Healthy birth weight results in higher vitamin A storage in neonate piglets administered high-dose supplements

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Abstract

A proposed intervention for newborn infants in countries with suspected vitamin A (VA) deficiency is to administer 50,000 IU retinyl palmitate at birth to reduce mortality risk. However, no studies have investigated birth weight effects. In this study, low birth weight (LBW; <1 kg, $n = 18$) and healthy birth weight (HBW) piglets (>1.5 kg, $n = 18$) from VA-depleted sows were dosed with 25,000 or 50,000 IU retinyl palmitate (26.2 or 52.4 μmol retinol equivalents) at birth to compare VA reserves. Blood was collected at varying times ($n = 3\text{--}5/\text{time}/\text{dose}$), and piglets were killed at 12 or 24 h for blood, liver, kidneys, spleen, lungs, adrenal gland, and intestinal contents. HBW piglets had significantly higher birth, death, and organ weights than LBW ($P < 0.0001$ for all). HBW and LBW piglets, which received VA, had higher liver and kidney VA concentrations (0.18 ± 0.09 , 0.24 ± 0.10 $\mu\text{mol}/\text{g}$ liver and 13.4 ± 4.1 , 14.2 ± 4.5 nmol/g kidney, respectively) than controls ($n = 10$) (0.051 ± 0.01 $\mu\text{mol}/\text{g}$ liver and 1.01 ± 0.43 nmol/g kidney) ($P = 0.0061$ and < 0.0001 , respectively). Total liver (9.75 ± 5.16 μmol) and kidney retinol (204 ± 79.1 nmol) were higher in HBW than LBW piglets ($P < 0.0001$). Extrahepatic tissues, except lung, had higher VA concentration than controls ($P < 0.0001$). Serum retinol and ester concentrations were higher in treated than control piglets ($P = 0.0028$, $P < 0.0001$, respectively), and significantly changed during the times sampled ($P = 0.022$, $P = 0.011$, respectively). Peak serum retinyl ester concentrations, which occurred at 3 h, were higher in piglets that received 50,000 IU (4.2 ± 4.4 $\mu\text{mol}/\text{L}$) than 25,000 IU (2.7 ± 2.3 $\mu\text{mol}/\text{L}$) ($P = 0.031$). Regardless of dose amount, HBW piglets stored more supplemental VA than LBW piglets when administered at birth.

Keywords: Newborns, retinol, swine

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Introduction

Birth weight is an anthropometric measurement that is used both clinically and globally as an indicator of newborn health.¹ Low birth weight (LBW) infants are classified as weighing <2.5 kg and the condition is usually caused by pre-term birth (<37 weeks).² Factors that contribute to LBW include the nutritional status of the mother during pregnancy, such as young age, malnutrition, hypertension, smoking, drug or alcohol abuse, and insufficient prenatal care.³ High prevalence of LBW, as well as birth defects, is common in developing countries, such as those in sub-Saharan Africa and Southeast Asia, which are the same regions that have a high prevalence of vitamin A (VA) deficiency.³

LBW infants are often underdeveloped physiologically and have minimal VA stores, because of highly regulated trans-placental transport.⁴ Preterm infants need VA for proper lung and retina development.⁵ Extremely LBW infants (defined as birth weight <1 kg) often have low plasma and

tissue concentrations of VA.^{6,7} Supplementation trials in LBW infants have yielded contraindicated results. Indeed, a VA supplementation study in Guinea-Bissau resulted in increased mortality of VA-supplemented LBW females, but no effect on males when dosed with 25,000 IU (25 kIU) (26.2 μmol retinol equivalents) retinyl palmitate.⁸ However, 25 kIU was well-tolerated in both male and female South African LBW infants (42.9% males).⁹

Recent studies show that maternal VA status is positively associated with birth weight, length, and head circumference.¹⁰ In India, maternal night blindness increased the risk of LBW,¹¹ and maternal serum retinol concentrations <0.7 $\mu\text{mol}/\text{L}$ were associated with preterm delivery.¹² However, research regarding maternal VA supplementation shows little evidence for efficacy in prevention of LBW or factors associated with VA D. A systematic review revealed that antenatal VA supplementation did not prevent LBW or preterm births.¹³ In a randomized,

placebo-controlled trial in Bangladesh,¹⁰ weekly VA or β -carotene supplementation to mothers during pregnancy until three months post-partum did not reduce the overall incidence of preterm birth or fetal growth restriction that contribute to LBW. Further, birth weights of infants whose mothers had received the supplements were not different from those whose mothers had received the placebo.¹⁰

Historically, VA deficiency has been treated by government-regulated supplementation with preformed VA. The World Health Organization (WHO) recommends preformed VA supplements to infants and young children in areas of high VA deficiency prevalence, but there is no dosing recommendation regarding birth weight. WHO currently recommends that children 6–11 months old receive a one-time dose of 100,000 IU VA and that those 12–59 months old receive 200,000 IU VA every 4–6 months to mitigate mortality risk.¹⁴ Previously, WHO recommended that infants receive 50,000 IU (50 kIU) VA (52.4 μ mol retinol equivalents) at immunization contacts at age 6, 10, and 14 week,¹⁵ but it is no longer recommended that children under six months receive supplementation based on little or no impact on childhood mortality rates.¹⁶ A proposal to orally administer 50 kIU VA at birth is currently undergoing population and mechanistic evaluation.

Although VA status of infants can be determined by dose response or stable isotope tests,¹⁷ studies that determine organ uptake of newly ingested VA are not feasible in LBW human infants due to lack of appropriate methodology. Piglets are an appropriate model due to similar physiology and size, compared with rodent models. Thus, LBW and healthy birth weight (HBW) piglets from VA-depleted sows were orally dosed with 25 kIU or 50 kIU VA and killed 12 or 24 h after dosage to evaluate VA distribution to various tissues. These VA doses were chosen based on previous evidence that 25 kIU was well-tolerated and 50 kIU was a previous recommendation by WHO for infants <6 months. We hypothesized that HBW piglets would absorb more VA than LBW, due to more mature gastrointestinal and organ development.

Materials and methods

Animal care and dosing

Approval was obtained from the University of Wisconsin-Madison Animal Care and Use Committee and included a recommendation that not all blood samples be obtained from the same piglets within the 24-h period of the study. Sows ($n=11$; *Sus scrofa domestica*, crossbreeds of Large White and Landrace) were housed at the Swine Research and Teaching Center (Arlington, WI). The sows had 3.3 ± 2.7 (mean $\pm$ SD) overall parities and one prior VA-depleted parity before entering the study. Sows were fed a VA-free wheat diet throughout gestation and lactation.¹⁸

Male and female piglets ($n=36$) meeting study criteria (<1.0 kg or >1.5 kg) were paired from the same sow and randomly dosed orally with 25 kIU or 50 kIU retinyl palmitate (26.2 or 52.4 μ mol retinol equivalents) at ~12-h post farrowing. The dose was supplied by WHO as 50 kIU “cut and squeeze” softgel capsules (retinyl palmitate, Strides

Acrolab, India). The 25 kIU doses were diluted with soybean oil to administer approximately the same volume of oil as the 50 kIU group. Doses (200 μ L) were administered orally with a positive displacement pipet, which was placed deep into the piglets’ throats before discharge. Blood was collected at 1.5 and 5 h for piglets killed at 12 h, and 3 and 7 h for 24-h kill piglets. A group of control piglets ($n=10$), weighing >1.0 and <1.5 kg were dosed with 250 μ L soybean oil and killed at 24 h. Liver, lungs, kidney, adrenal gland, spleen, and blood were collected from all piglets after killing. Intestinal contents were collected by saline wash on a subset of piglets ($n=20$) from a 10-cm section of the intestine, which was 10 cm distal from the pylorus.

Piglet tissue analysis

Liver¹⁹ and serum²⁰ were analyzed using published methods. Kidney was analyzed using an adaptation of published methods.²¹ Kidney (0.5 g) was ground with sodium sulfate (3–4 g) in a mortar, and C-23 β -apo-carotenol was added to determine extraction efficiency. Dichloromethane was used to extract retinol and retinyl esters and filtered into a 25-mL volumetric flask; 5 mL was dried under nitrogen and dissolved in 100 μ L 50:50 (v:v) methanol:dichloromethane; 20 μ L was injected. The HPLC system included a Resolve C₁₈ (5 μ m, 3.9 $\times$ 300 mm) reverse-phase column, Waters 600 binary pump (Milford, MA), Shimadzu Chromatopac C-R7A plus and SPD-10 AVP UV-Vis detector (Kyoto, Japan). Solvent and runtime were as described.²⁰

Lung (5 g), adrenal gland (0.5 g), and spleen (2 g) were run using an adaptation to published methods.²¹ The samples were extracted three times with 20 mL (lung) or 10 mL (adrenal and spleen) dichloromethane. The extract was dried in a round-bottom flask using a rotary evaporator. The residue was redissolved in dichloromethane three times (2, 1, and 1 mL) and transferred to a glass tube (13 $\times$ 100 mm). The combined solution was dried under nitrogen and redissolved in 100 μ L 50:50 (v:v) methanol:dichloromethane; 50 μ L was injected onto a Waters HPLC system with a 2707 autosampler, 1525 binary pump, and 2996 photodiode array detector (Milford, MA). Solvent A consisted of 92.5:7.5 (v:v) acetonitrile:water and solvent B consisted of 85:10:5 (v:v) acetonitrile:methanol:dichloroethane (10 mmol/L ammonium acetate in each solvent).

Intestinal contents (0.5 mL) were added to 0.5 mL ethanol with 0.1% butylated hydroxytoluene as an antioxidant. C23- β -apo-carotenol (200 μ L) was added as an internal standard, mixed with a vortex, and centrifuged. The sample was extracted three times with 1 mL hexanes. Organic layers were pooled, dried under nitrogen, and resuspended in 200 μ L 50:50 (v:v) methanol:dichloroethane; 25 μ L was injected onto a Waters C-18 3- μ m, 3.9 $\times$ 300-mm reversed-phase column (Milford, MA) equipped with a guard column. Solvent A consisted of 92.5:7.5 (v:v) acetonitrile:water (10 mmol/L ammonium acetate) and solvent B consisted of 80:10:10 (v:v) acetonitrile:methanol:dichloroethane. The HPLC system and specifications were the same as described for lung.

Quantification

Retinol and retinyl esters (e.g. linoleate, oleate, palmitate, and stearate) in all tissues were identified by comparing their retention times with synthesized or isolated standards, as well as characteristic spectra, and quantified using retinol and retinyl butyrate, respectively, based on the $E_{1\text{cm}}^{1\%}$ of retinol (i.e. 1845 in alcohol). Both retinol concentration and total retinol reserves in each tissue collected were calculated. All VA values in tissues were calculated based on tissue wet weight. The percent retention of VA dose in piglet tissues for treatment groups was calculated: total amount of retinol in the treatment group minus the control, divided by the ingested VA amount.

Statistical analysis

SAS software (version 9.2, Cary, NC) was used. Values are presented as means $\pm$ SD. ANOVA was used to test differences. Levene's test for homogeneity was used to determine unequal variances. Repeated measure tests were applied to organ data sets with unequal variances. Three-way ANOVA was used to evaluate the effect of the VA dose according to dose amount, birth weight, and time. To account for the inter-piglet variation in the serum retinol concentrations, the peak value for each piglet was assigned a value of 1 and other times for the same piglet were normalized as a fraction of the peak and these values were further used in the statistical evaluation of the data. $P \leq 0.05$ was considered significant and interactions with $P < 0.1$ were considered important.

Results

Birth and final weights

By design, LBW piglets weighed 0.93 ± 0.06 kg and HBW piglets weighed 1.79 ± 0.29 kg at birth ($P < 0.0001$). Control piglets weighed 1.30 ± 0.19 kg and their weight was significantly different from both LBW and HBW ($P < 0.0001$). There was no difference between birth and final weights of the piglets from the VA treatment groups; however, piglets were significantly heavier in 24-h kill groups for both birth and final weights than the 12-h kill groups ($P = 0.044$ and 0.035 , respectively). All organs collected were significantly heavier in HBW piglets than LBW piglets (Table 1, $P < 0.0001$).

Table 1 Organ weights from healthy (HBW) and low birth weight (LBW) piglets.^a

Organ ^b	HBW (g)	LBW (g)
Liver	51.5 ± 9.67	20.7 ± 5.12
Lungs	25.9 ± 5.19	13.6 ± 2.83
Kidneys	14.9 ± 2.62	7.97 ± 1.40
Adrenals	0.41 ± 0.10	0.27 ± 0.063
Spleen	2.47 ± 0.67	1.08 ± 0.30

^aOrgan weights are mean $\pm$ SD.

^bHBW organ weights were significantly higher than those of LBW piglets for all organs ($P < 0.0001$).

Figure 1 Mean ($\pm$ SD), $n = 4$ or 5 /group. Liver vitamin A concentrations and reserves in all piglets. Means were analyzed using PROC MIXED and LSMEANS were used to make comparisons among groups. (a) Liver vitamin A concentration in treated piglets was significantly higher than control piglets ($P = 0.0061$). When treated piglets only were compared liver concentration was significantly higher at 24 h ($P = 0.036$). (b) Liver vitamin A reserves in treated piglets were significantly higher than control piglets ($P = 0.0001$). When treated piglets only were compared, vitamin A reserves were significant higher at 24 h ($P = 0.0015$), and in HBW piglets ($P = 0.0002$), no interaction existed. Unlike letters are significantly different. Trt, treatment

Liver retinol

Retinol includes the sum of the free form and all identifiable retinyl esters. Overall, both HBW and LBW piglets' liver retinol concentrations were significantly higher than control piglets that received no dose (Figure 1a, $P < 0.0001$). Piglets had higher liver concentration at 24 h than at 12 h ($P = 0.036$). However, no significant differences occurred between dose, weight, or time groups. All dosed piglets were above the VA deficiency cutoff of $0.07 \mu\text{mol/g}$ and 34 (94.4%) were above $0.1 \mu\text{mol/g}$ liver, a biologically relevant cutoff,¹⁷ but the control piglets were all VA-deficient. Correcting for organ weight, total liver retinol (Figure 1b) in both HBW and LBW piglets was significantly higher than the controls ($P < 0.0001$). HBW piglets had significantly higher total liver retinol ($9.75 \pm 5.16 \mu\text{mol}$) compared with LBW piglets ($4.91 \pm 2.01 \mu\text{mol}$) ($P = 0.0002$). All piglets killed at 24 h had significantly higher total liver retinol ($9.33 \pm 5.30 \mu\text{mol}$) than those killed at 12 h ($5.43 \pm 2.64 \mu\text{mol}$) ($P = 0.0015$), likely due to the impact of a subsequent meal. A trend existed for an interaction between dose amount and time ($P = 0.087$), indicating that both the dose level and the

sampling time influenced the total amount recovered in the liver.

Extrahepatic tissues

All piglets treated with VA had significantly higher kidney retinol concentration and total kidney retinol than control piglets, which did not receive VA (Table 2, $P < 0.0001$). There were no differences in retinol concentration among dose, time, or weight groups. HBW piglets had significantly higher total kidney retinol (203.8 ± 79.1 nmol) than LBW piglets (114.8 ± 34.0 nmol) ($P = 0.0002$).

Spleen retinol concentration and total retinol were significantly higher in VA-treated piglets (2.82 ± 2.28 nmol/g, 4.93 ± 4.69 nmol/spleen, respectively) than controls that did not receive VA (0.60 ± 0.29 nmol/g, 1.56 ± 0.66 nmol/spleen, respectively) (Table 2, $P < 0.0001$, $P = 0.0017$, respectively). Birth weight was important ($P = 0.055$), and an interaction between birth weight and time sampled may have influenced spleen retinol concentration and total uptake ($P = 0.086$).

Lung VA concentration and total lung VA did not differ among dose, time, or weight groups of the VA-supplemented piglets at the times of kill but there was a trend for total VA in HBW piglets to be higher (Table 2, $P = 0.076$).

Adrenal gland VA was higher in treated piglets than controls for both VA concentration and total VA (Table 2, $P < 0.0001$, $P = 0.018$, respectively). Dose level and time did not affect adrenal gland VA concentration. A trend for a difference in LBW piglets having higher VA concentration than HBW piglets existed ($P = 0.066$).

Serum retinol and retinyl esters

VA-treated piglets had significantly higher serum retinol and retinyl ester concentrations than control piglets (Figure 2; $P = 0.011$, $P < 0.0001$, respectively). Piglets dosed with 50 kIU had significantly higher serum retinyl esters (3.79 ± 3.85 μ mol/L) than those dosed with 25 kIU (2.53 ± 2.24 μ mol/L) ($P = 0.05$), but serum retinol concentrations were not different between the two dose groups. Time was a significant factor for both serum retinol and retinyl esters ($P = 0.022$ and $P = 0.017$, respectively). Serum retinol concentrations at 1.5, 3, 5, 7, 12, and 24 h were 1.15 ± 1.12 , 0.36 ± 0.16 , 0.87 ± 0.73 , 0.48 ± 0.16 , 0.72 ± 0.68 , 0.62 ± 0.32 μ mol/L, respectively. Serum retinyl ester concentrations at 1.5, 3, 5, 7, 12, and 24 h were 1.25 ± 1.06 , 4.73 ± 2.46 , 4.00 ± 2.93 , 3.76 ± 4.64 , 2.20 ± 1.34 , 2.46 ± 3.06 μ mol/L, respectively. Piglet serum retinol concentration was highest at 1.5 h and retinyl esters were highest at 3 h. Normalization of serum retinol concentrations by piglet mitigated the significant difference by time (Figure 2b). Weight status did not influence serum retinol or retinyl ester concentration response to VA treatment.

Percentage of dose

Although the entire intestinal contents were not collected, 0.03–17% of the administered dose was recovered from the intestinal lumen at tissue harvest. The highest percentage of VA-dose retention was found in the liver. The percentage

Table 2 Extrahepatic total tissue retinol and concentrations in piglets administered 25 kIU and 50 kIU retinyl palmitate

Organ	Control	12 h ^b				24 h ^c			
		25 kIU		50 kIU		25 kIU		50 kIU	
		LBW	HBW	LBW	HBW	LBW	HBW	LBW	HBW
Total retinol (nmol/tissue) ^a									
Kidney	17.6 ± 10.6	122 ± 61.1^d	$203 \pm 49.6^{d,e}$	112 ± 17.6^d	$227 \pm 95.1^{d,e}$	108 ± 8.24^d	$176 \pm 113^{d,e}$	117 ± 32.5^d	$210 \pm 61.6^{d,e}$
Spleen	1.57 ± 0.66	2.45 ± 0.76^d	8.34 ± 7.21^d	1.43 ± 2.89^d	7.29 ± 8.75^d	3.99 ± 2.89^d	5.46 ± 3.62^d	5.57 ± 4.60^d	4.79 ± 1.87^d
Lung	15.6 ± 9.01	14.4 ± 7.80	24.3 ± 15.9	11.6 ± 6.84	25.3 ± 15.8	19.6 ± 6.83	24.1 ± 8.67	26.0 ± 28.9	36.4 ± 23.5
Adrenal	0.52 ± 0.43	5.19 ± 3.15^d	3.13 ± 1.26^d	2.56 ± 1.56^d	4.67 ± 1.82^d	3.91 ± 2.79^d	4.69 ± 5.06^d	3.66 ± 2.01^d	3.40 ± 1.57^d
Retinol concentration (nmol/g) ^a									
Kidney	1.01 ± 0.43	15.7 ± 6.81^f	12.8 ± 2.19^f	16.1 ± 2.62^f	15.6 ± 5.50^f	13.1 ± 3.01^f	11.2 ± 3.96^f	12.6 ± 4.40^f	14.0 ± 3.85^f
Spleen	0.60 ± 0.29	2.79 ± 0.83^f	3.38 ± 2.82^f	1.80 ± 1.30^f	3.43 ± 4.27^f	3.39 ± 2.76^f	1.72 ± 0.49^f	4.04 ± 3.25^f	2.01 ± 0.67^f
Lung	0.61 ± 0.36	1.21 ± 0.83	0.90 ± 0.50	1.08 ± 0.59	1.18 ± 0.78	1.40 ± 0.55	0.80 ± 0.25	1.58 ± 1.72	1.50 ± 1.05
Adrenal	1.01 ± 0.43	22.1 ± 12.6^f	8.90 ± 4.22^f	11.1 ± 5.92^f	11.6 ± 5.62^f	14.2 ± 12.1^f	9.64 ± 6.30^f	11.7 ± 7.12^f	8.13 ± 4.31^f

HBW: healthy birth weight; LBW: low birth weight.

^aAll values are means $\pm$ SD.

^b $n = 5$ /dose, time group.

^c $n = 4$ /dose, time group.

^dTotal tissue VA was higher in treated piglets than control piglets for kidney ($P < 0.0001$), spleen ($P = 0.0017$), and adrenal glands ($P = 0.018$). No differences in lung existed, although there was a trend for the total lung retinol in the HBW piglets to be higher ($P = 0.076$).

^eTotal tissue VA was higher in HBW piglets than LBW piglets ($P = 0.0002$).

^fVit A concentrations in treated piglets were higher in kidney, spleen, and adrenal tissues than control piglets ($P < 0.0001$ for all). No differences in lung existed.

Figure 2 (a) Serum retinol concentrations (means $\pm$ SD). Time was the only significant factor ($P = 0.022$) and treated piglets killed at 24 h had significantly higher serum retinol concentrations than control piglets ($P = 0.011$). Different superscripts indicate differences between times. (b) Serum retinol concentrations were normalized by assigning a value of 1 to the highest concentration and calculating lower concentrations as a ratio (means $\pm$ SD). No factors were significant. (c) Serum retinyl ester concentrations (means $\pm$ SD). Trt and time were significant factors ($P = 0.05$ and $P = 0.017$, respectively). Serum retinyl esters in treated piglets at 24 h were significantly higher than 24 h control piglets ($P < 0.0001$). Unlike letters are significantly different. Trt: treatment

of dose stored in liver for the 25 kIU and 50 kIU groups did not differ at 24 h (Table 3). HBW piglets stored a higher percentage of administered VA than LBW piglets ($P = 0.0006$), and this represented approximately 200% more dose stored in the HBW piglets than in the LBW piglets (Table 3). All piglets had higher retinyl ester accrual at 24 h than 12 h ($P = 0.0097$), which likely represents some of the dose being retained in the intestinal wall awaiting new chylomicron formation with subsequent meals. There was no difference in percent storage of VA dose between piglets dosed with 25 kIU or 50 kIU, but an interaction between dose level and time existed ($P = 0.014$) and a trend for an interaction with dose, time, and weight existed ($P = 0.064$).

Discussion

Currently, the only evidence-based WHO recommendation for VA supplementation is for 12- to 59-month-old children to receive 200,000 IU retinyl palmitate every 4–6 months and those who are 6–11 mo to receive one 100,000 IU dose.¹⁴ Although a proposal to give 50 kIU VA as retinyl palmitate to newborn infants is undergoing evaluation, there are no guidelines regarding birth weight. This study compared 25 kIU and 50 kIU VA supplements between LBW and HBW piglets born to VA-depleted sows. Newborn infant dosing regimens were explored because a study in Indonesia documented a remarkable 0.36 relative risk in infant mortality for those infants who received 50 kIU VA at birth compared with a placebo.²² A study with LBW infants administered 25 kIU VA found that the dose was well-absorbed and no adverse effects were noted.²³ Our study found that both the 25 kIU and 50 kIU doses were efficacious because all organs from HBW and LBW piglets, except lung at the times sampled, were higher in piglets receiving the VA dose than control piglets receiving no VA. However, it must be noted that no difference was detected between the 25 kIU and 50 kIU doses in any of the tissues sampled likely indicating a threshold for VA uptake and storage.

Some studies have used the sow-piglet model to investigate VA supplementation and storage in liver, but few have investigated the storage of VA in extrahepatic tissues post-supplementation. Vitamin A is crucial for both pre- and post-natal lung development and is stored primarily as retinyl esters.^{24–26} Lung and kidney retained <1% of the doses administered in this study and thus likely do not represent major storage sites for drawing on long-term VA needs of high-dose supplements given at birth. The low retention in the lung confirms prior studies in rats.^{25,27} Supplementing rats with retinyl palmitate alone did not increase fractional uptake of ³H-VA in lung, but uptake was increased with co-treatment of acidic retinoids.²⁷ The highest retention in the liver found in the HBW piglets at 24 h (i.e. 37.4%) was similar to neonatal rats (~40%).²⁷ The rank of VA concentration in organs analyzed was liver > kidney > adrenal glands > spleen > lung for all treated piglets within one day after dosage. The rank of spleen and lung differed from piglets killed 10 days after supplementation,²¹ where lung had a higher retinol concentration than spleen. The higher spleen concentration at one day in the current study is likely due to the scavenging of chylomicra after the large doses given in this study.²⁸ In a prior swine study,²¹ spleen acquired more chylomicron-derived 3,4-didehydroretinol in a 4-h timeframe than the lung. The retinol values from the high doses of VA would have had more time to equalize with total body retinol in the Sun *et al.* study considering the 10-day wait period before organ harvest.²¹ Further, lung retinol concentrations likely peaked earlier than 12 h in the current study considering that the first pass of chylomicron-delivered VA, which deliver large amounts to the lung,^{19,21,29} would have cleared before 12 h.

Both VA deficiency and LBW prevalence are high in developing countries, due in part to widespread maternal

Table 3 Percentage of oral doses of vitamin A retained in tissues of HBW and LBW piglets.^a

Group	Percentage of dose retained							
	Liver ^{b,c}		Lung ^d		Kidney ^e		Adrenal ^d	
	HBW	LBW	HBW	LBW	HBW	LBW	HBW	LBW
12 h, 25 kIU	22.4 ± 12.1 ^c	8.29 ± 4.47	0.05 ± 0.06	0.02 ± 0.02	0.69 ± 0.19 ^{e,f}	0.38 ± 0.23 ^e	0.004 ± 0.002	0.013 ± 0.009
24 h, 25 kIU	21.8 ± 9.21 ^{b,c}	11.7 ± 7.56 ^b	0.05 ± 0.03	0.03 ± 0.03	0.59 ± 0.43 ^{e,f}	0.33 ± 0.03 ^e	0.010 ± 0.016	0.007 ± 0.008
12 h, 50 kIU	7.85 ± 2.78 ^c	2.21 ± 1.77	0.07 ± 0.06	0.02 ± 0.03	0.39 ± 0.18 ^f	0.17 ± 0.03	0.005 ± 0.002	0.001 ± 0.002
24 h, 50 kIU	37.4 ± 3.82 ^{b,c}	9.10 ± 3.82 ^b	0.11 ± 0.08	0.08 ± 0.11	0.36 ± 0.12 ^f	0.18 ± 0.06	0.003 ± 0.002	0.003 ± 0.002

HBW: healthy birth weight; LBW: low birth weight.

^aAll values are means ± SD, *n* = 4–5/group.

^bFor liver, percentage of dose retained was significantly higher at 24 h than 12 h (*P* = 0.01).

^cFor liver, percentage of dose retained was higher in HBW piglets than LBW (*P* = 0.0006); an interaction between treatment × time existed (*P* = 0.037), and a trend occurred for treatment × time × weight (*P* = 0.064).

^dA trend existed for lung to have a higher percentage of dose retained by treatment (*P* = 0.074) and birth weight (*P* = 0.08). No significant differences occurred for adrenal gland.

^eFor kidney, the percentage of dose retained was higher in 25 kIU-dosed piglets than 50 kIU-dosed piglets (*P* = 0.002).

^fFor kidney, the percentage of dose retained was higher in HBW piglets than LBW piglets (*P* = 0.001).

malnutrition.³⁰ The condition of LBW is usually the result of the infant being born preterm (before 37 weeks). These infants have increased susceptibility to morbidities of the eye, respiratory system, and gastrointestinal tract.⁵ VA deficiency is problematic in newborn LBW infants who have an underdeveloped respiratory tract, which increases their susceptibility for infection. Lack of VA transfer to the fetus from mothers with low VA status further impairs growth and development of LBW infants.³¹ In a study with South African LBW infants, there were no differences in respiratory disease development within the first year of life between LBW infants given three 25 kIU VA doses and those given placebos within eight days of life.⁹ In addition, LBW and very LBW prevalence is influenced by risk of disease and lack of prenatal care in some developing countries.³⁰ Healthy newborn infants from VA-sufficient mothers are already born with limited VA stores due to tight regulation of placental transfer,⁴ thus it is not surprising that LBW infants born to mothers who are VA-deficient are compromised. Even in breastfed infants, preterm infants were found to have significantly lower plasma retinol concentrations than term infants at four weeks of age.³²

LBW, small-for-gestational age, and preterm birth are associated with increased odds of wasting, stunting, and underweight,³³ which may negatively impact gut health. Abnormal morphology and delayed development of the small intestine may impact VA absorption. Preterm infants have abnormal intestinal morphology and decreased mucosal DNA, as well as decreased protein content and enzymatic activity.³⁴ A rat study found that inducing VA deficiency in late pregnancy resulted in less growth of intestinal villi in the small intestine of fetuses.³⁵ The difference in liver retinol storage between LBW and HBW piglets may be due to length of the absorptive intestinal tract or stellate cell development. Little is known about the specific development of stellate cells, other than that their production arises from mesenchymal stem cells.³⁶

LBW infants are often given perinatal nutrition or special formulas, rather than breast milk from their mothers.³⁰

In this study, piglets were not given any additional supplements other than the VA dose ~12 h after birth. LBW and HBW piglets relied on their VA-depleted mother for milk throughout the study, which could not be equalized. It was not feasible to measure milk consumption; therefore, a combination of diminished intestinal development and possibly lower milk intake by the LBW piglets could have contributed to variance in this study.

The intestinal contents were collected to provide a snapshot of unabsorbed VA dose during the study. VA doses are more efficacious when given as a lipid bolus, in addition to that which is delivered in milk.³⁷ However, young infants may not be able to absorb these doses as efficiently as older children,³⁸ and very LBW infants need special nutritional management.³⁷ The percentage of dose found in extrahepatic organs did not differ between treatment or time groups, similar to VA concentration. The higher recovery in the piglets that were killed at 24 vs. 12 h likely reflects more uptake of the VA dose during subsequent meals. Considering unabsorbed dose was detected in the intestinal contents, absorbed retinol was likely still present in the intestinal cells waiting for another meal to be esterified and packaged into new chylomicra for circulation and storage.^{20,39,40} Fat from a previous meal contributes to early chylomicra release from the intestine.³⁹ Fat-soluble vitamins are also retained when high doses are administered.

Serum retinol concentrations did not follow any obvious pattern. This could be because the piglets were not the same at each time point and normalization of the data mitigated any statistical findings of these data. On the other hand, retinyl esters peaked by 7 h and there was a treatment effect such that the 50 kIU dose resulted in a higher peak retinyl ester concentration than the 25 kIU dose. However, this did not result in greater accrual of VA in all analyzed organs. Birth weight had an overall greater effect on outcomes in this study than dose level.

VA deficiency and LBW continue to be problems in many countries. While certain factors increase the risk and prevalence of LBW, it is difficult to plan LBW status

into prospective studies. In this newborn piglet model, serum retinol and retinyl ester concentrations combined with percentage of dose stored described the destination and distribution of newly administered VA supplementation. Birth weight affected VA absorption, distribution, and liver storage in newborn piglets, and this finding should be taken into account when determining a regimen for newborn infants.

Author contributions: EKH conducted research, analyzed samples, analyzed data, and wrote the first draft of the manuscript. EH analyzed samples. SAT designed the research, provided input for the statistical analysis, and revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

- Christian P. Micronutrients, birth weight, and survival. *Annu Rev Nutr* 2010;**30**:83-104
- Gardosi J. Fetal growth: towards an international standard. *Ultrasound Obstet Gynecol* 2005;**26**:112-4
- Lindsay KL, Gibney ER, McAuliffe FM. Maternal nutrition among women from Sub-Saharan Africa, with a focus on Nigeria, and potential implications for pregnancy outcomes among immigrant populations in developed countries. *J Hum Nutr Diet* 2012;**25**:534-46
- Spiegler E, Kim YK, Wassef L, Shete V, Quadro L. Maternal-fetal transfer and metabolism of vitamin A and its precursor β -carotene in the developing tissues. *Biochim Biophys Acta* 2012;**1821**:88-98
- Mactier H, Weaver LT. Vitamin A and preterm infants: what we know, what we don't know, and what we need to know. *Arch Dis Child Fetal Neonatal Ed* 2005;**90**:F103-8
- Shenai JP, Chytil F, Jhaveri A, Stahlman MT. Plasma vitamin A and retinol-binding protein in premature and term neonates. *J Pediatr* 1981;**99**:302-5
- Ambalavanan N, Wu TJ, Tyson JE, Kennedy KA, Roane C, Carlo WA. A comparison of three vitamin A dosing regimens in extremely-low-birth-weight infants. *J Pediatr* 2003;**142**:656-61
- Benn CS, Fisker AB, Napirna BM, Roth A, Diness BR, Lausch KR, Ravn H, Yazdanbakhsh M, Rodrigues A, Whittle H, Aaby P. Vitamin A supplementation and BCG vaccination at birth in low birthweight neonates: two by two factorial randomised controlled trial. *BMJ* 2010;**340**:c1101
- Coutsoudis A, Adhikari M, Pillay K, Kuhn L, Coovadia HM. Effect of vitamin A supplementation on morbidity of low-birth-weight neonates. *S Afr Med J* 2000;**90**:730-6
- Christian P, Klemm R, Shamim AA, Ali H, Rashid M, Shaikh S, Wu L, Mehra S, Labrique A, Katz J, West KP Jr. Effects of vitamin A and β -carotene supplementation on birth size and length of gestation in rural Bangladesh: a cluster-randomized trial. *Am J Clin Nutr* 2013;**97**:188-94
- Tielsen JM, Rathmathullah L, Katz J, Thulasiraj RD, Coles C, Sheeladevi S, Prakash K. Maternal night blindness during pregnancy is associated with low birthweight, morbidity, and poor growth in South India. *J Nutr* 2008;**138**:787-92
- Radhika MS, Bhaskaram P, Balakrishna N, Ramalakshmi BA, Devi S, Kumar BS. Effects of vitamin A deficiency during pregnancy on maternal and child health. *BJOG* 2002;**109**:689-93
- van den Broek N, Dou L, Othman M, Neilson JP, Gates S, Gülmezoglu AM. Vitamin A supplementation during pregnancy for maternal and newborn outcomes. *Cochrane Database Syst Rev* 2010;**11**:CD008666
- World Health Organization. Guideline: Vitamin A supplementation in infants and children 6-59 months of age. http://www.who.int/nutrition/publications/micronutrients/guidelines/vas_6to59_months/en/ (2011, accessed 29 August 2014)
- Surles RL, Mills JP, Valentine AR, Tanumihardjo SA. One-time graded doses of vitamin A to weanling piglets enhance hepatic retinol but do not always prevent vitamin A deficiency. *Am J Clin Nutr* 2007;**86**:1045-53
- World Health Organization. Guideline: Vitamin A supplementation for infants 1-5 months of age. http://www.who.int/nutrition/publications/micronutrients/guidelines/vas_infants_1-5/en/ (2011, accessed 11 January 2014)
- Tanumihardjo SA. Vitamin A: biomarkers of nutrition for development. *Am J Clin Nutr* 2011;**94**:658-65
- Dever JT, Surles RL, Davis CR, Tanumihardjo SA. α -Retinol is distributed through serum retinol-binding protein-independent mechanisms in the lactating sow-nursing piglet dyad. *J Nutr* 2011;**141**:42-7
- Heying E, Grahm M, Pixley KV, Rocheford T, Tanumihardjo SA. High-provitamin A carotenoid (orange) maize increases hepatic vitamin A reserves of offspring in a vitamin A-depleted sow-piglet model during lactation. *J Nutr* 2013;**143**:1141-6
- Riabroy N, Tanumihardjo SA. Oral doses of α -retinyl ester track chylomicron uptake and distribution of vitamin A in a male piglet model for newborn infants. *J Nutr* 2014;**144**:1188-95
- Sun T, Surles RL, Tanumihardjo SA. Vitamin A concentrations in piglet extrahepatic tissues respond differently ten days after vitamin A treatment. *J Nutr* 2008;**138**:1101-6
- Humphrey JH, Agoestina T, Wu L, Usman A, Nurachim M, Subardja D, Hidayat S, Tielsen J, West KP Jr, Sommer A. Impact of neonatal vitamin A supplementation on infant morbidity and mortality. *J Pediatr* 1996;**128**:489-96
- Coutsoudis A, Adhikari M, Pillay K, Coovadia HM. Absorption of high-dose enteric vitamin A in low-birth-weight neonates. *S Afr Med J* 1996;**86**:1337-9
- Mactier H. Vitamin A for preterm infants; where are we now? *Semin Fetal Neonatal Med* 2013;**18**:166-71
- Ross AC, Ambalavanan N, Zolfaghari R, Li N-Q. Vitamin A combined with retinoic acid increases retinol uptake and lung retinyl ester formation in neonatal rats. *J Lipid Res* 2006;**47**:1844-51
- Shenai JP, Chytil F. Vitamin A storage in lungs during perinatal development in the rat. *Biol Neonate* 1990;**57**:126-32
- Wu L, Ross AC. Acidic retinoids synergize with vitamin A to enhance retinol uptake and STRA6, LRATA, and CYP26B1 expression in neonatal lung. *J Lipid Res* 2010;**51**:378-87
- Huettinger M, Retzek H, Eder M, Goldenberg H. Characteristics of chylomicron remnant uptake into rat liver. *Clin Biochem* 1988;**21**:87-92
- Ross AC, Li N. Lung retinyl ester is low in young adult rats fed a vitamin A-deficient diet after weaning, despite neonatal vitamin A supplementation and maintenance of normal plasma retinol. *J Nutr* 2007;**137**:2213-8
- Chapter 11: Low birth weight. In: *Care of the newborn in developing countries*. Oxford University Press, pp.137-53. http://www.oxfordjournals.org/our_journals/tropej/online/care.html (accessed 20 March 2014)
- Ghembriskal K, Burns L, Burden TJ, Harbige L, Costeloe K, Powell JJ, Crawford M. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. *Early Hum Dev* 1994;**39**:177-88
- Henriksen C, Helland IB, Rønnestad A, Grønn M, Iversen PO, Drevon CA. Fat-soluble vitamins in breast-fed preterm and term infants. *Eur J Clin Nutr* 2006;**60**:756-62

33. Christian P, Lee SE, Donahue Angel M, Adair LS, Arifeen SE, Ashorn P, Barros FC, Fall CHD, Fawzi WW, Hao W, Hu G, Humphrey JH, Huybregts L, Joglekar CV, Kariuki SK, Kolsteren P, Krishnaveni GV, Liu E, Martorell R, Osrin D, Persson LA, Ramakrishnan U, Richter L, Roberfroid D, Sania A, Ter Kuile FO, Tielsch J, Victora CG, Yajnik CS, Yan H, Zeng L, Black RE. Risk of childhood undernutrition related to small-for-gestational age and preterm birth in low-and middle-income countries. *Int J Epidemiol* 2013;**42**:1340–55
34. McClure RJ, Newell SJ. Randomised controlled study of clinical outcome following trophic feeding. *Arch Dis Child Fetal Neonatal Ed* 2000;**82**:F29–F33
35. See AW, Kaiser ME, White JC, Claggett-Dame M. A nutritional model of late embryonic vitamin A deficiency produces defects in organogenesis at a high penetrance and reveals new roles for the vitamin in skeletal development. *Dev Biol* 2007;**316**:171–90
36. Asahina K, Tsai SY, Li P, Ishii M, Maxson RE Jr, Sucov HM, Tsukamoto H. Mesenchymal origin of hepatic stellate cells, submesothelial cells, and perivascular mesenchymal cells during mouse liver development. *Hepatology* 2009;**49**:998–1011
37. Ben X-M. Nutritional management of newborn infants: Practical guidelines. *World J Gastroenterol* 2008;**14**:6133–9
38. Lewis JM, Bodansky O, Cohlman SQ. Comparative absorption, excretion, and storage of oily and aqueous preparations of vitamin A. *J Pediatr* 1947;**31**:496–508
39. Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN. Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *Am J Clin Nutr* 1996;**63**:36–41
40. Silva KD, Wright JW, Williams CM, Lovegrove JA. Meal ingestion provokes entry of lipoproteins containing fat from the previous meal: possible metabolic implications. *Eur J Nutr* 2005;**44**:377–83

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