Oral Doses of α-Retinyl Ester Track Chylomicron Uptake and Distribution of Vitamin A in a Male Piglet Model for Newborn Infants1–3

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Abstract

α-Retinol has utility in determining chylomicron trafficking of vitamin A to tissues given that it will not be recirculated in blood on retinol binding protein (RBP). In this study, α-retinol was used as a chylomicron tag to investigate short-term uptake from high-dose supplements given to piglets as a model for neonates. The distribution of orally administered α-retinol doses in liver and extrahepatic tissues was assessed at varying times after dosing. Male piglets (n = 24 per group) from vitamin A–depleted sows were orally given 26.2 or 52.4 μmol of α-retinyl acetate, the molar equivalent of 25,000 and 50,000 IU of vitamin A, respectively. Tissues were collected and analyzed by HPLC. Lung (6.46 ± 2.94 nmol/g), spleen (22.1 ± 11.3 nmol/g), and adrenal gland (17.0 ± 11.2 nmol/g) α-retinol concentrations peaked at 7 h after dosing, and, by 7 d, α-retinol was essentially cleared from these tissues (<0.25 ± 0.12 nmol/g). This demonstrates that the lung, spleen, and adrenal gland receive substantial vitamin A from chylomicra to maintain concentrations. Conversely, storage of α-retinol in the liver reached a plateau at 24 h (1.72 ± 0.58 μmol/liver) and was retained through 7 d (2.10 ± 0.38 μmol/liver) (P > 0.05). This indicates that α-retinol was not substantially utilized locally in the liver nor transported out from the liver via RBP. In serum, the majority of α-retinol was in the ester form, which confirms that α-retinol does not bind to RBP but does circulate. α-Retinyl esters were detectable at 7 d in the serum but were not different from baseline. Collectively, these data suggest that crucial immune organs need constant dietary intake to maintain vitamin A concentrations because α-retinol was quickly taken up by tissues and decreased to baseline in all tissues except long-term storage in the liver. J. Nutr. 144: 1188–1195, 2014.

Introduction

Vitamin A deficiency is estimated to affect 190 million preschool-age children and 19.1 million pregnant women (1). Children suffering from vitamin A deficiency have a high risk of irreversible blindness and death from infectious diseases. Supplementation programs are a common approach to address this issue (2). Unfortunately, the efficacy of supplements to change the vitamin A status and improve liver vitamin A reserves of premature and young infants has not been determined because of a lack of appropriate methodology. Accurate evaluation of the dosing regimens and assessment of total-body reserves of vitamin A in infants who are the recipients of these dosing regimens is needed. Animal models can be used to evaluate liver reserves and vitamin A distribution (3), which are not feasible in humans except in special circumstances (4).

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3 Supplemental Figures 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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The lungs, and possibly other tissues, require frequent exposure to dietary vitamin A to maintain tissue concentrations (3). Using 3,4-didehydroretinol as an oral tracer that binds to retinol binding protein (RBP),4 the estimated chylomicron contribution to lung and spleen 3,4-didehydroretinol was 63% and 280% higher, respectively, than the maximum exposure from the 3,4-didehydroretinol–RBP complex in a 4-h period (6). One way to evaluate the benefit of vitamin A dosing regimens targeted to vulnerable infants is to have a better understanding of the maintenance of vitamin A concentrations in these crucial organs.

The spleen and lungs are active in the immune response (7,8), and vitamin A plays an essential role in immunity (9). The spleen is involved in both innate and adaptive immune processes in humans (7). The lung has constant exposure to the environment through respiration and is therefore 1 of the first defenses to inhaled antigens (8). The adrenal cortex is important in the stress response and is part of the hypothalamic–pituitary–adrenal (HPA) axis (10). Infants are continuously under environmental stress, especially in developing countries. A study that explored the regulation...
of HPA-axis function by retinoids concluded that adequate vitamin A status contributes to low HPA-axis activity (11).

Dietary vitamin A is obtained from animal foods in the form of retinyl esters and plant sources of provitamin A carotenoids. Almost all retinyl esters are hydrolyzed to retinol in the gastrointestinal tract and absorbed by intestinal epithelial cells. Once inside the enterocytes, retinol bound to cellular RBP type II is esterified by lecithin-retinol acyltransferase to form retinyl esters, which are then packaged into chylomicrons, circulated to the lymphatic system, and passed through the thoracic duct into the bloodstream (12). As the chyloicmicra lose their TGs and other constituents, most of the retinyl esters remain within, and the particles become chylomicron remnants, which are cleared mainly by the liver. Chylomicra deliver vitamin A to essential organs through extrahepatic release and direct uptake of the remnants, which may be important in the delivery of vitamin A to mammary tissue, bone marrow, adipose tissue, and spleen (13).

α-Carotene contains both β- and α-ionone rings, connected by a polylene chain. When α-carotene is centrally cleaved, retinal and α-retinol are produced and subsequently reduced. In a previous study conducted with α-carotene in Mongolian gerbils, α-retinol hepatic concentration was identical to the difference in hepatic retinol concentration between the α-carotene and a control group, consistent with central cleavage of α-carotene in the intestinal brush border (14). α-Retinol was not detected in serum, supporting previous observations that it does not bind to RBP (15,16). Thus, α-retinol has utility in determining chylomicra trafficking of vitamin A to tissues, given that it will not be recirculated in blood on RBP and has similar structure and polarity to retinol. In this study, α-retinol was used to determine short-term uptake to evaluate supplementation practices to newborn infants in a piglet model by assessing the distribution of orally administered α-retinol in extrahepatic tissues at varying times after dosing. Piglets are a good model for human infants because of their similar size, gastrointestinal anatomy, and vitamin A requirements (17).

Materials and Methods

Animals and sample collection. Approval for animal use was obtained from the University of Wisconsin–Madison Animal Care and Use Committee, and all animal procedures adhered to the public health service policy on humane care and use of laboratory animals. The College of Agriculture and Life Sciences facilities are Association for Assessment and Accreditation of Laboratory Animal Care accredited and frequently inspected internally and externally to ensure compliance.

Male piglets (n = 54) were obtained from 10 sows (Sus scrofa domestica) that were housed at the Swine Research and Teaching Center in Arlington, Wisconsin. The sows were fed vitamin A–depleted feed (18) for 2 gestation and lactation cycles to deplete liver reserves of vitamin A. Six piglets were killed at baseline (no dose), and the remaining piglets were randomly assigned to orally receive 26.2 or 52.4 μg vitamin A. Six piglets were killed at baseline (no dose), and the remaining piglets were given vitamin A at 12, 24, 48, and 72 h. Blood samples were taken at 1, 2, 4, 8, 12, and 24 h (6 piglets per time). Because of blood volume constraints, animal approval was contingent on piglets not being used at all blood-draw time points. Thus, there were 4 time subgroups of 6 piglets at each dose amount. Subgroup 1 had blood samples taken at 1, 2, 4, and 7 h, subgroup 2 had blood samples taken at 1, 1.5, 5, 8, and 12 h, subgroup 3 had blood samples taken at 2, 6, 12, and 24 h, and subgroup 4 had blood samples taken at 3 and 10 h and 7 d. Subsequently, the piglets (n = 6 per time) were killed by stunning with electricity followed by exsanguination at 7, 12, and 24 h and 7 d for organ (i.e., liver, adrenal gland, kidney, lung, spleen, and intestinal mucosa) collection. All samples were stored at −80°C until analysis. Purified retinyl butyrate was added to all samples as internal standard to determine extraction efficiency.

Analysis of serum retinol, α-retinol, retinyl esters, and α-retinyl esters. Serum (500 μL) was analyzed using previously published procedures (14,18) with modification. Ethanol (500 μL with 0.1% butylyated hydroxytoluene) was added, and extraction occurred 3 times with 1 mL of hexanes. The HPLC detection was at 311 nm for α-retinol and α-retinyl esters and 325 nm for retinol and retinyl esters. The mobile phases were acetonitrile:water (85:15, v:v; solvent A) and acetonitrile: dichloroethane:methanol (70:20:10, v:v:v; solvent B), both containing 10 mmol/L ammonium acetate as a modifier at 1 mL/min: 1) 100% solvent A (25 min hold); 2) 10 min-linear gradient to 100% solvent B; 3) 23-min hold; 4) 5-min reverse gradient to 100% solvent A; and 5) 10-min hold.

Piglet tissue sample analysis. The liver (0.5–0.7 g) and adrenal glands (0.2–0.7 g) were ground with sodium sulfate (3 times tissue weight) in a mortar. The tissue was extracted repeatedly with dichloromethane to 25 mL; 5 mL was dried under nitrogen. The residues were reconstituted in 200 μL of methanol:dichloroethane (50:50, v:v); 25 μL was injected and analyzed as above.

Lungs (4.5–5.5 g), spleen (1.3–2.3 g), and adrenal glands (0.2–0.7 g) were ground with sodium sulfate (3 times tissue weight) in a mortar. The tissue was extracted repeatedly with dichloromethane to ~60 mL. The extract was dried in a round-bottom flask using a rotary evaporator. The residue was dissolved in 1 mL of dichloromethane (3 times) and transferred to a glass tube (13 × 100 mm). The combined solution was dried under nitrogen and redissolved in 200 μL of methanol:dichloroethane (50:50, v:v); a 25-μL aliquot was injected and analyzed as above. For mucosal scrapings (range: 0.1–0.8 g), proteins were denatured with 2 mL of ethanol (0.1% butylated hydroxytoluene); 2 mL of deionized water was added to assist phase separation. Samples were mixed with a vortex, sonicated for 10 min, and extracted 3 times with 1 mL of hexanes. Each sample was redissolved in 50 μL of methanol:dichloroethane (50:50, v:v); a 35-μL aliquot was injected and analyzed as above.

Statistical analysis. Animal data were analyzed using Statistical Analysis System software (version 9.4, 2002–2012; SAS Institute). Outcomes of interest (i.e., piglet weights, serum and tissue retinol, α-retinol, retinyl ester, and α-retinyl ester concentrations) were evaluated using 2-factor ANOVA. Homogeneity of variance was assessed using Levene’s test. If both or either test was significant, the data were analyzed using 2-factor ANOVA. If expected, final weights differed by time (P < 0.0001); 7-d piglets were heavier (3.05 ± 0.46 kg) than all other times. Final weights did not show a dose × time point interaction (P = 0.17).

In the 50kIU group, the concentration of α-retinol in the liver was stable at 7 and 12 h, peaked at 24 h, and decreased by 7 d. For the 25kIU group, the concentration of α-retinol increased after dose, remained steady throughout the study, and did not differ from 7 h through 7 d (Fig. 1A). Total recovered liver α-retinol was higher in the 50kIU group than the 25kIU group beginning at 24 h and was maintained through 7 d (Fig. 1B).
concentration of retinol (retinol + all identifiable esters) did not differ at any time point between the 25kIU and 50kIU groups (Supplemental Fig. 1A). However, the 12-h time was lower than the 24-h time, which may be due to the fact that the piglets were not the same at each time point or that the piglets would have been administered colostrum during this period, causing fluctuation. Total liver retinol had the same relation (Supplemental Fig. 2A).

In the kidney, a biphasic curve was noted in α-retinol concentration and total α-retinol for both dose groups during the early time points, especially in the 50kIU group (Supplemental Figs. 1B and 2B). α-Retinol entered the lung rapidly, peaked at 7 h, was higher in the 50kIU group only at 7 h, and decreased by 7 d to baseline in both groups (Fig. 3A). The patterns did not differ between concentration and total α-retinol/lung (Fig. 3B). Lung retinol concentration (Supplemental Fig. 1C) and total retinol (Supplemental Fig. 2C) were identical between groups and were higher at the beginning of the study than at the end, which is likely due to the rapid growth of this tissue after birth.

The α-retinol pattern in the spleen was very similar to the lung but at a much higher 7-h peak concentration (3.4 times higher in the spleen than the lung). The 50kIU group only had a higher α-retinol concentration at 7 h after dose, but the clearance by 12 h was identical in both groups and α-retinol was not detected at 7 d (Fig. 4). Spleen retinol concentration was higher in the 25kIU group at 12 and 24 h than the 50kIU group but was similar at all other times (Supplemental Fig. 1D). Total retinol was lower in the 50kIU group at 24 h (Supplemental Fig. 2D). The response in the adrenal gland followed the same pattern as that in the spleen. The adrenal gland acquired a high amount of α-retinol very quickly, especially in the 50kIU group in which the response was significantly different from 7 to 24 h between the 25kIU and 50kIU groups. The α-retinol was depleted by 7 d in both dose groups (Fig. 5). Adrenal gland retinol concentrations did not differ between dose groups at any time but was elevated at 7 h in the 25kIU group compared with 7 d (Supplemental Figs. 1E and 2E). In the 50kIU group, α-retinyl esters from the intestinal mucosa peaked (7 h) before the free alcohol form (24 h) and were still detectable at 7 d but were not different from baseline (Fig. 6).

As expected, serum α-retinol was predominantly in the ester form. As in the tissue, most of the activity occurred within the first 24 h (Fig. 7). α-Retinol (the alcohol form) was not detected in the first 4 h. The peak concentration of α-retinol was at 8 h ($0.13 \pm 0.08 \mu\text{mol/L}$) and 16 h ($0.32 \pm 0.16 \mu\text{mol/L}$) for the 25kIU and 50kIU groups, respectively. The combined α-retinyl
Ester concentrations peaked at 24 and 7 h for the 25kIU and 50kIU groups, respectively (Supplemental Fig. 3). α-Retinyl esters began to enter the bloodstream at 1.5 h in the 50kIU group and 2 h in the 25kIU group. When the data were normalized by piglet to account for multiple samples taken on individual piglets but different time courses by subgroup, ester concentrations remained elevated through 24 h and peaked between 5 and 8 h (Fig. 7).

Serum retinol concentrations were high in the control piglets at birth but rapidly dropped, continuously increased with time (P < 0.0001), and were not different by treatment (Table 1). Serum retinol esters were relatively high throughout the study (0.28 ± 0.15 μmol/L), likely as a result of frequent suckling of retinol-rich colostrum within the first 24 h. Although individual piglets were not the same at varying times due to blood drawing restrictions, the α-retinyl and retinyl esters tracked identically in the blood (Supplemental Fig. 3).

**Discussion**

The distribution of orally administered α-retinol in extrahepatic tissues was determined in a piglet model for newborn infants in doses identical to those used previously in newborn infants (20,21). The WHO recommends that high-dose vitamin A capsules be administered to children aged 6–59 mo (22) but has resisted making recommendations for younger children based on lack of evidence to reduce mortality risk (23). This study adds to the evidence that crucial organs need a constant supply of retinol from chylomicra to maintain concentrations. The early α-retinyl ester peak in all organs confirms that it arrived on chylomicra, and the clearance by 7 d demonstrates that it was not recirculated by the liver on RBP, although the liver retained the α-retinol in the ester form. The uptake of α-retinol by all tissues from chylomicra also confirms the observation that the (stimulated by retinoic acid 6) STRA6 receptor, which requires interaction with RBP for retinol release, is not necessary to maintain many different tissue vitamin A concentrations (24).

In all organs, a biphasic response was noted that appeared to be more pronounced in the 50kIU group. Although the specific responses at different times were not always significant, the biphasic response was likely caused from a second release and circulation from the intestine from a subsequent meal. Fat from a previous meal can contribute to the early postprandial lipemia (25). Except for the dosing period, the piglets were not food deprived and were allowed to suckle during the experiment, which explains the changes in retinol in all organs and high circulating serum retinyl esters. Colostrum is a rich source of retinol during early lactation (26). Furthermore, the second peak of α-retinyl esters was due to some α-retinol from these large doses being retained in the small intestine, as confirmed with mucosal analysis, while waiting for the next surge of FAs from a previous meal.

**FIGURE 3** Time course (0–168 h) of lung α-retinol concentration (A) and total α-retinol (B) in groups of piglets given 26.2 or 52.4 μmol of α-retinyl acetate, the molar equivalent of 25,000 and 50,000 IU of vitamin A, respectively. Piglets at time point 0 (n = 6) served as baseline (no dose). Values are means ± SDs, n = 6. Means without a common letter differ, P < 0.05. trt, treatment; 25kIU, 25,000 IU; 50kIU, 50,000 IU.

**FIGURE 4** Time course (0–168 h) of spleen α-retinol concentration (A) and spleen total α-retinol (B) in groups of piglets given 26.2 or 52.4 μmol of α-retinyl acetate, the molar equivalent of 25,000 and 50,000 IU of vitamin A, respectively. Piglets at time point 0 (n = 6) served as baseline (no dose). Values are means ± SDs, n = 6. Means without a common letter differ, P < 0.05. trt, treatment; 25kIU, 25,000 IU; 50kIU, 50,000 IU.
subsequent meal. This phenomenon was demonstrated using retinyl palmitate as a marker of ingested fat for the first meal of 2 sequential meals in humans (27). In the analysis of the intestinal mucosa, free \( \alpha \)-retinol and a wide array of \( \alpha \)-retinyl esters were still elevated at 24 h and detectable at 7 d. This corroborates a study in rats that identified cells similar to hepatic stellate cells in the mucosa of rat intestine, and these cells were able to absorb and store vitamin A (28).

Piglet lungs, spleen, and adrenal glands acquired substantial amounts of chylomicron-derived \( \alpha \)-retinol after dosage. The concentrations of \( \alpha \)-retinol were higher than those of retinol in the kidneys and spleen at 7 and 24 h and much higher in lungs and adrenal glands. This indicates that the oral dose of \( \alpha \)-retinol was delivered to these tissues on chylomicra within hours of dosage and taken up by the tissues either by passive diffusion through the cellular membranes or through lipoprotein lipase activity (29). This work adds to the evidence of chylomicron distribution among various extrahepatic organs in rats (3), in which radiolabeled retinyl esters in chylomicra from donor rats were reintroduced into other rats. In contrast, our study used pharmacologic doses of a nonradioactive tracer that does not bind to RBP (14,15) and has similar polarity to retinol, and the study quantified changes in concentration at multiple time points after oral administration.

\( \alpha \)-Retinol tracks chylomicron and lipoprotein trafficking of vitamin A without the need for radioactivity, which has been used to study vitamin A metabolism in rats (3,29–31) and humans (32). Chylomicron-delivered retinyl esters may serve a unique functional role in some extrahepatic tissues (29).

The decrease of \( \alpha \)-retinol in the extrahepatic tissues to near 0 concentrations by 7 d indicates that \( \alpha \)-retinol was utilized locally, released from these tissues, or catabolized. In rats, \( \alpha \)-retinol was utilized for growth and had 40–50% of the bioactivity of retinol when fed as an daily equimolar supplement to retinol over time (33). The quick clearance of \( \alpha \)-retinol from

![FIGURE 5](https://academic.oup.com/jn/article-abstract/144/8/1188/4571766)

**FIGURE 5** Time course (0–168 h) of adrenal \( \alpha \)-retinol concentration (A) and total \( \alpha \)-retinol (B) in groups of piglets given 26.2 or 52.4 \( \mu \)mol of \( \alpha \)-retinyl acetate, the molar equivalent of 25,000 and 50,000 IU of vitamin A, respectively. Piglets at time point 0 (\( n = 6 \)) served as baseline (no dose). Values are means \( \pm \) SDs, \( n = 6 \). Means without a common letter differ, \( P < 0.05 \). trt, treatment; 25kIU, 25,000 IU; 50kIU, 50,000 IU.

![FIGURE 6](https://academic.oup.com/jn/article-abstract/144/8/1188/4571766)

**FIGURE 6** Time course (0–168 h) of mucosal \( \alpha \)-retinol concentration (A), \( \alpha \)-retinyl ester concentration (B), and total \( \alpha \)-retinol concentration (C) in each group of piglets given 26.2 or 52.4 \( \mu \)mol of \( \alpha \)-retinyl acetate, the molar equivalent of 25,000 and 50,000 IU of vitamin A, respectively. Piglets at time point 0 (\( n = 6 \)) served as baseline (no dose). Values are means \( \pm \) SDs, \( n = 6 \). Means without a common letter differ, \( P < 0.05 \). trt, treatment; 25kIU, 25,000 IU; 50kIU, 50,000 IU.
the spleen in the 50kIU group may have caused the lower retinol concentration in the spleen at 12 and 24 h. The spleen scavenges chylomicra through mechanisms that are different from the liver, likely mediated by powerful nonspecific scavenger cell pathways (34). It is likely that the large doses of α-retinol caused atypically formed chylomicra with α-retinyl esters, which were filtered through these scavenger pathways in the spleen. Peak concentrations of α-retinol in the liver (at 24 h) were higher than in any of the other tissues sampled, consistent with the central role of the liver in vitamin A storage and metabolism. Total liver α-retinol amounts were substantial, even at 7 d, compared with retinol, demonstrating that it is effectively stored. Approximately 4–5% of the total oral dose of α-retinol was present in the liver at 7 d after dosing. Low retention is consistent with studies in older vitamin A–deficient piglets in which only 11% and 17% of 25kIU and 50kIU retinyl acetate doses, respectively, were stored in the liver (17). This is in contrast to rats with adequate vitamin A stores in which 62–76% was quickly taken up by the liver from radioactive vitamin A–labeled chylomicra (3,31), and 56–60% was retained 3–6 d after dosage (3). This retention fits with human data in which 71–76% of a radioactive retinol dose administered to Zambian children was retained after 3 d (32). Vitamin A status affects the amount of dose that can be stored, and these piglets were vitamin A deficient during this study, as demonstrated by the fact that they all had retinol concentrations < 0.1 μmol/g liver at the time they were killed (35). Furthermore, it is likely that some of the α-retinol dose bypassed absorption in these newborn piglets.

Concentrations of α-retinol in the liver decreased by 7 d because of piglet growth, but total liver α-retinol was maintained over time. This indicates that α-retinol may have been released from extrhepatic tissues and taken up by the liver, in which it accumulated because α-retinol is not released on RBP of liver origin. The nonplanarity of the α-ionone ring (15) likely prevents it from associating with the β barrel of RBP (36). During adequate vitamin A status in a rat model, 61–71% of cumulative α-retinol doses were recovered in the liver (33), supporting the current study that α-retinol accumulates and is not released on RBP. Frequent doses of α-retinol supported rat growth (33), but, when given as a bolus dose, α-retinol had only 2% growth-promoting activity (37). This further supports the benefit of frequent vitamin A intake.

Serum α-retinyl esters were elevated through 24 h and still detectable at 7 d in many of the piglets, which may indicate that the esters were repackaged in the liver with VLDLs and recirculated (38). This is supported by work in rats in which α-retinyl esters were circulating through 15 d after administration of repeated high doses that increased liver concentrations to ~2 μmol α-retinol equivalents/g liver (33), which is almost 10 times higher than liver concentrations in this study.

In a lactating sow-nursing piglet dyad, chylomicron-derived α-retinyl esters were a major constituent in the milk (18). The nursing piglet total α-retinol (754 ± 232 nmol/liver) 3 d after a single 33-μmol α-retinol dose to the sows was similar to the 25kIU (26.4 μmol) group at 24 h (661 ± 188 nmol/liver), indicating more efficient uptake and storage through the sow’s milk. The sow’s milk met daily retinol requirements, illustrated by the fact that α-retinol was not utilized in the liver as in the rat study (33), but did not supply ample retinyl ester storage because the piglets were vitamin A deficient. The WHO has not recommended high-dose vitamin A supplementation to postpartum women because there is weak evidence that it prevents maternal and infant morbidity and mortality (39).

Few dispute the benefits of vitamin A supplementation to preschool-age children in the prevention of mortality and overt vitamin A deficiency (40), but perhaps food-based approaches to

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**TABLE 1** Serum retinol concentrations in newborn piglets after oral dosage with 26.2 (25,000 IU) or 52.4 (50,000 IU) μmol of α-retinyl acetate

<table>
<thead>
<tr>
<th>Treatment (IU)</th>
<th>7 h</th>
<th>12 h</th>
<th>24 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>25,000 μmol/L</td>
<td>0.22 ± 0.04e</td>
<td>0.33 ± 0.08e</td>
<td>0.51 ± 0.06e</td>
<td>0.63 ± 0.09e</td>
</tr>
<tr>
<td>50,000 μmol/L</td>
<td>0.27 ± 0.04e</td>
<td>0.32 ± 0.04e</td>
<td>0.46 ± 0.03e</td>
<td>0.66 ± 0.16e</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs, n = 6 per time. Two-factor ANOVA showed a difference by time (P < 0.0001), but treatment (P = 0.95) and the interaction (P = 0.41) were not significant. Means without a common letter differ, P < 0.05.
the global vitamin A problem need to be considered (41) along with supplementation programs to mitigate morbidity and maintain optimal health. Daily vitamin A intake is obviously more appropriate than periodic boluses. However, many mammals are born vitamin A depleted, especially if the mother has depleted stores. Careful consideration needs to be given to newborn dosing regimens, especially in cultures in which breastfeeding rates are low or the vitamin A–rich colostrum is not fed to the infant. Mortality is currently the only measure that is used, which is multifactorial. Therefore, other markers, such as immune response, should be considered in program development.

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