One-time graded doses of vitamin A to weanling piglets enhance hepatic retinol but do not always prevent vitamin A deficiency

Rebecca L Surles, Jordan P Mills, Ashley R Valentine, and Sherry A Tanumihardjo

ABSTRACT

Background: Vitamin A supplements are administered to infants in developing countries at immunization contacts; doses of 50,000 IU vitamin A are recommended. Doses of 100,000 IU are given to children aged 0.5–1 y. The efficacy of these doses has not been adequately determined.

Objective: We aimed to quantify liver vitamin A after the administration of vitamin A doses to piglets. Piglets are a good model for infants because of their similar size, gastrointestinal anatomy, and vitamin A requirements.

Design: Castrated male piglets born to sows fed a vitamin A–depleted diet throughout 1 (parity A) or 3 (parity B) pregnancy and lactation cycles were randomly assigned to receive 1 of 4 oral vitamin A doses (ie, 0, 25,000, 50,000, or 100,000 IU) at weaning (days 9–14). A vitamin A–depleted diet was fed until the piglets were killed on day 10. Serum retinol was measured on days 1, 2, 4, 7, and 10. The modified relative dose response was measured before supplementation and at the time of killing, and liver vitamin A concentration was measured.

Results: In both parities, 25,000 IU did not result in a mean liver retinol reserve > 0.07 μmol/g liver (the deficiency cutoff). The 50,000 IU dose increased mean reserves above 0.07 μmol/g only in parity A. Liver vitamin A reserves with the 100,000 IU treatment were only 5% above those with the 50,000 IU treatment. The modified relative dose-response test reflected differences in liver vitamin A stores in parity B, and the 0 IU group differed significantly from the 100,000 IU group (P = 0.011).

Conclusion: This piglet model suggests that, for supplementation to infants <6 mo old, a 50,000 IU dose is likely to be more efficacious in mitigating deficiency than is a 25,000 IU dose.

KEY WORDS Vitamin A status, infant nutrition, supplementation

INTRODUCTION

Children with vitamin A deficiency have a greater risk of irreversible blindness and dying of infectious diseases than do children who are vitamin A replete. Globally, >250 million children under the age of 5 y have vitamin A deficiency (1), and supplementation programs are commonly used to address this issue (2). Studies found little or no improvement in infant vitamin A status after supplementation with either low-dose β-carotene (3) or 25,000 IU vitamin A in the form of retinyl palmitate (4, 5). At about the same time, the recommendation was made to increase the dose to 50,000 IU (2, 6).

The World Health Organization currently recommends that infants receive 50,000 IU vitamin A at immunization contacts at age 6, 10, and 14 wk, that children 6–11 mo old receive 100,000 IU vitamin A, and that those 12–59 mo old receive 200,000 IU vitamin A every 4–6 mo (1).

Multicentered (4) and randomized (4, 5) trials of infant supplementation programs have been inconsistent, and the value of infant supplementation itself has been debated because of the greater occurrence of bulging fontanelle in vitamin A–dosed infants than in those not receiving vitamin A (7). In Zimbabwe, a large combined maternal and infant vitamin A supplementation trial showed similar infant mortality rates up to age 12 mo in the treated and placebo group infants born to HIV-negative mothers (8). In Nepal, infants ≤5 mo old who were given 50,000 or 100,000 IU vitamin A, depending on their age, had 4-mo mortality rates that did not differ significantly from those of infants receiving a placebo (9). In Ghana, India, and Peru, infants receiving three 25,000-IU doses within 14 wk at immunization contacts had mortality rates at 9 mo of age that did not differ significantly from those of infants receiving a placebo (10). In contrast, a study in India showed a 22% reduction in mortality up to age 3 mo in infants treated with two 24,000-IU capsules within 48 h of birth (11), and Indonesian infants had a 63% reduction in mortality up to age 12 mo if they had received a 50,000-IU capsule at birth (12).

The efficacy of these supplements in changing the vitamin A status and improving the liver vitamin A reserves of young infants has not been ascertained, because of a lack of appropriate methods. The gold standard for determining vitamin A status is a liver sample. Retinol concentrations < 0.07 μmol retinol equivalents (RE)/g liver are considered deficient. Because liver biopsy is not a feasible method for determining the vitamin A status of humans—except in special circumstances (13)—

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indirect measures such as the serum retinol concentration and the modified relative dose-response (MRDR) test (14) are used. The serum retinol concentration is maintained under homeostatic control and does not indicate vitamin A deficiency until liver reserves are below the deficiency cutoff. The MRDR test is an indicator that offers more information on vitamin A status than do serum retinol concentrations alone (15), but, as currently applied in the field, the MRDR test cannot provide a quantitative estimate of changes in liver vitamin A reserves in response to an intervention.

The objective of the present study was to determine the efficacy of graded vitamin A doses in quantitatively improving liver vitamin A reserves in a piglet model. The swine model has been applied to evaluate vitamin A supplementation programs in lactating women (16–18) and to validate and refine vitamin A status assessment methods (19, 20). Swine are a good model because of the similarity of their anatomy and physiology to those of humans. Weanling piglets are similar in size to infants who are <6 mo old, which allows the use of vitamin A doses identical to those used in vitamin A supplementation programs aimed specifically at infants.

MATERIALS AND METHODS

Sows and milk collection

Approval for the use of the animals was obtained from the University of Wisconsin (UW)–Madison Animal Care and Use Committee. Sows (n = 15; crossbreeds of Large White and Landrace) were housed at the Swine Research and Teaching Center (Arlington, WI) during vitamin A depletion. The sows had a mean (±SD) of 2.4 ± 0.5 parities when assigned to this study. The sows were crossbred with Duroc boars, and, when pregnancy was confirmed, the sows’ diet was changed from a standardized fortified diet (16) to a diet that did not contain added preformed vitamin A. They were maintained on this diet for 3 consecutive gestation and lactation cycles. The diet for the first cycle (126 d) was wheat-based. This strategy resulted in a gradual vitamin A depletion in the sows. Because litter size is variable, more sows than necessary were put on the vitamin A–depleted diets to ensure enough male piglets for these studies.

The experimental timeline is depicted in Figure 1. Milk was collected on postpartum days 1, 3, and 6 for measurement of vitamin A concentrations during transition. To collect milk, piglets were isolated from the sows for 1 h. Sows were injected intramuscularly with 40 IU oxytocin, and one teat was milked by hand (≈30 mL) (20). The milk was gently mixed to ensure homogeneity, and 500-μL aliquots were put into analysis tubes, which were stored on dry ice until they were transferred to a −80 °C freezer.

Piglets, dosing, and blood and liver collection

Castrated male piglets were selected from 6 sows in the first parity (parity A) and from 7 sows in the third parity (parity B) (n = 28 and 36 piglets for parity A and B, respectively). Because of differences in parturition, 4 of the 15 sows on the depletion diet were the same for parities A and B. The piglets were brought to the UW-Madison Livestock Laboratory when they were 9–14 d old (11.4 ± 1.1 d); they were weaned to a diet with no added vitamin A (Table 1) for 1 wk, and then the MRDR test was conducted (19). The MRDR test involves giving a standard 5.3-μmol dose of 3,4-didehydroretinyl acetate and taking a single blood sample 4–7 h after the dose. The ratio of 3,4-didehydroretinol to retinol (MRDR value) is determined by HPLC. An MRDR value > 0.060 is considered abnormal for both infants and piglets.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Ingredient composition of piglet diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ingredients</td>
</tr>
<tr>
<td>Corn</td>
<td>g/kg</td>
</tr>
<tr>
<td>Lactose</td>
<td>200.0</td>
</tr>
<tr>
<td>Oat groats</td>
<td>300.0</td>
</tr>
<tr>
<td>1-lysine mHCL</td>
<td>3.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>1.5</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>19.35</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.9</td>
</tr>
<tr>
<td>Iodized sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Antibiotics(^1)</td>
<td>5.75</td>
</tr>
<tr>
<td>Formulated vitamin and mineral mix(^2)</td>
<td>15</td>
</tr>
</tbody>
</table>

\(^1\) Provides the following antibiotics as mg/kg diet: tiamulin hydrogen fumarate, 38.5; and chlorotetracycline, 440.

\(^2\) Composition: 50% corn, 20% vitamin concentrate (Vitamin Mix; Teklad, Madison, WI) [vitamin preparation: vitamin D (as cholecalciferol), 0.26 g/kg; vitamin E (as dl-α-tocopheryl acetate), 72.0 g/kg; menadione sodium bisulfite complex, 5.40 g/kg; biotin, 7.50 g/kg; folic acid, 0.50 g/kg; niacin, 5.53 g/kg; pantothenic acid, 12.87 g/kg; riboflavin, 7.84 g/kg; thiamin B-12, 13.64 g/kg; and corn, 874.5 g/kg], and 30% University of Wisconsin mineral mix (final feed concentration: iron, 57 mg/kg; selenium, 0.3 mg/kg; zinc, 135 mg/kg; iodine 0.45 mg/kg; and copper, 2.3 mg/kg).

The experimental timeline is depicted in Figure 1.
repeated; then the piglets were killed, and their livers were collected. Serum and livers were stored on dry ice until they were transferred to a −80 °C freezer until they were analyzed.

**Milk, serum, and liver HPLC analysis**

Milk (500 μmol) was analyzed for retinol by using a slight modification of a previously described method (16, 20). After saponification and extraction, the residue was reconstituted with 200 μL 50:50 (by vol) methanol:dichloroethane; 25 μL was injected onto a Resolve C18, 5-μm, 3.9 × 150–mm reversed-phase column (Waters, Milford, MA) equipped with a guard column. The HPLC system consisted of a binary pump, an autosampler, and a photodiode array detector (model nos. 1525, 717, and 996, respectively; Waters). Serum (200 μL) and liver (1 g) were analyzed for retinol and retinyl esters, respectively, according to published procedures (18, 20).

**Statistical analysis**

A repeated-measures analysis of variance (ANOVA) with fixed effects was applied by using SAS PROC MIXED software (version 8.2, SAS Institute, Cary, NC) to determine the main effects of parity and of the time since birth on sow milk retinol concentration and the effects of vitamin A treatment, parity, and the time since dosing on piglet serum retinol and 3,4-didehydroretinol concentrations. PROC MIXED allows unequal variances among groups. To account for potential differences by parity group, sow was a random effect in the model. The influence of parity was evaluated by using 3-factor and 2-factor ANOVAs when appropriate. The least-squares means (LSMs) were determined by LSM differences when the interaction terms were significant. When the treatment × day interactions were significant, a Bonferroni-corrected test of effect slices (SAS OnlineDoc 9.1.3) was used to determine which days were significant. When the parity × treatment interaction term was significant, 1-factor ANOVA was used to evaluate differences in response to treatment, and Tukey’s test was applied for multiple comparisons. Retention of the vitamin A supplemental doses and the 3,4-didehydroretinol dose was calculated. The vitamin A liver reserves of the treatment groups were corrected for the placebo group and divided by the vitamin A dose amount. The 3,4-didehydroretinol retention was estimated by calculating the total amount in the liver and dividing by the standard amount of 3,4-didehydroretinyl acetate given to all piglets (5.3 μmol). A 2-factor ANOVA was used to determine the differences in the percentage retention of the doses administered by parity and by treatment. Values are presented as means ± SDs. Main treatment effects and interaction terms that included time were considered significant at P < 0.05; interactions that did not include time and 3-factor interactions at P < 0.1 were considered significant.

**RESULTS**

**Sow milk vitamin A concentrations**

Milk vitamin A concentrations did not differ significantly between the 2 lactation cycles (P = 0.55). A repeated-measures ANOVA showed that the sow milk vitamin A concentration decreased significantly (P < 0.0001) with time, as the milk transitioned from colostrum on day 1 (4.4 ± 1.7 μmol retinol/L) to more mature milk on day 3 (2.3 ± 0.85 μmol/L) and day 6 (1.2 ± 0.40 μmol/L). The day 6 concentration in milk was similar to that in mature milk found in previous studies (16, 20). A parity × days since birth interaction was not significant (P = 0.71).

**Piglet body and liver weight**

At weaning (experimental day 11), piglet body weights did not differ by treatment or parity, nor was there a significant treatment × parity interaction (P > 0.62) (Table 2). At the time of the killing of the piglets (experimental day 28), piglet body weight was significantly (P < 0.0001) different by parity but not by treatment (P = 0.51), and the treatment × parity interaction was not significant (P = 0.57). Piglet body weight differed significantly by sow in both parity A (P = 0.02) and parity B (P = 0.0003). On day 28, the difference in piglet body weight by sow was marginal in parity A (P = 0.088) and significant in parity B (P < 0.0003). On day 28, mean body weight for the piglets from parities A and B was 7.1 ± 1.4 and 5.8 ± 1.4 kg, respectively (P = 0.0006). On the day the piglets were killed, piglet liver weights were significantly (P < 0.0001) greater in animals from parity A (265.7 ± 51.7 g) than in those from parity B (191.1 ± 57.4 g), but they did not differ significantly by treatment in either parity (P > 0.74). One piglet from parity B (0 IU treatment group) died before the experiment was over, but no adverse

**TABLE 2**

Characteristics of piglets from 2 parities of sows fed a vitamin A–depleted diet at the time of treatment allocation

<table>
<thead>
<tr>
<th>Parity</th>
<th>0 IU</th>
<th>25 000 IU</th>
<th>50 000 IU</th>
<th>100 000 IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity A piglets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Age (d)</td>
<td>18.6 ± 0.5</td>
<td>18.6 ± 0.5</td>
<td>18.6 ± 0.5</td>
<td>18.6 ± 0.5</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>3.6 ± 1.0</td>
<td>3.6 ± 0.7</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Serum retinol (μmol/L)</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Prior parities of sows (n)</td>
<td>2.3 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.3 ± 0.5</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Parity B piglets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Age (d)</td>
<td>18.5 ± 1.4</td>
<td>18.6 ± 1.1</td>
<td>18.3 ± 1.4</td>
<td>18.4 ± 1.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>3.5 ± 1.0</td>
<td>3.5 ± 0.9</td>
<td>3.7 ± 0.9</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>Serum retinol (μmol/L)</td>
<td>0.9 ± 0.3</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>0.9 ± 0.8</td>
</tr>
<tr>
<td>Prior parities of sows (n)</td>
<td>4.4 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.3 ± 0.5</td>
</tr>
</tbody>
</table>

1 All values are x ± SD. The piglets were given 0-, 25 000-, 50 000-, or 100 000 IU (0, 26.2, 52.4, or 105 μmol) supplements of retinyl acetate. No significant differences were noted among the treatment groups.

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events, such as vomiting or diarrhea, were observed after vitamin A treatment.

Liver vitamin A reserves

The RE/g liver concentration in each treatment group was determined at the time of killing (Figure 2). Significant main effects of parity (P = 0.038) and treatment (P < 0.0001) existed, but the parity × treatment interaction was not significant (P = 0.56). Of the piglets from parity A, liver vitamin A concentrations in those receiving the 50,000- and 100,000-IU treatments increased above 0.07 μmol RE/g liver, the current deficiency cutoff. Of the piglets from parity B, only the 100,000-IU treatment group had mean reserves that increased above 0.07 μmol retinol equivalents/g.

FIGURE 2. Hepatic retinol concentrations (μmol/g liver) in piglets dosed with either 0, 25,000, 50,000, or 100,000 IU vitamin A. Significant main effects of parity (P = 0.038) and treatment (P < 0.0001) existed, but the parity × treatment interaction was not significant (P = 0.56). Of the piglets from parity A, liver vitamin A concentrations in those receiving the 50,000- and 100,000-IU treatments increased above 0.07 μmol/g liver, the current deficiency cutoff. Of the piglets from parity B, only the 100,000-IU treatment group had mean reserves that increased above 0.07 μmol retinol equivalents/g.

FIGURE 3. Total hepatic retinol of piglets dosed with 0, 25,000, 50,000, or 100,000 IU vitamin A. Significant main effects of parity and treatment existed (P < 0.0001 for both), but the parity × treatment interaction was not significant (P = 0.12). Total liver reserves (RE/liver) were also determined for piglets from both parities (Figure 3). Significant main effects of parity and treatment existed (P < 0.0001 for both), but the parity × treatment interaction was not significant (P = 0.12). The piglets from parity A retained more of the treatment dose than did the more vitamin A–depleted piglets from parity B. Total liver reserves increased only 5% more between the 50,000- and 100,000-IU dose groups.

Modified relative dose-response test and serum 3,4-didehydroretinol concentrations

Significant main effects of parity (P < 0.0001) and treatment (P = 0.0066) existed, and the parity × treatment interaction was significant (P = 0.061). In the piglets from parity A, the baseline (P = 0.85) and final (P = 0.39) MRDR tests were not affected overall by treatment (Table 3). In piglets from parity B, whose mothers had greater vitamin A depletion, baseline MRDR test values did not differ significantly (P = 0.33), but the post-vitamin A treatment values did differ significantly (P = 0.013) among the treatments. With the use of LSM differences with a Tukey adjustment, the control group (0-IU dose) had a response marginally higher than that of the 50,000-IU dose group (P = 0.072) and significantly higher than that of the 100,000-IU dose group (P = 0.011). The increases in MRDR values were consistent with reduced liver vitamin A reserves in the groups from parity B.

The repeated blood drawing allowed the 3,4-didehydroretinol concentration to be followed from the first MRDR test (at 4 h) to 7 d after dosing (Table 4). A review solely of the change in 3,4-didehydroretinol concentration with time can show its use as a vitamin A tracer, independent of the influence of serum retinol. A 3-factor ANOVA showed no significant difference by parity
TABLE 3
Baseline and final modified relative dose-response (MRDR) values in piglets from 2 parities of sows fed a vitamin A–depleted diet including 0-, 25 000-, 50 000-, or 100 000- IU (0, 26.2, 52.4, or 105 μmol) supplements of retinyl acetate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parity A piglets</th>
<th></th>
<th>Parity B piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline MRDR</td>
<td>Final MRDR</td>
<td>Baseline MRDR</td>
</tr>
<tr>
<td>0 IU</td>
<td>0.042 ± 0.040</td>
<td>0.053 ± 0.030</td>
<td>0.038 ± 0.032</td>
</tr>
<tr>
<td>25 000 IU</td>
<td>0.044 ± 0.030</td>
<td>0.037 ± 0.020</td>
<td>0.024 ± 0.028</td>
</tr>
<tr>
<td>50 000 IU</td>
<td>0.044 ± 0.030</td>
<td>0.049 ± 0.027</td>
<td>0.015 ± 0.010</td>
</tr>
<tr>
<td>100 000 IU</td>
<td>0.032 ± 0.039</td>
<td>0.033 ± 0.017</td>
<td>0.026 ± 0.036</td>
</tr>
</tbody>
</table>

1 All values are ± SD.
2 n = 7/group.
3 n = 9/group except for Final MRDR, in which n = 8 for the 0-IU group.
4 A main effect of parity (P < 0.0001) and treatment (P = 0.0066) existed, and the parity × treatment interaction was significant (P = 0.061). Because of this significant interaction term, the parities were analyzed separately. The effect of treatment using a one-way ANOVA on MRDR values among parity A piglets was not significant, but that for parity B piglets was significant (P = 0.013). Values in this column with different superscript letters are significantly different, P < 0.05 (Tukey’s test).

(P = 0.73), but significant differences were seen in the 3,4-didehydroretinol concentration by treatment group and by time after dosing (P < 0.0001 for both). The parity × day (P < 0.0001), the parity × treatment (P = 0.078), and parity × treatment × day (P = 0.098) interactions were significant, but the treatment × day interaction was not significant (P = 0.16). After treatment, the 3,4-didehydroretinol concentration was consistently higher in the control group from both parities than in the groups treated with vitamin A, and it decreased in all of the treatment groups with time.

Serum retinol concentrations

A 3-factor ANOVA found significant differences by parity (P = 0.012), treatment (P = 0.0023), and day (P < 0.0001). The parity × treatment interaction was not significant (P = 0.76), but the parity × day (P < 0.0001), treatment × day (P < 0.0001), and parity × treatment × day (P = 0.073) interactions were significant. Because of these significant interactions, the data were analyzed separately. Serum retinol concentrations for piglets from parity A (Figure 4) were marginally affected by treatment (P = 0.064), and there was a significant difference by time (P < 0.0001) and a significant treatment × day interaction (P = 0.005). With the use of a Bonferroni-corrected test of effect slices by day, the only treatment × day effect that was significant was that for day 1 (P = 0.044). This effect represents a slight increase in the serum retinol concentration during the time that the vitamin A doses were equilibrating with body vitamin A. No other treatment × day effects were noted.

The main effects of treatment and time on serum retinol concentrations for piglets from parity B (Figure 4) were significant (P = 0.033 and < 0.0001, respectively). A significant (P = 0.0002) treatment × day interaction also existed. With the use of

TABLE 4
3,4-Didehydroretinol concentrations in the serum of piglets followed for 7 d after an oral dose of 3, 4-didehydroretinyl acetate (5.3 μmol).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IU</td>
<td>41 ± 40</td>
<td>18 ± 6.1</td>
<td>11 ± 8.1</td>
<td>10 ± 6.6</td>
<td>5.2 ± 5.2</td>
<td></td>
</tr>
<tr>
<td>25 000 IU</td>
<td>45 ± 30</td>
<td>7.6 ± 4.4</td>
<td>1.8 ± 3.1</td>
<td>3.9 ± 3.8</td>
<td>1.1 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>50 000 IU</td>
<td>48 ± 31</td>
<td>3.5 ± 4.4</td>
<td>1.1 ± 2.9</td>
<td>2.6 ± 6.9</td>
<td>1.6 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>100 000 IU</td>
<td>17 ± 20</td>
<td>3.3 ± 4.2</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>Parity B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 IU</td>
<td>28 ± 18</td>
<td>38 ± 27</td>
<td>28 ± 20</td>
<td>14 ± 12</td>
<td>20 ± 13</td>
<td></td>
</tr>
<tr>
<td>25 000 IU</td>
<td>26 ± 27</td>
<td>6.6 ± 5.4</td>
<td>3.5 ± 3.2</td>
<td>1.1 ± 2.9</td>
<td>5.5 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>50 000 IU</td>
<td>15 ± 9.9</td>
<td>8.2 ± 4.6</td>
<td>2.2 ± 3.7</td>
<td>0.90 ± 2.7</td>
<td>5.2 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>100 000 IU</td>
<td>25 ± 34</td>
<td>7.4 ± 9.1</td>
<td>2.2 ± 1.4</td>
<td>0.50 ± 1.4</td>
<td>1.9 ± 3.0</td>
<td></td>
</tr>
</tbody>
</table>

1 All values are ± SD. A 3-way ANOVA showed no significant difference by parity (P = 0.73) but a significant difference by treatment group (P < 0.0001) and day (P < 0.0001). The parity × day (P < 0.0001), parity × treatment (P = 0.078), and parity × treatment × day (P = 0.098) interactions were significant. The treatment × day interaction was not significant (P = 0.16). The 3,4-didehydroretinol concentration in the 0-IU group was consistently higher than that in the vitamin A–treated groups (P < 0.05). The 3,4-didehydroretinol concentrations were the highest on day 0, when they differed significantly from all other times (P < 0.05). Values on days 1 and 2 and on days 2, 4, and 7 did not differ significantly (P > 0.05).
2 n = 7/group.
3 n = 9/group, except for the 0-IU group in which n = 8.
Retention of administered doses

The percentage retention of both the graded vitamin A doses and the 3,4-didehydroretinyl acetate doses was analyzed by parity and group (Table 5). Significantly ($P = 0.0002$) more of the vitamin A dose was stored in the livers of the piglets from parity A than in the livers of piglets from parity B, as evidenced by the lower percentage retention in the parity B piglets. There also was a significant ($P = 0.002$) effect of treatment group, but no significant parity × treatment interaction occurred ($P = 0.76$).

Storage of the standard 3,4-didehydroretinol dose for the MRDR test consistently was significantly ($P < 0.0001$) greater in the piglets from parity A than in those from parity B, and a significant ($P < 0.0001$) difference by treatment group existed, but the parity × treatment interaction was not significant ($P = 0.14$). The percentage range of the 3,4-didehydroretinol dose stored was 23 ± 14%–51 ± 15% for piglets from parity A and 16 ± 7.1%–29 ± 11% for piglets from parity B. The lowest amount of 3,4-didehydroretinol dose stored for both parities was that for the control groups. This finding concurs with the observation that more 3,4-didehydroretinol was circulating in the serum of the control groups that generated higher MRDR values (Table 3).

**DISCUSSION**

In areas where vitamin A deficiency is prevalent, high doses of vitamin A are currently given to postpartum mothers in hopes of increasing the vitamin A status of the mothers and their nursing infants (1). Subsequent to maternal supplementation, infants are given multiple vitamin A doses throughout the first 2 y of life. These practices continue, even without adequate evidence of their efficacy, which results from the difficulty of assessing total-body vitamin A reserves in infants. This study used piglets, which are born with low hepatic vitamin A reserves (18, 21–23) and which weigh approximately the same as 3–5-mo-old infants at the 50th percentile of weight-for-age (24), to determine quantitative improvements in liver vitamin A reserves after a single dose of vitamin A in the 0-IU dose group ($P < 0.05$). The serum retinol concentrations in the different treatment groups did not differ significantly at day 10 (Figure 4). Thus, both treatment response and time after treatment are important in interpreting changes in serum retinol concentration.

**TABLE 5**

Retention of administered doses of retinyl acetate and 3,4-didehydroretinyl acetate expressed as alcohol equivalents in liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Retention of retinol</th>
<th>Retention of didehydroretinol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parity A piglets</td>
<td>Parity B piglets</td>
</tr>
<tr>
<td>0 IU</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>25 000 IU</td>
<td>38 ± 20</td>
<td>11 ± 11</td>
</tr>
<tr>
<td>50 000 IU</td>
<td>35 ± 16</td>
<td>17 ± 15</td>
</tr>
<tr>
<td>100 000 IU</td>
<td>21 ± 14</td>
<td>11 ± 4.9</td>
</tr>
</tbody>
</table>

1 All values are $\bar{x} \pm$ SD. The retinol values are compared against the 0-IU group, and the didehydroretinol is calculated from that recovered from the 5.3-μmol standard dose.

2 Using a 2-way ANOVA, the percentage retention of retinol in parity A was significantly higher than that in parity B ($P < 0.0002$), and there was a significant effect of treatment group ($P = 0.002$), but the parity × treatment interaction was not significant ($P = 0.76$).

3 Using a 2-way ANOVA, the percentage retention of 3,4-didehydroretinol differed significantly by parity ($P < 0.0001$) and by treatment group ($P < 0.0001$), but the parity × treatment interaction was not significant ($P = 0.14$).

4 $n = 7$ / group. Supplemented groups were compared with the 0-IU group.

5 $n = 9$ / group except in the 0-IU group, in which $n = 8$. 

FIGURE 4. Serum retinol concentrations in piglets dosed with 0 (○), 25 000 (△), 50 000 (△), or 100 000 (○) IU vitamin A in the form of retinyl acetate. A 3-factor ANOVA showed a significant difference for parity ($P = 0.012$), treatment ($P = 0.0023$), and day ($P < 0.0001$). The parity × day ($P < 0.0001$), treatment × day ($P < 0.0001$), and parity × treatment × day ($P = 0.073$) interactions were significant. In piglets from parity A, the main effect of treatment on serum retinol concentrations was marginal ($P = 0.064$), but the effect of time after dosing ($P < 0.0001$) and the treatment × day interaction ($P = 0.005$) were significant. Significant treatment × day effect (day 1), $P = 0.044$ (Bonferroni-corrected test of effect slices by day). No significant differences between treatments existed. In piglets from parity B, the main effects of treatment and time on serum retinol concentrations were significant ($P = 0.033$ and $< 0.0001$, respectively). The treatment × day interaction was also significant ($P = 0.0002$). Significant treatment × day effect, $P < 0.0006$ (day 1) and $P = 0.022$ (day 7). The only difference between treatments using LSM differences occurred on d 1 when the mean serum retinol concentration was significantly higher in the 50 000-IU group than in the 0-IU group ($P < 0.05$).
dose of vitamin A. These results suggest that few infants would be vitamin A adequate after receiving a single large dose, especially if the mother is vitamin A depleted. In addition, the 100 000-IU vitamin A dose may be too large to be effectively handled in the gastrointestinal system of young infants, as suggested by the fact that total liver vitamin A reserves increased only 5% more with the 100 000-IU dose than with the 50 000-IU dose. These findings explain the conflicting results from human trials (8–12) and provide a reason for the fact that some investigators have found smaller doses to be more effective in reducing infant morbidity and mortality (25, 26).

In utero, vitamin A pathways are tightly regulated to ensure normal fetal development. Studies have found a reduction in maternal nightblindness (27) and improvements in infant serum retinol concentrations (28, 29) after vitamin A supplementation during pregnancy. Because of the risk of teratogenicity during pregnancy, low-dose supplementation has been used (30–32). Groups given daily vitamin A supplementation during pregnancy tended to have a lower incidence of depleted vitamin A liver stores, as assessed by the MRDR test (31, 32). Analysis of fetal livers from nonhuman primates found an amplification of early vitamin A hepatic reserves when mothers were fed excessive amounts of vitamin A in laboratory diets (33), which indicates continuous transfer to the fetus. The piglets from parity A of vitamin A–adequate sows were able to store more of the supplemented vitamin A than were piglets from parity B of vitamin A–depleted sows. This difference suggests that the vitamin A dose was utilized by vitamin A–deprived tissues in the piglets from parity B through chylomicron delivery (34), and, thus, less vitamin A was available for long-term storage in the liver. Because of this finding, more emphasis on increasing vitamin A stores during pregnancy may be warranted. In addition, a greater proportion of the 3,4-didehydroretinol dose was found in the livers of the treatment groups than in those of the control groups from both parities. Along with chylomicron delivery, this could indicate a "priming" of the vitamin A regulatory pathways by the vitamin A supplements (35) that were received 10 d before, which meant that the vitamin A–treated piglets were able to retain more of the subsequent 3,4-didehydroretinol dose.

In some communities, prenatal care is sparse or nonexistent, and many programs have tried high-dose postpartum supplementation (36) and direct supplementation to infants (8–11). Liver vitamin A reserves of piglets from sows dosed with single high-vitamin A doses that were equivalent to 200 000 or 400 000 IU (according to differences from women in body weight) were elevated over a placebo dose (18) but did not differ from each other. Because the percentage retention of the vitamin A doses decreased in both parities in the present study and because liver reserves increased only 5% between the 50 000- and 100 000-IU dose groups, it can be concluded that more is not necessarily better in the defining of dose sizes. Combined programs for mothers and infants may ensure adequate liver vitamin A reserves, but a single intervention strategy could result in a failure of many infants to attain an adequate vitamin A status.

In piglets from parity A, no treatment effect for serum retinol concentration was observed; however, there was a significant treatment × day interaction on the day after the doses were administered. In contrast were the piglets from parity B, in whom a treatment effect was observed but not evident on the final day of the experiment. The drop in serum retinol concentration 10 d after treatment may be due to the body’s attempt to conserve hepatic retinol by decreasing the utilization rate of retinol and thereby setting a new lower homeostatic setpoint in the serum (37). A similar finding was seen in an earlier study in piglets that were fed a vitamin A–depleted diet after being weaned (18). Serum retinol concentrations decreased after 2 wk regardless of vitamin A status. A lack of dietary vitamin A will trigger a response to slow the utilization rate to retain vitamin A. Previous studies in piglets (18), rats (37), and gerbils (38) have shown this phenomenon, which sometimes results in a slight increase in serum retinol concentrations before the lower setpoint is obtained, as was seen for the 7- and 10-d data of the piglets from parity B.

MRDR values were affected by treatment but did not differ among parity A piglets because of the lack of power due to the small group size, although a trend was noted. The more vitamin A–depleted parity B piglets showed increasing MRDR values as liver vitamin A reserves decreased. Furthermore, the MRDR values and liver reserves in these piglets showed that infants may still be deficient after supplementation. Even in the piglets given the higher doses, stores were barely over the deficiency cutoff of 0.07 μmol/g liver. This finding concurs with recent data in Tanzanian infants: 43% and 47% of those infants were considered vitamin A–deficient according to the MRDR test after the high- and low-dose supplements, respectively (39). The fact that the test was conducted 3 h after dosing and not at 4 h after dosing— the time recommended for infants—strengthens the conclusion that some infants are still vitamin A deficient after supplementation. A 3-h MRDR test would be more sensitive to very low liver reserves of vitamin A at the individual level than would a later test (19). Measurements only of serum retinol concentrations or of differences in childhood morbidity and mortality (4, 12, 25) would mean that the ability of vitamin A supplementation to increase liver vitamin A reserves cannot be assessed. The MRDR values obtained in the vitamin A–deficient piglets from parity B in the present study are similar to those obtained in infants who were recipients of repeated 25 000-IU supplements (4), which confirms that not all infants are vitamin A replete after supplementation. The liver vitamin A concentration data in the present study showed that 71% of the piglets were still vitamin A deficient after a 25 000-IU dose, and 24% remained deficient after a 50 000- or 100 000-IU dose. This evaluation of the efficacy of vitamin A doses currently used in infants may explain negative effect trials. Because of these low liver reserves, a single illness could easily deplete the liver vitamin A stores by decreasing intake and absorption and increasing losses in the urine (40).

Side effects of supplementation are rare in infants, and many studies show improvement in morbidity or mortality with dosing (11, 12). The most common acute side effect in infants given vitamin A doses is bulging of the anterior fontanelle (7). However, one study found the incidence of bulging fontanelle to be similar to that in a placebo group (41). Comparison of long-term effects in infants reported to have had a vitamin A–induced bulging fontanelle and effects in infants with normal fontanelles after supplementation found no adverse effects on growth and development (42). However, maternal and infant supplementation programs in HIV-endemic areas need further evaluation. Among HIV-exposed infants who were not infected with HIV during pregnancy and delivery, both postpartum maternal and neonatal vitamin A supplementation were associated with a doubling of the mortality rate by age 2 y (43).
Because the liver vitamin A reserves of most piglets in this study did not reach adequacy, the current recommendation to repeat vitamin A administration at the immunization contacts at 6, 10, and 14 wk seems appropriate (6), but it still may not prevent deficiency (4, 39). Promoting the use of provitamin A–containing foods and providing them to women could be effective in improving the vitamin A status of the women and, perhaps, of their nursing infants. Provitamin A–containing foods were as effective as retinyl palmitate in treating nightblindness in pregnant women (27). A recent study in Mongolian gerbils showed that dark-orange maize can contribute as much vitamin A to the liver as β-carotene supplements contribute during vitamin A depletion (44). As developing countries search for sustainable strategies to improve community vitamin A status, biofortification of staple crops (44, 45) and promotion of the consumption of vegetables (46) are viable options that can be safely introduced alongside vitamin A supplementation programs.

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