No improvement in suboptimal vitamin A status with a randomized, double-blind, placebo-controlled trial of vitamin A supplementation in children with sickle cell disease1–3

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ABSTRACT

Background: Suboptimal vitamin A status is prevalent in children with type SS sickle cell disease (SCD-SS) and is associated with hospitalizations and poor growth and hematologic status. The supplemental vitamin A dose that optimizes suboptimal vitamin A status in this population is unknown.

Objective: The efficacy of Recommended Dietary Allowance (RDA) doses (based on age and sex) of vitamin A (300, 400, or 600 µg retinyl palmitate/d) or vitamin A + zinc (10 or 20 mg zinc sulfate/d) compared with placebo to optimize vitamin A status was assessed in children aged 2.0–12.9 y with SCD-SS and a suboptimal baseline serum retinol concentration (<30 µg/dL).

Design: In this randomized, double-blind, placebo-controlled trial, vitamin A status (serum retinol, prealbumin, retinol-binding protein, and relative-dose-response test) and disease-related illness events were assessed.

Results: Twelve months of vitamin A supplementation at the doses recommended for healthy US children (based on age and sex) failed to improve serum retinol values in either group (vitamin A: n = 23; vitamin A + zinc: n = 18) compared with placebo (n = 21). By 12 mo, the increase (±SD) in serum retinol (3.6 ± 2.8 µg/dL) in those taking 600 µg vitamin A/d was significantly different from the decrease (±SD: −2.8 ± 2.4 µg/dL) in those taking 300 µg/d, which possibly suggests a dose-response relation (P < 0.05) with RDA doses.

Conclusions: Compared with placebo, 12 mo of vitamin A supplementation at the RDA for healthy children did not improve serum retinol values in children with SCD-SS, which possibly suggests that higher doses are needed. However, the existence of alternative explanations emphasizes the need for future research. Am J Clin Nutr 2012;96:932–40.

INTRODUCTION

Vitamin A is an essential nutrient in humans required for immune function, growth, development, reproduction, and vision (1). The adverse health effects of vitamin A deficiency (serum retinol <20 µg/dL) are numerous, including an increased risk of morbidity and mortality, reduced resistance to infection, impaired cellular differentiation, and higher rates of anemia, xerophthalmia, and blindness (2). In US children with type SS sickle cell disease (SCD-SS)4, suboptimal vitamin A status (serum retinol <30 µg/dL) is prevalent and is associated with increased hospitalizations and poor growth and hematologic status (3). In addition, the adequacy of vitamin A dietary intake declines with age in children with SCD-SS (4). Collectively, these data suggest that vitamin A supplementation is necessary throughout childhood in those with SCD. However, no study has investigated the supplemental vitamin A dose needed to optimize vitamin A status in this population.

The aims of this study were to determine the efficacy of Recommended Dietary Allowance (RDA) doses (for healthy US children based on age and sex) of vitamin A or vitamin A + zinc compared with placebo to optimize vitamin A status and reduce the number of hospitalizations and other illness events in children with SCD-SS. It was hypothesized that in children with SCD-SS: 1) RDA-level vitamin A supplementation would optimize vitamin A status, 2) vitamin A supplementation would be significantly associated with a reduction in the rate of hospitalizations, and 3) vitamin A + zinc supplementation would be associated with a further reduction in the rate of hospitalizations.

SUBJECTS AND METHODS

Subjects with SCD-SS aged 2.0–12.9 y were recruited in Pennsylvania from the Sickle Cell Center at The Children’s Hospital of Philadelphia.1

1 From the Divisions of Gastroenterology, Hepatology and Nutrition (KAD, JIS, DAK, BSZ, and VAS) and Hematology (KO-F), Department of Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, PA; the Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA (KAD, KO-F, BSZ, and VAS); and the Department of Nutritional Sciences, The Pennsylvania State University, University Park, PA (MHG).

2 Supported by the Comprehensive Sickle Cell Center (SU54 HL070596), Clinical Translational Research Center (UL1RR024134), K12 Mentored Career Development Award (KL2RR024132), and Nutrition Center at the Children’s Hospital of Philadelphia.

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4 Abbreviations used: CHOP, Children’s Hospital of Philadelphia; DA-PT, dark adaptation pupillary threshold; ED, Emergency Department; HACU, Hematology Acute Care Unit; hs-CRP, high-sensitivity C-reactive protein; LME, longitudinal mixed effects; RBP, retinol-binding protein; RDA, Recommended Dietary Allowance; RDR, relative dose response; SCD, sickle cell disease; SCD-SS: type SS sickle cell disease.

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Hospital of Philadelphia (CHOP) and Lehigh Valley Hospital and in New Jersey from the CHOP Voorhees Specialty Care Center and Newark Beth Israel Medical Center. Exclusion criteria included chronic transfusion therapy or a transfusion within the past 2 mo, hydroxyurea therapy, history of stroke, liver enzymes >3 times the reference range, height >2.0 SDs above the age and sex mean (>98th percentile, CDC 2000 reference standards; 5), participation in another intervention study, pregnancy, and other chronic conditions known to affect growth, dietary intake, or nutritional status. In addition, subjects taking daily vitamins or commercial nutritional supplements containing vitamin A were not eligible for the study. However, subjects willing to discontinue supplementation with the approval of their medical care team were eligible after a 2-mo washout period.

This protocol was approved by the Institutional Review Boards at CHOP and the Newark Beth Israel Medical Center. In accordance with Institutional Review Board guidelines, written informed consent was obtained from the subjects’ parents or guardians, and verbal assent was obtained from subjects >6 y of age.

Study design

All subjects completed a screening visit, and, if eligible, baseline and 3-, 6-, 9-, and 12-mo visits. The initial recruitment date was 16 November 2004. Before the screening visit, a chart review was completed to document an SCD-SS diagnosis based on electrophoresis and to conduct a preliminary assessment of inflammatory status; dietary intake; height; and weight. Those eligible completed records were analyzed by the Minnesota Nutrition Data System, and calculated nutrient intakes were compared with age- and sex-specific Dietary Reference Intakes, expressed as a percentage of the estimated energy requirement for resting energy expenditure was 1.4, which corresponds to the ratio of total energy expenditure and standardized techniques (6), and the mean was used for analysis. BMI was calculated (kg/m^2) from weight by using a digital scale (Sacletronix) and standing height by using a stadiometer (Holtain) at screening and, if enrolled, at baseline and 3, 6, 9, and 12 mo. Weight, height, and BMI were compared with CDC 2000 reference standards to generate age- and sex-specific z scores (5). At baseline and 3, 6, and 12 mo, skinfold thickness was measured (0.1 mm) at the triceps, biceps, subscapular, and suprailiac sites with a skinfold caliper (Holtain) to estimate subcutaneous fat stores by using the Brook (7) and Slaughter (8) age-specific equations. Midupper-arm circumference with a nonstretchable fiberglass tape (McCoy; baseline and 3, 6, and 12 mo) and triceps-skinfold-thickness measures were used to calculate upper-arm muscle area and upper-arm fat area (9). Resultant areas were compared with reference data from the National Center for Health Statistics to generate the z scores (10). Pubertal status according to the criteria of Tanner (11) was determined by using a validated self-assessment questionnaire (12) at baseline and 6 and 12 mo.

Dietary intake

Dietary intake was estimated from one 24-h recall at screening and from three 24-h recalls (2 weekdays and 1 weekend day) during the vitamin A–supplementation study at baseline and 3 and 12 mo (three 24-h recalls averaged for each assessment). Children and/or parents were interviewed by a research dietitian within 3 wk of the study visit. The first interview was conducted in person during the study visit, and 2 subsequent interviews were conducted by phone (13, 14). The quantity and size of each food portion were estimated by using a food-portion booklet (2D Food Portion Visual; Nutrition Counseling Enterprises). Completed records were analyzed by the Minnesota Nutrition Data System, and calculated nutrient intakes were compared with age- and sex-specific Dietary Reference Intakes, expressed as a percentage of the RDA (15). In children with SCD-SS, we previously showed (16) that the ratio of total energy expenditure to resting energy expenditure was 1.4, which corresponds to a low active physical activity level (17). Thus, energy intake adjusted for height, weight, age, sex, and BMI z score was expressed as a percentage of the estimated energy requirement for low active children.

Biochemistry

All blood samples were obtained after a minimum 10-h fast. Serum concentrations of retinol (at screening and, if enrolled, at baseline and 3, 6, and 12 mo) and retinol esters (baseline and 3, 6, and 12 mo) were measured by HPLC, and retinol-binding protein (RBP; screening and if enrolled baseline and 3, 6, and 12 mo) and prealbumin (baseline and 3, 6, and 12 mo) were measured by radial immunodiffusion assay (Green Laboratory, Pennsylvania State University). Plasma zinc was measured at baseline and at 3, 6, and 12 mo by atomic absorption spectrophotometry (18) by using a Perkin-Elmer 2380 atomic absorption spectrophotometry fitted with background deuterium lamp (Krebs Pediatric Nutrition Laboratory). A complete blood count without a differential count
at screening and, if enrolled, with a differential count at baseline and 3, 6, and 12 mo and reticulocyte count (baseline and 3, 6, and 12 mo) by using standard hematologic techniques and hemoglobin F (baseline and 3, 6, and 12 mo) by HPLC (19) were determined by the Clinical Hematology Laboratory at CHOP. The hepatic function panel (baseline and 6 and 12 mo) and γ-glutamyl transferase (screening and, if enrolled, baseline and 6 and 12 mo) were measured by the Clinical Chemistry Laboratory at CHOP. Serum high-sensitivity C-reactive protein (hs-CRP) was measured at screening and, if enrolled, at baseline and 3, 6, and 12 mo by particle-enhanced rate nephelometry (Behring BNII nephelometer) by the Clinical Immunology Laboratory at CHOP (Dade-Behring Diagnostics Inc).

The relative-dose-response (RDR) test assessed the vitamin A status at the baseline and 12-mo visits. After a fasting blood sample was collected, the subject was immediately given an oral dose of 600 μg vitamin A as retinyl palmitate in a flavored liquid solution. A small standardized meal containing moderate total fat and minimal vitamin A was given after dosing. After 5 h, a second blood sample was collected. The RDR was calculated from the fasting (A0) and 5-h (A5) values for serum retinol as: $RDR = (A_5 - A_0)/A_5 \times 100$. An RDR > 20% indicated

### TABLE 1

Characteristics, biochemistry, and dietary intakes for subjects with SCD-SS at screening by vitamin A status presented as serum retinol ($\mu$g/dL)$^1$

<table>
<thead>
<tr>
<th>Retinol group</th>
<th>Optimal, $\geq$30 $\mu$g/dL (n = 26)</th>
<th>Suboptimal, 20–29 $\mu$g/dL (n = 48)</th>
<th>Deficient, &lt;20 $\mu$g/dL (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 96)</td>
<td>6.8 ± 3.1$^2$</td>
<td>6.4 ± 3.6</td>
<td>6.8 ± 3.2</td>
</tr>
<tr>
<td>Sex (%) female</td>
<td>48</td>
<td>54</td>
<td>56</td>
</tr>
<tr>
<td>Height z score</td>
<td>$-0.4 \pm 1.0^4$</td>
<td>$-0.4 \pm 1.0^4$</td>
<td>$-0.3 \pm 1.0^4$</td>
</tr>
<tr>
<td>Weight z score</td>
<td>$-0.5 \pm 1.0^4$</td>
<td>$-0.3 \pm 1.0^4$</td>
<td>$-0.5 \pm 1.0^4$</td>
</tr>
<tr>
<td>BMI z score</td>
<td>$-0.4 \pm 1.0^4$</td>
<td>$-0.2 \pm 1.0^4$</td>
<td>$-0.4 \pm 0.9$</td>
</tr>
<tr>
<td>Vitamin A status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinol ($\mu$g/dL)</td>
<td>26 ± 8</td>
<td>36 ± 5</td>
<td>24 ± 3$^e$</td>
</tr>
<tr>
<td>RBP ($\mu$g/mL)</td>
<td>21 (13, 58)$^{5,6}$</td>
<td>29 (17, 58)$^7$</td>
<td>23 (18, 41)$^8$</td>
</tr>
<tr>
<td>Hemoglobin, inflammatory, and liver status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.2 (4.6, 11.8)$^{10}$</td>
<td>8.7 (6.7, 11.8)$^4$</td>
<td>8.1 (6.2, 10.9)$^{11,12}$</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>23.8 (13.4, 37.7)$^4$</td>
<td>25.2 (19.4, 37.7)$^4$</td>
<td>23.9 (17.5, 31.9)$^4$</td>
</tr>
<tr>
<td>Platelets ($\times 10^3/\mu$L)</td>
<td>467 ± 120$^4$</td>
<td>460 ± 150$^{12}$</td>
<td>475 ± 81</td>
</tr>
<tr>
<td>WBCs ($\times 10^3/\mu$L)</td>
<td>13.0 (6.7, 27.8)$^4$</td>
<td>13.3 (8.7, 23.9)$^4$</td>
<td>13.0 (6.7, 25.1)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>2.2 (0.1, 55.3)$^{13,14}$</td>
<td>2.3 (0.2, 23.1)$^4$</td>
<td>1.9 (0.1, 23.4)$^{14,15}$</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>20 (10, 95)$^{10}$</td>
<td>17 (11, 29)$^4$</td>
<td>21 (11, 54)$^4$</td>
</tr>
<tr>
<td>Dietary intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1431 (469, 3135)$^{15,16}$</td>
<td>1226 (594, 3119)$^{12}$</td>
<td>1414 (469, 2848)$^{16}$</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>45 (15, 123)$^{13,14}$</td>
<td>41 (15, 123)$^{13,14}$</td>
<td>46 (18, 107)$^{16}$</td>
</tr>
<tr>
<td>Protein (% of RDA)</td>
<td>230 (87, 539)$^{16}$</td>
<td>222 (88, 483)$^{10}$</td>
<td>233 (87, 344)$^{16}$</td>
</tr>
<tr>
<td>EER, low active (%)</td>
<td>94 (38, 227)$^{14,16}$</td>
<td>89 (50, 209)$^{10}$</td>
<td>94 (38, 197)$^{16}$</td>
</tr>
<tr>
<td>Vitamin A (IU/d)</td>
<td>1876 (159, 14,729)$^{15}$</td>
<td>1748 (159, 4362)$^{12}$</td>
<td>2100 (481, 14,729)$^{2}$</td>
</tr>
<tr>
<td>Vitamin A (% of RDA)</td>
<td>107 (11, 534)$^{13,14}$</td>
<td>111 (11, 354)$^{12}$</td>
<td>119 (17, 534)$^{16}$</td>
</tr>
</tbody>
</table>

$^1$ Significant differences were assessed using ANOVA or Kruskal-Wallis tests. When differences were detected, Bonferroni adjustment of normally distributed variables was made or a Wilcoxon’s rank-sum test with Bonferroni correction of the $P$ for nonnormally distributed variables was used. Significantly different from optimal: $^5P < 0.05$, $^6P < 0.01$. Significantly different from suboptimal: $^1P < 0.05$, $^2P < 0.01$. Significantly different from optimal: $^3P < 0.05$, $^4P < 0.01$. EER, estimated energy requirement; GGT, γ-glutamyl transferase; hs-CRP, high-sensitivity C-reactive protein; IU, International Units; RBP, retinol-binding protein; RDA, Recommended Dietary Allowance; SCD-SS, type SS sickle cell disease; WBCs, white blood cells.

$^2$ Mean ± SD (all such values).

$^3$ Median: range in parentheses (all such values).

$^4$ Optimal, $\geq$30 $\mu$g/dL.

$^5$ Suboptimal, 20–29 $\mu$g/dL.

$^6$ Deficient, <20 $\mu$g/dL.

$^7$ $n = 95$.

$^8$ $n = 25$.

$^9$ $n = 25$.

$^{10}$ $n = 10$.

$^{11}$ $n = 22$.

$^{12}$ $n = 11$.

$^{13}$ $n = 11$.

$^{14}$ $n = 20$.

$^{15}$ $n = 17$.

$^{16}$ $n = 87$.

$^{17}$ $n = 23$. 

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a positive test result, which indicated inadequate hepatic stores of vitamin A, ie, marginal vitamin A status (20–22).

**Dark adaptation**

Afferent pupillary reflex was measured at baseline and at 6 and 12 mo by using a dark adaptometer (LKC Technologies Inc), and the dark adaptation pupillary threshold (DA-PT) in luminance [log candela (cd)/m²] was assessed. After a camera flash, the subject sat in a darkened room for 10 min to adapt the eyes to the dark. Next, the dark adaptometer was held over the left eye while a very dim light was shown until the pupil in the right eye constricted. The reading at which the pupil constricted was recorded as the pupillary reflex (23). More negative scores reflect the dark. Next, the dark adaptometer was held over the left eye subject sat in a darkened room for 10 min to adapt the eyes to established as supplements taken reviewed by the research team, and the level of adherence was questionnaire at the 3-, 6-, 9-, and 12-mo visits. All information was about supplement-use patterns was also obtained by question-naire for those enrolled in the vitamin A supplementation study. All admissions to an inpatient unit, the Hematology Acute Care Unit (HACU; a short-stay medical unit), or Emergency Department (ED) were obtained by chart review for the 12 mo before enrollment (baseline visit) and prospectively over the 12-mo study. Information on all illness events, including specific diagnosis of pain and/or fever episodes, acute chest syndrome, infection, and other illness events, were recorded.

**Adherence to intervention**

Three methods were used to assess adherence with the study protocol. All families were asked to return monthly calendars, which tracked the days on which the supplement was taken, and the supplement bottles (residual volumes were measured) every 3 mo in exchange for new bottles for the next 3 mo. Information about supplement-use patterns was also obtained by questionnaire at the 3-, 6-, 9-, and 12-mo visits. All information was reviewed by the research team, and the level of adherence was established as supplements taken ≥85% of the time or <85% of the time based on the supplement adherence questionnaire, because this is the only method for which complete data were obtained. Questions regarding how many days per week the supplement was and was not taken were assessed.

**Statistical analyses**

The primary outcome of the study was the number of hos-pitalizations over 12 mo among children with SCD-SS. On the basis of our previous data in children with SCD-SS (3), with the use of an ANOVA model in nQuery Advisor software, the following values were used to determine that 48 subjects were needed overall (n = 16 in each group): the difference in means characterized by a variance of means = 0.305, SD = 1, power = 0.8, α = 0.017 (used to adjust the type I error rate for the 3 planned pairwise comparisons). This translated into an effect size of 0.3054. In the ANOVA model, the effect size was the variance of the means divided by the within-group variance (square of the SD) and represented an index of the separation expected among the observed means. It was expected that 35% of subjects would not continue to meet the study eligibility criteria over the 12-mo period and, accordingly, 66 subjects would be needed—22 in each arm of the study.

All variables were tested for normality, and nonparametric tests were used as appropriate. To determine associations between variables, Pearson’s correlation coefficients or Spearman’s rank correlations were performed, as appropriate. For multiple comparisons, group differences at baseline and over time (change score) were determined by using an ANOVA or Kruskal-Wallis tests. When differences were detected, Bonferroni adjustment for normally distributed variables or Wilcoxon’s rank-sum test with Bonferroni correction of the α for nonnormally distributed variables was used.

Longitudinal-mixed-effects (LME) analyses (25) were used to examine change over time in serum retinol, hospitalization, and disease events and whether patterns of change were different among the 3 groups. These analyses were made using the intention-to-treat model where all subjects are included regardless of adherence to the study protocol. Similar to multiple linear regression analysis, LME analysis allows for multiple observations per subject. LME assumes that observations measured from the same subject are dependent and, therefore, the regression coefficients vary across subjects and are considered to be random. Also, it allows for unequal intervals between visits, uses data from all subjects, even when some study visits were missed, and accommodates both fixed and random effects. Parameter estimates, as in regression analysis, indicate the contribution of the independent variable to the model. For these LME analyses, the subject was treated as a random effect and measurement and time as fixed effects.

All statistical analyses were performed by using STATA 12.0 (StataCorp). The results were considered significant at P < 0.05 (unless otherwise indicated), and data are presented as means ± SDs (normal distribution) or medians and ranges (skewed distribution).

**RESULTS**

At screening, 96 children with SCD-SS were evaluated. Characteristics, biochemistry, and dietary intake for screening participants are presented in Table 1. Overall, children with
SCD-SS had suboptimal growth status as indicated by negative z scores for height, weight, and BMI, which did not differ by retinol group. Those with deficient vitamin A status had significantly lower RBP compared with suboptimal and optimal groups (P < 0.01). Vitamin A status was positively associated with measures of hemoglobin (r = 0.47, P < 0.0001) and hematocrit (r = 0.45, P < 0.0001). In comparison with the optimal retinol group, those in the suboptimal and deficient groups had significantly higher γ-glutamyl transferase (P < 0.01). A total of 27%, 50%, and 23% of subjects had serum retinol concentrations in the optimal, suboptimal, and deficient ranges, respectively (Figure 1). No significant differences were found among retinol groups in any assessment of dietary intake.

At baseline, 62 subjects with SCD-SS and suboptimal vitamin A status were randomly assigned to receive vitamin A (n = 23), vitamin A + zinc (n = 18), or placebo (n = 21), and 56 were classified as Tanner stage 1 and 6 as Tanner stage 2. Of the 52 subjects completing the study, 44 were Tanner stage 1, 6 were stage 2, and 2 were stage 3. The subjects’ characteristics and biochemistry results at baseline and 12 mo are presented in Table 2. Growth and nutritional status were suboptimal in children with SCD-SS, as indicated by negative z scores for height, weight, BMI, upper-arm muscle area, and upper-arm fat areas. At baseline, vitamin A status as serum retinol was positively associated with percentage body fat (r = 0.18, P < 0.02) and with z scores for weight (r = 0.26, P < 0.05), BMI (r = 0.31,

### Table 2

Characteristics and biochemistry for subjects with SCD-SS and suboptimal vitamin A status at baseline and 12 mo

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin A</td>
<td>Placebo</td>
</tr>
</tbody>
</table>
| Age (y)                  | 7.5 ± 2.9 | 7.6 ± 2.4 | 7.8 ± 3.2 | 8.3 ± 2.9
| Sex (% female)           | 52       | 40     | 58        | 58       |
| Height z score           | -0.4 ± 0.9 | -0.3 ± 0.9 | -0.6 ± 1.0 | -0.4 ± 0.9 |
| Weight z score           | -0.7 ± 1.1 | -0.5 ± 0.8 | -0.7 ± 1.1 | -0.5 ± 0.9 |
| BMI z score              | -0.7 ± 1.0 | -0.5 ± 0.8 | -0.4 ± 1.1 | -0.5 ± 0.9 |
| UAMA z score             | -0.6 (−2.9, 4.5) | -0.6 (−2.6, 0.5) | -0.5 (−3.1, 1.0) | -0.6 (−2.2, 1.3) |
| UAFA z score             | -1.0 (−2.2, 0.5) | -1.1 (−2.0, 0.2) | -1.1 (−1.7, 1.1) | -1.1 (−2.4, 0.8) |
| Body fat (%)             | 12.8 ± 4.3 | 11.7 ± 3.1 | 12.9 ± 3.9 | 14.6 ± 4.8 |
| Vitamin A and zinc status |          |        |          |          |
| Retinol (µg/dL)          | 17.6 ± 4.0 | 18.2 ± 3.3 | 19.4 ± 4.9 | 18.9 ± 4.5 |
| RBP (µg/mL)              | 33.5 ± 8.3 | 33.7 ± 9.6 | 33.3 ± 9.5 | 33.3 ± 5.7 |
| Prealbumin (µg/mL)       | 118 ± 22 | 115 ± 18 | 122 ± 20 | 122 ± 20 |
| RDR (%)                  | 1.1 ± 1.1 | 2.9 ± 6.6 | 4.4 ± 5.8 | 2.3 ± 5.6 |
| Zinc (µg/dL)             | 79.5 (63.2, 100.5) | 75.9 (63.5, 94.6) | 81.8 (62.7, 106.6) | 76.8 (62.9, 97.0) |

**Hematologic and inflammatory status**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/mL)</td>
<td>8.2 (6.2, 9.8)</td>
<td>7.9 (6.6, 10.0)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>23.4 (19.3, 29.1)</td>
<td>23.3 (19.1, 30.5)</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>12.2 ± 5.0</td>
<td>14.3 ± 5.1</td>
</tr>
<tr>
<td>HgbF (%)</td>
<td>6.9 (0.5, 22.9)</td>
<td>7.6 (2.2, 18.4)</td>
</tr>
<tr>
<td>Platelets (x10³/µL)</td>
<td>429 (239, 682)</td>
<td>484 (173, 490)</td>
</tr>
<tr>
<td>WBCs (x10³/µL)</td>
<td>12.0 (5.4, 22.7)</td>
<td>13.2 (8.7, 26.8)</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>4.7 (0.4, 16.3)</td>
<td>1.0 (0.4, 45.1)</td>
</tr>
<tr>
<td>GGTT (U/L)</td>
<td>20 (8, 50)</td>
<td>23 (11, 43)</td>
</tr>
</tbody>
</table>

**Notes:**

1. Significant group differences at baseline and over time (change score) were investigated by using an ANOVA or Kruskal-Wallis test. No differences were detected. For supplemental vitamin A, subjects in the vitamin A or vitamin A + zinc groups received 300 µg/d (1000 IU) if aged 2.0–3.9 y at baseline, 400 µg/d (1333 IU) if aged 4.0–8.9 y, and 600 µg/d (2000 IU) if aged 9.0–12.9 y. For supplemental zinc, subjects in the vitamin A + zinc group received 10 mg/d if aged 2.0–8.9 y and 20 mg/d if aged 9.0–12.9 y. Vitamin A and zinc were supplied as vitamin A palmitate and ZnO, respectively.

2. Mean ± SD (all such values).

3. n = 22.

4. Median; range in parentheses (all such values).

5. n = 16.

6. n = 20.

7. n = 14.

8. n = 19.

9. n = 15.

10. n = 18.

11. n = 12.

12. n = 21.

13. n = 1.
was negatively associated with the change in serum retinol, which is an indirect measure of vitamin A stored in the liver, Allowance doses for healthy children failed to optimize serum retinol at baseline and after 12 mo of vitamin A supplementation compared with placebo in 51 children with type SS sickle cell disease and suboptimal serum retinol in those taking 600 μg (2000 IU) vitamin A/d was significantly different from that in the placebo group and from the decrease in those taking 300 μg (1000 IU) vitamin A/d was significantly different from that in the placebo group, which possibly suggests a dose-response relation (Table 3) with RDA doses (based on age and sex). The change in the RDR test, which is an indirect measure of vitamin A stored in the liver, was negatively associated with the change in serum retinol concentrations over 12 mo (r = −0.53, P < 0.003). The change in the RDR by group (P > 0.05) was 0.7 ± 13.4% for vitamin A (n = 18), −0.9 ± 9.7% for vitamin A + zinc (n = 10), and −0.3 ± 8.2% for placebo (n = 15). No subject in any group has an RDR >20% at baseline or 12 mo.

Subjects with SCD-SS at baseline had an adequate median (range) overall dietary intake of vitamin A [107% (15%, 617%) of RDA; 2349 (279, 18,900) IU/d], zinc [123% (28%, 458%) of RDA; 71 (1.4, 13.7) mg/d], and protein [243% (43%, 542%) of RDA; 51 (10, 92) g]. Also adequate were energy intake [1607 (358, 2608) kcal] and the estimated energy requirement for low active children [102% (23%, 185%)]. No differences were observed among groups at baseline or over 12 mo in any assessment of dietary intake.

Sixty-four percent of the subjects were adherent to taking the oral daily supplement ≥85% of the time, and 36% reported <85% adherence to supplement intake. No significant differences in adherence among the vitamin A, vitamin A + zinc, and placebo groups were found. In addition, no association between adherence and change in serum retinol was found.

In the vitamin A, vitamin A + zinc, and placebo groups, respectively, DA-PT did not differ at baseline [−1.7 (−2.1, −0.9), −1.7 (−2.1, −0.9), and −1.3 (−2.9, −0.9) cm/d; n = 21, 15, 19] or among groups (mean values or change from baseline) in response to 12 mo of vitamin A supplementation [−1.7 (−2.9, −0.9), −2.1 (−2.9, −1.3), and −2.1 (−2.9, −1.3) cm/d; n = 17, 11, 18]. The change in DA-PT per group (P > 0.05) was −0.4 (−1.2, 0.8) cm/d for vitamin A (n = 15), −0.4 (−1.2, 0.8) cm/d for vitamin A + zinc (n = 11), and 0 (−1.6, 0.4) cm/d for placebo (n = 17). The percentages of subjects in the vitamin A, vitamin A + zinc, and placebo groups, respectively, that had a DA-PT of >−1.24 cm/d² (suggestive of abnormal dark adaptation) were 48%, 27%, and 58% at baseline and 41%, 18%, and 33% by 12 mo.

Admissions to an inpatient unit, HACU, and/or ED and associated illness events for the 12 mo before enrollment and subsequent 12 mo of the study are shown in Table 4. For the 12 mo before the baseline visit, subjects in the vitamin A group had significantly more days in the HACU than did those in the vitamin A + zinc and placebo groups (P < 0.01), and subjects in the placebo group had significantly fewer vaso-occlusive events in the HACU than did those in the vitamin A and vitamin A + zinc groups. On the basis of an LME analysis, vitamin A supplementation alone or in combination with zinc for 12 mo did not significantly reduce the number of hospitalizations or HACU or ED visits or associated disease events in any group.

**DISCUSSION**

The main findings from this study were that suboptimal vitamin A status was prevalent in children with SCD-SS. At screening, 73% had a serum retinol concentration <30 μg/dL with a surprising 23% in the deficient range (<20 μg/dL)—a level that includes depletion of liver stores (2). Additionally, 12 mo of vitamin A supplementation at the RDA dose for healthy children did not improve vitamin A status compared with placebo in children with SCD-SS. At 12 mo, the increase in serum retinol in those taking 600 μg (2000 IU) vitamin A/d was significantly different from the decrease in those taking 300 μg/d (1000 IU). Collectively, these data may suggest that the dose needed to replete children with SCD-SS is higher than that recommended for healthy US children. This is one interpretation of the data and possibility of alternative conclusions, such as...
altered liver metabolism or SCD-specific mechanisms emphasizes the need for future research.

It has been recognized for several decades that children with SCD, especially those with the hemoglobin SS genotype, have poor growth and delayed maturation (26). Increased nutrient requirements and/or poor nutritional status have been documented in children with SCD, which suggests that chronic undernutrition contributes to the growth failure and delayed maturation (16, 27). Only one nutrition-intervention study in children with SCD (28) addressed this potentially remediable problem, showing that zinc supplementation improved growth. Several nutrient deficiencies have been reported in subjects with SCD and were associated with adverse disease outcomes (3, 29–31). In children, vitamin A deficiency has been associated with higher rates of mortality and morbidity (32) and with poor growth and development (33). Vitamin A supplementation reduced mortality from infectious disease up to 30% in community-based studies (34) and morbidity, particularly from measles, gastrointestinal diseases, and malaria (32). The current study is the first prospective investigation of the effect of vitamin A supplementation on important clinical outcomes, such as hospitalizations, number and length of nonhospitalized illness events, and growth, nutrition, and hematologic status in children with SCD. The results show that vitamin A supplementation at the dose recommended for healthy children failed to improve vitamin A status and in this sample failed to change hospitalizations or growth. This outcome may suggest a higher vitamin A requirement than anticipated, possibly because of chronic inflammation and/or possible stool or urine loss. In addition, alternative conclusions may also exist. The dose of vitamin A needed to optimize vitamin A status in children with SCD-SS remains unclear.

The median dietary intake of vitamin A (% RDA) for all subjects (n = 60) at baseline was 107% with a wide range, spanning from 15% to 617%, which suggests adequate vitamin A dietary intake in agreement with previous research (3). Of interest, the adequacy of dietary vitamin A intake in SCD declines starting at age 9 y and lasts into young adulthood (4). In the current study, subjects with SCD-SS were aged 2.0–12.9 y, and future studies should prospectively determine whether the observed decline in dietary intake affects vitamin A status in teenagers and young adults with SCD.

We previously showed that children with SCD are at risk of zinc deficiency (28, 29). Because zinc is involved in the absorption, mobilization, transport, and metabolism of vitamin A (35), it was important to investigate the contribution of zinc status in the etiology of vitamin A deficiency, which has not been previously addressed in children with SCD. Zinc deficiency reduces hepatic RBP synthesis, resulting in lower concentrations of the circulating transport protein in plasma and lower serum retinol concentrations (36). Zinc is also important in the conversion of retinol to retinal, because the zinc-dependent enzyme alcohol dehydrogenase is required (35). Compared with vitamin A alone, only vitamin A + zinc supplementation corrected vitamin A deficiency in a large proportion of otherwise healthy Bangladeshi children (37), which suggests a synergistic effect. Results from the current study differ; suboptimal vitamin A status did not improve in either the vitamin A or the vitamin A + zinc.
group, likely because of inadequate levels of supplement (alternative conclusions may exist). Future research should test the efficacy of different doses of vitamin A and/or zinc to improve vitamin status in children with SCD.

Children with SCD often come to the ED or HACU or are admitted to the hospital for care because of pain or fever for a sickle cell acute illness event. Many times the patient is treated in the ED or the HACU (<24-h stay) and discharged to home care. HACU visits are expected admissions for children with SCD because of frequent pain and other acute events, and the HACU was established to handle such admissions. If the child is not well enough to be discharged home, then he or she is transferred to the inpatient unit. More common diagnoses for a child with SCD who visits the HACU or ED or is admitted as an inpatient include the following: pain events, fever, acute chest syndrome, priapism, splenic sequestration/splenectomy, and dactilitis infections. In a study conducted in children with SCD-SS from 1995 to 1997, we previously showed that those with suboptimal serum retinol concentrations had a significantly greater number of hospitalizations and days spent in the hospital over 12 mo than did those with adequate serum retinol status (3). This suggests that vitamin A supplementation may significantly reduce hospitalizations, as do other beneficial therapies such as hydroxyurea and transfusion therapy in children with SCD (38, 39). However, in the current study conducted from 2004 to 2008, because vitamin A supplementation at the doses recommended for US healthy children did not improve vitamin A status in children with SCD, there was no subsequent change in the rate of hospitalizations or any disease events. Of interest, as shown in Table 5, subjects in the current as compared with the previous study were older, had significantly lower serum retinol (µg/dL), and had significantly fewer total hospitalizations, days hospitalized, and fever episodes. This suggests worsening vitamin A status with age and a change in the hospitalization patterns over the decade between studies.

Vitamin A metabolism is complex and poorly understood in those with SCD. At the time this study was conducted, the best measures of vitamin A status were assessed, including serum retinol, prealbumin, RBP, and the RDR test. However, the serum retinol concentration is not the optimal vitamin A status indicator to measure response to an intervention because it is homeostatically controlled and does not reflect liver vitamin A stores until liver reserves are substantially depleted. Liver vitamin A concentration is the best indicator of vitamin A status because it is the primary tissue storage site; however, liver biopsy tissue is rarely available from clinical indications. The RDR test is based on the principle that when liver stores of retinol are high, the serum retinol concentration is not changed by an oral dose of vitamin A; however, when reserves are low, the serum retinol concentration increases markedly, reaching a peak 5 h after the oral dose. Although the RDR test gives an indication of vitamin A liver reserves (the higher the response, the lower the vitamin A liver stores), it does not provide a sufficient quantitative estimate of total-body vitamin A. Dose-finding vitamin A–supplementation studies in children with SCD and suboptimal vitamin A status are needed to determine total-body vitamin A status, liver stores, and potential losses in the urine and stool.

This study was designed to test the efficacy of RDA doses (based on age and sex) of vitamin A or vitamin A + zinc compared with placebo to optimize vitamin A status. This study was not designed to investigate the dose of vitamin A supplementation required to improve vitamin A status regardless of age (a dose-finding study), and future studies should address this important question. In our sample, age was not associated with serum retinol at baseline or 12 mo. In addition, these data may suggest that the dose needed to improve serum retinol in this population is higher than the RDA. This is one interpretation of the data. Another interpretation is that, compared with healthy children, those with SCD may be different, and the fact that the RDR was normal at baseline in all subjects and remained normal 12 mo later may suggest that their vitamin A stores may be adequate and that serum retinol and RBP are lower because of alterations in liver metabolism perhaps related to SCD. An alternative conclusion is that higher doses may not result in normalized concentrations if inflammation reduces the plasma retinol set point. If this is the case, then plasma retinol should increase if the inflammation state is effectively treated. In our sample, 45% (26 of 58) at baseline and 30% (14 of 47) at 12 mo had an hs-CRP >3 mg/L. Adjustment for hs-CRP did not result in differences among groups in response to supplementation. In summary, serum retinol values were often suboptimal in children with SCD-SS, and 12 mo of vitamin A supplementation at the RDA dose for healthy children failed to optimize vitamin A status in this population. This may suggest that the dose needed to improve serum retinol is higher than the RDA. However, alternative conclusions exist and future dose-finding and liver vitamin A status studies are needed.

We are grateful to the subjects and their families for study participation and to our many colleagues, including the CHOP Clinical Translational Research

### Table 5

Characteristics, biochemistry, and hospitalizations for subjects with SCD-SS in the pilot study in comparison with a randomized controlled trial

<table>
<thead>
<tr>
<th>Nutritional status</th>
<th>Pilot (n = 44)</th>
<th>Randomized controlled trial (n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum retinol (µg/dL)</td>
<td>22.9 ± 4.2</td>
<td>18.4 ± 4.1</td>
</tr>
<tr>
<td>Dietary vitamin A (% of RDA)</td>
<td>99 (20, 897)</td>
<td>106 (15, 617)</td>
</tr>
<tr>
<td>EER, low active (%)</td>
<td>109 ± 31</td>
<td>108 ± 31</td>
</tr>
<tr>
<td>Growth status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height z score</td>
<td>−0.2 ± 0.8</td>
<td>−0.5 ± 0.9</td>
</tr>
<tr>
<td>Weight z score</td>
<td>−0.5 ± 0.8</td>
<td>−0.7 ± 1.0</td>
</tr>
<tr>
<td>BMI z score</td>
<td>−0.7 ± 1.0</td>
<td>−0.6 ± 0.9</td>
</tr>
<tr>
<td>Hematologic status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/mL)</td>
<td>7.9 ± 0.9</td>
<td>8.0 ± 0.9</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>23.3 ± 3.0</td>
<td>23.5 ± 2.9</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>13.1 ± 4.8</td>
<td>13.4 ± 4.6</td>
</tr>
<tr>
<td>HgbF (%)</td>
<td>10.3 ± 6.3</td>
<td>8.7 ± 5.5</td>
</tr>
<tr>
<td>Hospitalization (n/yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hospitalizations</td>
<td>2.8 ± 2.0</td>
<td>1.6 ± 2.0</td>
</tr>
<tr>
<td>Days hospitalized</td>
<td>6.8 ± 5.8</td>
<td>3.0 ± 4.5</td>
</tr>
</tbody>
</table>

1 Significant differences were assessed by using a Student’s t test or Wilcoxon’s rank-sum test: *Significantly different from pilot, P < 0.01. EER, estimated energy requirement; HgbF, fetal hemoglobin; RDA, Recommended Dietary Allowance; SCD-SS, type SS sickle cell disease.

2 Previously published data from Schall et al, 2004 (3).

3 Mean ± SD (all such values).

4 Values are medians; ranges in parentheses.
REFERENCES


