficient liver vitamin A stores at discharge to render their RDR test result negative. We also assumed, on the basis of previous serum retinol measurements in this population (9), that some children entering the study would have subclinical vitamin A deficiency and that this would be reflected in the RDR test results at discharge in the placebo group.

SUBJECTS AND METHODS

Study population and recruitment

Children from 3 mo to 10 y of age with a principal diagnosis of pneumonia (confirmed by X-ray) and admitted as inpatients to the pediatrics ward of the Universidad Peruana Cayetano Heredia (UPCH) Hospital from 4 July 1994 to 31 October 1995 were eligible for inclusion in the study, as described previously (8). The criteria for exclusion from the study included previously diagnosed immunodeficiency, regular use of vitamin A supplements, a weight-for-height below the 70th percentile of the National Center for Health Statistics reference standards (available on the Internet: http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/wtstat.txt), a history of asthma, a diagnosis of tuberculosis, and the presence of underlying chronic disease. Informed written consent was solicited by one of the study nurses from a parent or guardian. A diagnosis of tuberculosis was made 48 h after admission to the study on the basis of a purified protein derivative skin test and a medical history.

Forty-seven subjects were admitted to the vitamin A group and 49 to the placebo group. Three subjects in the placebo group had tuberculosis and were excluded from the analysis. One subject who should have been excluded from the study (because of Down syndrome) was inadvertently admitted to the placebo group but was subsequently excluded from the data analysis. Thus, data from 47 subjects in the vitamin A group and 45 in the placebo group were available for analysis. Because of a limited sample volume or problems with blood collection, the number of serum retinol or C-reactive protein (CRP) measurements varied at each time point. Retinol measurements were available for 46 and 42, 45 and 44, and 40 and 36 subjects available in the vitamin A and placebo groups, respectively. CRP measurements were available for 47 and 45, 46 and 43, and 40 and 33 subjects in the vitamin A and placebo groups, respectively. RDR and serum CRP data at discharge (the key variables for this analysis) were available for 45 subjects in the vitamin A group and 41 subjects in the placebo group. Both variables were available at all 3 time points for 37 subjects in the vitamin A group and for 29 subjects in the placebo group, and only this limited subset was used in Figure 1. The research protocol was reviewed and approved by the human subjects committees at both the University of Alabama at Birmingham and the UPCH.

Vitamin A and placebo

Oral vitamin A and placebo preparations were given in a double-blind fashion according to a randomized block design as described previously (8). No signs of acute toxicity were observed. Aquasol A (Astra Pharmaceuticals, Westborough, MA), a water-miscible preparation of retinol containing 50 000 IU/mL (15 mg/mL), was used as the treatment, and an identical placebo syrup was prepared by the University of Alabama Hospital Pharmacy by using the formula provided by the manufacturer. In the treatment group, children < 1 y of age received 100 000 IU (2 mL, 30 mg) vitamin A on admission to the study and 50 000 IU (1 mL, or 15 mg) on the second day of hospitalization. Children aged ≥ 1 y received 200 000 IU (4 mL, or 60 mg) on the first day and 100 000 IU (2 mL, or 30 mg) on the second day. Children in the placebo groups received the appropriate volume of placebo syrup on both days.

Baseline data collection

On admission to the study, baseline data were collected for each subject from a parent or guardian (Table 1). On the basis of baseline data, a presumptive diagnosis of viral (upper respiratory signs or symptoms, wheezing and rales, and hyperinflation, interstitial infiltrate, or both on chest X-ray) or bacterial (no wheezing; crepitant rales, bronchial breath sounds, or both; and consolidation, effusion, or both on chest X-ray) pneumonia was made.

Severity score

We developed a numerical severity score based on 8 principal clinical outcome variables (collected during hospitalization) to monitor clinical recovery, as described previously (8). In summary, the aggregate score incorporates heart rate adjusted for age, respiratory rate adjusted for age, temperature, presence of retractions, central cyanosis, 2 indicators of appetite, and blood oxygen saturation. The score ranged from 0 (least severe) to 24 (most severe).

Antibiotic treatment regimens

Antibiotic treatment regimens are standardized within the pediatrics unit at the UPCH. Decisions regarding the selection of antibiotics and dosage were not controlled for by study personnel and were made by the attending physicians.

Serum CRP, retinol, and the RDR test

Serum CRP was measured by radial immunodiffusion with the use of kits from The Binding Site (Birmingham, United Kingdom). Serum retinol was measured by HPLC, essentially as described previously (9). The RDR test was performed as described previously.

### TABLE 1

<table>
<thead>
<tr>
<th>Admission characteristic</th>
<th>Vitamin A group (n = 45)</th>
<th>Placebo group (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (n [%])</td>
<td>23 [51]</td>
<td>23 [56]</td>
</tr>
<tr>
<td>Age (mo)^2</td>
<td>25.5 (13.5, 47.5)</td>
<td>24.5 (11.8, 53.0)</td>
</tr>
<tr>
<td>Height-for-age z score^1</td>
<td>-0.57 ± 1.46</td>
<td>-0.28 ± 2.17</td>
</tr>
<tr>
<td>Weight-for-age z score^1</td>
<td>-0.65 ± 1.08</td>
<td>-0.21 ± 1.40</td>
</tr>
<tr>
<td>Weight-for-height z score^1</td>
<td>-0.34 ± 1.04^1</td>
<td>0.090 ± 0.87</td>
</tr>
<tr>
<td>Clinical diagnosis (n [%])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral pneumonia</td>
<td>14/45 [31]</td>
<td>11/40 [28]</td>
</tr>
<tr>
<td>Bacterial pneumonia</td>
<td>31/45 [68]</td>
<td>29/40 [72]</td>
</tr>
<tr>
<td>Temperature (°C)^2</td>
<td>37.4 ± 0.91</td>
<td>37.5 ± 1.0</td>
</tr>
<tr>
<td>Severity score^2</td>
<td>4.5 ± 3.7</td>
<td>3.4 ± 2.8</td>
</tr>
<tr>
<td>Duration of hospitalization (d)^3</td>
<td>2.83 (1.83, 4.83)</td>
<td>2.83 (1.83, 4.25)</td>
</tr>
</tbody>
</table>

^1 Median; 25th and 75th percentiles, respectively, in parentheses. ^2 ± SD. ^3 Significantly different from the placebo group, P = 0.044 (Kruskal-Wallis one-way ANOVA).
Venous blood was collected from fasting subjects at 0 h, immediately before the subjects were given an oral dose of 3.5 μmol water-miscible retinol (Aquasol A; Astra Pharmaceuticals). Children remained at the clinic, and a second blood sample was taken from them 5 h later. The RDR test results were calculated by using these 2 serum retinol concentrations as follows: RDR = [(5 h − 0 h)/5 h] × 100%. An RDR value ≥ 20% was considered positive and an indicator of depleted liver vitamin A stores. Serum retinol and CRP concentrations were measured at admission, discharge (a median of 3 d after admission), and follow-up (14 d after admission). The RDR test was performed on the day of discharge. Discharge decisions were made in the late afternoon. The subjects remained one more night in the hospital the day of discharge. Discharge decisions were made in the late afternoon. The subjects remained one more night in the hospital and the RDR tests were done the following morning.

Statistical analysis

SPSS software (version 4.01; SPSS Inc, Chicago) and SIGMA-STAT (version 2.03; Jandel Scientific, San Rafael, CA) were used for the statistical analysis. Kruskal-Wallis one-way analysis of variance (ANOVA) was used to compare continuous data between 2 groups. Pearson’s chi-square test was used to compare categorical data, except when the expected number of observations in a particular cell was <5, in which case Fisher’s exact test was used. Comparisons of continuous variables (eg, serum retinol concentration) between groups at different times were made by analysis of covariance. Serum CRP concentrations were log10 transformed at admission and discharge for analysis. Serum retinol and CRP were compared at admission, discharge, and follow-up by repeated-measures two-factor ANOVA with the use of linear estimation to estimate missing data points, when necessary. Two-factor ANOVA was used to compare serum retinol in the placebo group, categorized by RDR test results and CRP category. The same approach was used for subjects in the vitamin A group. The 2 groups were not combined into a single analysis by three-factor ANOVA because of the imbalanced cell sizes. Comparisons of discrete variables (eg, positive compared with negative RDR test results) between groups over time were made by logistic regression. In both cases, the time since admission and group × time interaction terms were always included in the model. Significant differences are indicated by P values <0.05 for both the group and group × time interaction variables; if one of these variables was not identified, it was not significant. When other factors (eg, baseline weight-for-height) were included in the analysis, they are indicated in the text.

RESULTS

Baseline characteristics of the placebo and control groups

As shown in Table 1, the 2 study groups were well-balanced at baseline, when sex, age, anthropometric measures, clinical diagnosis, and disease severity scores were compared. Only weight-for-height was significantly different (P = 0.044) between the groups.

Serum retinol

Serum retinol concentrations were low at admission and increased by the time of discharge (a median of 3 d after admission) to concentrations that apparently remained stable until the follow-up visit (14 d after admission) (Figure 1). When serum retinol concentrations were analyzed by two-factor repeated-measures ANOVA, the means differed by time (P < 0.001), but no significant differences were seen between the treatment groups (P = 0.61) and no interaction between time and treatment was found (P = 0.29). This analysis was performed with the use of data from subjects for whom serum retinol concentrations were available at each time point (n = 46 and 42, 45 and 44, and 40 and 36 subjects in the vitamin A and placebo groups, respectively). Essentially identical results were found when the 37 subjects in the vitamin A group and the 29 subjects in the placebo group for whom data were available at all time points were analyzed. The mean increase in serum retinol between admission and discharge did not differ significantly between the vitamin A (0.96 ± 0.43 μmol/L; n = 43) and placebo (0.75 ± 0.62 μmol/L; n = 41) groups (P = 0.07, by Student’s t test) nor did the mean increase in serum retinol between admission and follow-up differ significantly between the vitamin A (0.82 ± 0.51 μmol/L; n = 39) and placebo (0.72 ± 0.53 μmol/L; n = 33) groups (P = 0.41). In addition, the percentage of subjects with serum retinol values <0.70 μmol/L at admission was high and did not differ significantly between the vitamin A (96%; n = 44 of 46) and placebo (86%; n = 36 of 42) groups (P = 0.14, by chi-square test). The percentage decreased to 16% (n = 7 of 45) in the vitamin A group and to 18% (n = 8 of 44) (P = 1.00) in the placebo group at discharge and to 18% (n = 7 of 40) and 14% (n = 5 of 36) (P = 0.91) at follow-up, respectively.
CRP concentrations at admission and discharge, although not at follow-up; this association was not modified by vitamin A status. The correlation coefficient between serum retinol and $\log_{10}$ serum CRP was 0.639 ($P < 0.0001$; $n = 88$) at admission and 0.417 at discharge ($P < 0.0001$; $n = 88$), whereas at follow-up the association was no longer significant ($r = 0.211$, $P = 0.078$; $n = 72$). At discharge, the association of serum CRP with serum retinol was significant in both the vitamin A ($r = -0.340 \pm 0.578$, $P = 0.0003$; $n = 45$) and placebo ($r = -0.274 \pm 0.762$, $P = 0.026$; $n = 41$) groups, and the slope of the prediction lines did not differ significantly between groups ($P = 0.65$, by Student’s $t$ test). The serum CRP concentration was consistently the strongest predictor of serum retinol at all time points. No other variables (including admission temperature, severity score at admission, diagnosis of bacterial or viral pneumonia, weight-for-age $z$ score, weight-for-height $z$ score, height-for-age $z$ score, hematocrit, age, and sex) were associated with serum retinol at admission ($P > 0.05$). Other variables (including treatment group, discharge temperature, severity score at discharge, diagnosis of bacterial or viral pneumonia, weight-for-age $z$ score, weight-for-height $z$ score, height-for-age $z$ score, age, and sex) were also not associated with serum retinol at discharge ($P > 0.05$). Of the variables tested at follow-up ($\log_{10}$ serum CRP, treatment group, diagnosis of bacterial or viral pneumonia, weight-for-age $z$ score, weight-for-height $z$ score, height-for-age $z$ score, age, and sex), only age had a significant ($P = 0.018$) and positive association with serum retinol.

The mean serum retinol concentration (1.08 $\pm$ 0.44 $\mu$mol/L; $n = 52$) in children with elevated CRP at discharge was lower than that in children with a normal CRP concentration (1.45 $\pm$ 0.59 $\mu$mol/L; $n = 34$) ($P = 0.0013$). As shown in Figure 2, an elevated CRP concentration was associated with lower serum retinol in both the vitamin A and placebo groups, whereas serum retinol concentrations did not differ significantly by treatment group. Children with elevated CRP at follow-up also had lower serum retinol concentrations (0.88 $\pm$ 0.30 $\mu$mol/L; $n = 14$) than did children with normal CRP values (1.17 $\pm$ 0.45 $\mu$mol/L; $n = 58$) ($P = 0.027$).

**CRP in subjects with bacterial pneumonia**

Severity of disease and the level of induction of the acute phase response in children with pneumonia is determined, in part, by the etiologic agent of the pneumonia. Because the percentage of subjects with a laboratory diagnosis of bacterial or viral pneumonia was low (13% in both cases), we examined the association of a clinical diagnosis of viral or bacterial pneumonia with indicators of disease severity and serum CRP concentration. Children admitted with viral pneumonia were much younger, on average (14 $\pm$ 10 mo; $n = 27$), than were the children with bacterial pneumonia (44 $\pm$ 34 mo; $n = 62$) ($P = 0.000027$). There was no significant difference between the groups based on sex: 63% of the subjects with viral pneumonia ($n = 17$ of 27) were female, whereas 40% of the subjects with bacterial pneumonia ($n = 25$ of 63) were female ($P = 0.072$). Severity of disease, as measured by the clinical severity score, did not differ significantly between the groups on admission [3.7 $\pm$ 3.0 in the viral group ($n = 27$) compared with 4.1 $\pm$ 3.3 in the bacterial group ($n = 63$); $P = 0.57$], nor were there significance differences in body temperature or the percentage of subjects with fever ($\geq$ 38°C) on admission (data not shown). However, subjects with bacterial pneumonia had a slightly higher white blood cell count (12 812 $\pm$ 7113 cells/mm$^3$; $n = 48$) than did subjects with viral pneumonia (9513 $\pm$ 3527; $n = 23$; $P = 0.040$). In addition, CRP concentrations were significantly higher in the

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**FIGURE 2.** Mean ($\pm$ SE) serum retinol concentrations at discharge in children hospitalized with pneumonia by treatment group (vitamin A or placebo) and by presence [C-reactive protein (CRP) concentration $\geq 10$ mg/L, $\square$] or absence (CRP < 10 mg/L; $\square$) of an active acute phase response. Serum retinol was lower in the subjects with elevated CRP (two-factor repeated-measures ANOVA, $P = 0.0009$) but was not significantly different by treatment group ($P = 0.50$). No interaction was seen between CRP and treatment group ($P = 0.68$). In the vitamin A group, 17 and 28 subjects had normal and elevated CRP concentrations, respectively. In the placebo group, 15 and 25 subjects had normal and elevated CRP concentrations, respectively.

**Serum CRP**

Severity of disease, as measured by several clinical indicators and an aggregate clinical severity score, improved over time during the period of hospitalization, as previously described (8). Serum CRP concentrations were very high in both the vitamin A and placebo groups at admission and decreased dramatically by discharge and remained low at follow-up (Figure 1). When serum $\log_{10}$ CRP concentrations were analyzed by two-factor repeated-measures ANOVA, the means differed by time ($P < 0.001$) but no significant difference ($P = 0.56$) between the treatment groups and no interaction between time and treatment ($P = 0.36$) were observed. This analysis was performed with the use of all data available at each time point ($n = 47$ and 45, 46 and 43, and 40 and 33 subjects in the vitamin A and placebo groups, respectively), but essentially identical results were found when the 37 subjects in the vitamin A group and the 29 subjects in the placebo group for whom data were available at all time points were analyzed. Mean serum CRP values did not differ significantly between groups at any time point. Similarly, the percentage of subjects with CRP values $\geq 10$ mg/L was high at admission; 100% ($n = 47$ of 47) in the vitamin A group and 96% ($n = 43$ of 45) ($P = 0.24$, by chi-square test) in the placebo group. CRP values remained elevated in most of the subjects at discharge: 61% ($n = 28$ of 46) of the subjects in the vitamin A group and 60% ($n = 26$ of 43) ($P = 0.86$) of the subjects in the placebo group had CRP values $\geq 10$ mg/L. Even at follow-up, 25% ($n = 10$ of 40) of the vitamin A group and 15% ($n = 5$ of 33) ($P = 0.46$) of the placebo group had elevated CRP concentrations.

**Serum retinol and CRP concentrations**

As expected from previous work (9), serum retinol concentrations correlated significantly (and negatively) with $\log_{10}$ serum CRP concentrations at admission and discharge, although not at follow-up; this association was not modified by vitamin A status.
subjects with bacterial pneumonia than in those with viral pneumonia [310 ± 234 mg/L (n = 62) compared with 96 ± 103 mg/L (n = 27); P = 0.000017]. Given this difference, it was not surprising that the children with bacterial pneumonia also had lower serum retinol concentrations (0.30 ± 0.24 μmol/L; n = 58) than did the children with viral pneumonia (0.45 ± 0.25 μmol/L; n = 27; P = 0.0085).

**Association between elevated serum CRP and apparent false-positive RDR test results**

The RDR test was performed on the day of discharge, when most of the subjects still had elevated serum CRP. We expected that a substantial percentage of children in the placebo group would have subclinical vitamin A deficiency and would thus have a positive RDR test result. In contrast, we expected that the high dose of vitamin A would eliminate any deficiency in the treatment group and that these subjects would all (within the reliability of the test) have negative RDR test results. However, although we found that 34% (n = 14 of 41) of the placebo group had a positive RDR test result, 16% (n = 7 of 45) of the vitamin A group also had a positive test result (Figure 3), a higher percentage than expected in the latter group. Furthermore, the difference between the groups was not statistically significant (P = 0.08). To determine whether the active acute phase response in some of these subjects might have interfered with the RDR test, we evaluated the association of elevated serum CRP (≥10 mg/L) with positive and negative RDR test results (Figure 3). Surprisingly, when subjects with elevated serum CRP were considered separately, the percentage of subjects in the vitamin A (21%; n = 6 of 28) and placebo (20%; n = 5 of 25) groups with a positive RDR test result was nearly identical (P = 0.83). However, of the subjects in the placebo group with normal CRP concentrations, 56% (n = 9 of 16) had a positive RDR test result compared with only 6% (n = 1 of 17) in the vitamin A group (P = 0.002). This result suggests that ~50% of the children in the placebo group had subclinical vitamin A deficiency at discharge, which was consistent with our expectations. These results suggest that the RDR test performed as expected in subjects with normal CRP concentrations but that the rate of false-positive results was high (~20%) in subjects with elevated CRP concentrations.

As a check on the validity of the RDR test in identifying subjects with subclinical vitamin A deficiency, we compared serum retinol concentrations in RDR-positive and RDR-negative subjects after adjustment for elevated serum CRP with use of two-factor ANOVA. As seen in Figure 4, the mean serum retinol concentration was significantly lower in the RDR-positive subjects from the placebo group (x ± SE: 0.85 ± 0.14 μmol/L) than in the RDR-negative subjects from that same group (1.49 ± 0.11 μmol/L).
Furthermore, the mean serum retinol concentration in subjects in the vitamin A group with a positive RDR test result (1.26 ± 0.24 μmol/L) did not differ significantly from that of the subjects with a negative RDR test result in the same group (1.31 ± 0.07 μmol/L). This lack of association of a positive RDR test result with lower serum retinol in the vitamin A group supports the conclusion that the positive RDR test results in this group were false positive and not associated with lower liver stores of vitamin A and may have resulted from changes in serum retinol induced by the acute phase response.

**DISCUSSION**

High-dose vitamin A supplements have been tested as therapeutic interventions in children with measles, diarrhea, and pneumonia (3). In the present hospital-based study in Lima, we found that vitamin A supplements had a modest, adverse effect on the recovery from pneumonia (8). Children who received vitamin A supplements had more severe disease during hospitalization, as indicated by a lower blood oxygen saturation, a higher prevalence of retractions, and greater auscultatory evidence of consolidation. As a result, the children in the vitamin A group were also more likely to require supplemental oxygen. Fortunately, no differences in the duration of hospitalization were seen. The question of whether the subjects in such intervention studies were vitamin A deficient naturally arises. In the present study, we attempted to answer this question by analyzing the results of the RDR test by using serum CRP to adjust for the possible confounding effects of the acute phase response.

Serum retinol concentrations were low at admission and increased at discharge and follow-up in both the placebo and vitamin A groups. The high percentage of subjects with serum retinol concentrations < 0.70 μmol/L (~90% at admission and from 14% to 18% at discharge and follow-up, respectively) suggested that some subjects in this study might have subclinical vitamin A deficiency. Treatment with vitamin A did not increase the mean serum retinol concentrations or decrease the percentage of children with serum retinol concentrations < 0.70 μmol/L relative to values in the placebo group. This finding does not support the hypothesis that these subjects had subclinical vitamin A deficiency. However, serum retinol is not a reliable measure of vitamin A status in persons who retain marginal liver vitamin A stores; thus, serum retinol may not be sufficiently sensitive to identify vitamin A deficiency in this setting (1–3).

Because the RDR test was developed to assess liver vitamin A stores in subjects with subclinical vitamin A deficiency, we reasoned that this test might show that a significant percentage of subjects in the placebo group had vitamin A deficiency at discharge. When subjects with an active acute phase response were examined alone, ~20% of subjects in both the vitamin A and placebo groups had a positive RDR test result. Because it is reasonable to assume that all of the subjects in the vitamin A group had adequate vitamin A stores at the time of hospital discharge, we concluded that ~20% of the subjects in this group had a false-positive RDR test result. Furthermore, because the percentage of subjects with a positive RDR test result was nearly identical in the placebo group, we further concluded that the RDR test is unable to differentiate among subjects of differing vitamin A status during an active acute phase response. A weakness with this conclusion is that we did not have independent confirmation of the vitamin A status of the children in the placebo group. However, when subjects with no active acute phase response were examined, we found that 56% of the placebo group had a positive RDR test result, a 10-fold greater rate than that seen in the vitamin A group. This finding strongly supports our presumption that subjects entering the hospital with pneumonia had subclinical vitamin A deficiency and also supports the conclusion that the RDR test may underestimate the prevalence of vitamin A deficiency when used during an active acute phase response in children with low liver vitamin A stores.

To buttress the conclusion that the RDR test was correctly predicting the vitamin A status in subjects with no active acute phase response, we examined serum retinol concentrations in these subjects, controlling for the RDR test result and the acute phase response. As expected, the subjects in the placebo group with a positive RDR test result had significantly lower serum retinol concentrations than did those with a negative RDR test result in that group. Furthermore, no association was seen between serum retinol and the RDR test result in the vitamin A group. This latter observation also supports the conclusion that the positive RDR test results in the vitamin A group were false positive and induced by the acute phase response and did not reflect underlying liver vitamin A stores.

Previous work shows that conditions other than the acute phase response can also interfere with the validity of the RDR test. Protein-energy malnutrition appears to interfere with the RDR test because of the inadequate synthesis or release of RBP from the liver (11). Zinc deficiency may interfere for the same reason (12). Protein-energy malnutrition was not a clinical problem in our subjects, as indicated by mean anthropometric z scores that were within 1 SD of that of the reference population (Table 1). Some of the subjects may have had a zinc deficiency because maternal and neonatal zinc concentrations in Lima are lower than those reported from well-nourished populations, and these concentrations increased with maternal zinc supplementation (13). It is thus plausible that correction for zinc status might have further increased the percentage of subjects identified as vitamin A deficient in our study.

After adjusting for the acute phase response, we found that nearly 60% of the subjects in the placebo group in the present study had subclinical vitamin A deficiency at discharge. Given the relatively short duration of hospitalization (a median of 3 d), we believe that this finding in the placebo group was representative of the vitamin A status of both groups before administration of the vitamin A treatment. We thus conclude that most children in the present study were deficient in vitamin A at the time of supplement administration. This finding is significant because the vitamin A group had more severe disease during their hospital stay than did the children in the placebo group (8). In addition to our finding, one other therapeutic clinical trial reported more severe disease in infants with respiratory disease who received vitamin A supplements (14), and an increased prevalence of acute lower respiratory tract infection was seen in subjects receiving vitamin A supplements in community studies, as indicated in a recent review (15). Although the mechanism underlying this difference in disease severity is not known, our results rule out the possibility that children in the present study were vitamin A replete at admission and that vitamin A supplements were thus being provided to non-deficient children.

To our knowledge, this is the first reported use of the RDR test in subjects with an active acute phase response. Our findings suggest that the RDR test cannot be used reliably in such subjects, just as serum retinol has proven to be an unreliable indicator of vitamin A deficiency in such subjects (16, 17). The need for a test...
of vitamin A status that can be used during an active acute phase response has been recognized by other investigators, who are testing alternative strategies (18, 19). Until such tests are available, this study emphasizes that neither serum retinol nor the RDR test is a reliable indicator of subclinical vitamin A deficiency during the acute phase response.

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REFERENCES