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High-Provitamin A Carotenoid (Orange) Maize Increases Hepatic Vitamin A Reserves of Offspring in a Vitamin A-Depleted Sow-Piglet Model during Lactation[1–4]

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Abstract

The relationship of dietary vitamin A transfer from mother to fetus is not well understood. The difference in swine offspring liver reserves was investigated between single-dose vitamin A provided to the mother post-conception compared with continuous provitamin A carotenoid dietary intake from biofortified (enhanced provitamin A) orange maize (OM) fed during gestation and lactation. Vitamin A-depleted sows were fed OM (n = 5) or white maize (WM) + 1.05 mmol retinyl palmitate administered at the beginning of gestation (n = 6). Piglets (n = 102) were killed at 0, 10, 20, and 28 d after birth. Piglets from sows fed OM had higher liver retinol reserves (P < 0.0001) and a combined mean concentration from d 10 to 28 of 0.11 ± 0.030 μmol/g. Piglets from sows fed WM had higher serum retinol concentrations (0.56 ± 0.25 μmol/L; P = 0.0098) despite lower liver retinol concentrations of 0.068 ± 0.026 μmol/g from d 10 to 28. Milk was collected at 0, 5, 10, 20, and 28 d. Sows fed OM had a higher milk retinol concentration (1.36 ± 1.30 μmol/L; P = 0.038), than those fed WM (0.93 ±1.03 μmol/L). Sow livers were collected at the end of the study (n = 3/group) and had identical retinol concentrations (0.22 ± 0.05 μmol/g). Consumption of daily provitamin A carotenoids by sows during gestation and lactation increased liver retinol status in weanling piglets, illustrating the potential for provitamin A carotenoid consumption from biofortified staple foods to improve vitamin A reserves. Biofortified OM could have a measurable impact on vitamin A status in deficient populations if widely adopted. J. Nutr. 143: 1141–1146, 2013.

Introduction

Vitamin A deficiency (VAD)[8] affects over 250 million people and contributes to morbidity and mortality in many developing nations (1). Pregnant and lactating women are especially at risk, because retinol requirements increase during this time (2). Chronic VAD during pregnancy results in low newborn vitamin A reserves. The most common method to alleviate VAD in countries with high risk is supplementation programs for lactating mothers and children up to 5 y of age. A prior recommendation for lactating mothers in high-risk VAD communities was 2 doses of 200,000 IU vitamin A within 6 wk of delivery, with at least 1 d between doses (3). However, because evidence is lacking for the impact of this intervention on childhood mortality, the WHO does not currently recommend this regimen as public health policy (4). Therefore, other sustainable methods are needed to improve population vitamin A status.

In a meta-analysis of 16 supplementation trials in children, vitamin A supplementation was associated with a 24% decrease in all-cause mortality and decreased prevalence of diarrhea, measles, night blindness, and xerophthalmia (5). Regarding supplementation to postpartum mothers, a study in Ghana, India, and Peru found greater breast milk retinol concentrations through 2 mo, but not 6 mo, indicating nonsustained improvement (6). Furthermore, supplementation programs, although common, can be expensive and require continuous external resources for their continuity (7).

Cereal grains with little vitamin A content are often staple foods for populations at greatest risk of VAD. Biofortification of staple crops has emerged as a potential long-term, sustainable approach to increase provitamin A content in high-staple food

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[3] Supplemental Table 1 is available from the "Online Supporting Material" link in the online posting and from the same link in the online table of contents at http://jn.nutrition.org.
[4] Presented at Experimental Biology 2012.
[8] Abbreviations used: OM, orange maize; RAE, retinol activity equivalent; RBP, retinol binding protein; UW, University of Wisconsin; VAD, vitamin A deficiency; WM, white maize.
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diets and a complement or alternative to supplementation in efforts to alleviate VAD (8). Maize (*Zea mays*) is a biofortification target due to its high consumption, particularly in Africa (9,10). Most provitamin A-enhanced maize contains mainly β-carotene, but some varieties have increased β-cryptoxanthin content (11,12). Provitamin A carotenoids must be cleaved in the intestine by β-carotene monooxygenase, allowing for regulation of carotenoid bioconversion to retinol (13). Thus, biofortification poses no risk of toxicity, due to increasing provitamin A carotenoids instead of preformed retinyl esters used in fortification programs (8). Maize biofortified with provitamin A improved the retinol status of depleted gerbils (9) and efficient bioconversion factors were obtained in 2 small human trials (14,15). However, to date, no studies to our knowledge have observed the impact of feeding a biofortified food during gestation and lactation on offspring retinol status.

Swine (*Sus scrofas domesticas*) were chosen for this study, because they have been used in previous research to model the impact of vitamin A interventions on the lactating woman-nursing infant dyad (16–18). Swine have physiological and gastrointestinal similarities to humans (19–23) and allow for direct determination of liver vitamin A concentrations, which are the best or gold standard indicator of vitamin A status (24). Although swine have limitations based on their metabolism of provitamin A carotenoids compared with humans (25), the purpose of this study was to determine the mother-to-fetus transfer during gestation and mother-to-newborn transfer through milk of vitamin A using liver reserves of the offspring as the main outcome with 2 interventions. Weanling piglets are more similar in size to infants and young children than alternative models, e.g., rodents. The objective was to compare the effect of a maternal high-provitamin A maize diet to a high-dose retinyl ester supplement at the beginning of gestation on the vitamin A status of their offspring. We hypothesized that continuous intake of provitamin A during gestation and lactation would enhance liver stores in the offspring more than a one-time, high-dose maternal vitamin A supplement.

Materials and Methods

Sow diet and milk collection. Approval for the ethical treatment and animal use was obtained from the University of Wisconsin (UW)-Madison Animal Care and Use Committee. Sows (n = 12) of the same crossbreed (Large White and Landrace) were housed at the UW-Madison Swine Research and Teaching Center in Arlington, WI. Sows were randomly allocated (n = 6/group) to either high-provitamin A orange maize (OM) feed or white maize (WM) feed with a 1.05-mmol retinyl palmitate oral dose at the beginning of gestation, with continuation of OM or WM throughout gestation and lactation. The provitamin A concentration of OM was determined weekly. One sow did not become pregnant, leaving 5 in the OM group for the duration of the study. Sows were between 2 and 5 overall parities, each having 2 or 3 parities during which a vitamin A-free diet was fed. Sow milk was collected 5 min after administering 1 mL oxytocin into the neck of each sow at birth, 5, 10, 20, and 28 d during the lactation phase.

Maize diets and supplement. Two different varieties of maize were used during this study: white commercial maize (DeLong) and orange biofortified maize. The OM was grown in West Lafayette, IN (Rocheford, Purdue) and in Arlington, WI (Pixley, UW Agricultural Research Center). Kernels were ground into meal using an industrial-sized mill. Feed consisted of 88% maize and a vitamin A-free premix (**Supplemental Table 1**). A nutritional supplement was provided by WHO (retinyl palmitate; Strides Arcolab) to make the 1.05-mmol retinyl palmitate dose in 5 mL soybean oil, which was administered orally to the sows on WM at breeding. Both maize feeds were analyzed by published methods weekly to determine the carotenoid composition throughout the study (26). Sows were fed 2.5 kg/d during gestation and 5.0 kg/d during lactation.

Sample collection. Piglet male-female pairs (n = 102) from sows fed each diet were randomly selected to be killed at d 0 (n = 26), 10 (n = 28), 20 (n = 28), and 28 (n = 20) after birth. The birth weights for piglets from sows fed OM and WM feeds were 1.55 ± 0.26 and 1.49 ± 0.31 kg, respectively, which did not differ by sex or treatment. Blood and liver samples were collected from piglets at each time point. Sow livers were collected from randomly selected sows (n = 3/group) at d 28 post-farrowing.

Serum and liver analyses. Piglet serum (500 μL) was analyzed for retinol using a standardized method with minor modifications (9). Retinyl acetate was the internal standard, 500 μL cold ethanol with 1% butylated hydroxytoluene was added and the sample was extracted 3 times with 1 mL hexanes. The Waters HPLC has been described (9). Solvent A was 95:5 (v:v) acetonitrile:water and Solvent B was 85:10:5 (v:v:v) acetonitrile: methanol:dichloroethane. Solvent A (100%) started at 2.0 mL/min from 0 to 3 min, with a change to 50% A and 50% B from 3 to 5 min and held until 6 min before reequilibrating with 100% A from 6 to 10 min.

Livers were analyzed using previously published methods (16). Three sections of liver (~1.5 g total) were randomly taken, homogenized by mortar and pestle with 2–3 g anhydrous sodium sulfate, and repeatedly extracted with dichloromethane to 50 mL. Five mL was dried under nitrogen and reconstituted in 100 μL 75:25 (v:v) methanol:dichloroethane; 50 μL was injected onto the same HPLC (9). Two mobile phases were used with modification (18): solvent A was 92.5:7.5 acetonitrile:water (v:v) and solvent B was 85:10:5 acetonitrile:methanol:dichloroethane (v:v:v); both with 0.365 g triethylamine/L as modifier. Retinol and retinyl ester values were summed to obtain the total vitamin A concentration (μmol/g liver) or corrected for liver weight for total liver reserves (μmol/liver) (18). Sow livers were also separately analyzed for carotenoids using the same extraction procedure and the carotenoid HPLC analysis (26).

Sow milk analysis. Milk was analyzed for retinol concentration by using a modification to a previously described method (12). Synthesized C23-*apo*-carotenol was used as an internal standard. After saponification and extraction, the residue was reconstituted in 100 μL 50:50 (v:v) methanol:dichloroethane and 25 μL was injected onto a Resolve C_{18} 5-μm, 3.9- × 300-mm reversed-phase column (Waters) equipped with a guard column. Milk fat was assayed using a published gravimetric method (27). Milk (1 mL) was analyzed for carotenoid concentration by using modifications to a published method (26). Then β-*apo* carotenal as internal standard, 2 mL ethanol with 0.1% butylated hydroxytoluene, and 800 μL 50:50 (wt:v) potassium hydroxide:water were added, mixed, and saponified for 8 min at 45°C, mixing at 4 min. Following saponification, 1.5 mL cold water was added and the sample was extracted 3 times with 1.5 mL hexanes. Organic layers were pooled, dried under nitrogen, and resuspended in 100 μL 50:50 (v:v) methanol: dichloroethane; 80 μL was injected onto a Waters carotenoid 3-μm, 4.6- × 250-mm reversed-phase column (Milford) equipped with a guard column. The HPLC system was described (9).

Statistical analysis. Values are means ± SDs. A repeated-measures ANOVA with mixed effects was used with SAS PROC MIXED software (version 9.2, SAS Institute) for the sow milk. An AUC analysis was performed on sow milk using a 2-tailed t test. A likelihood ratio test was used to test for unequal variance. The influence of treatment, day, and sex were evaluated by using a 3-factor ANOVA model in the piglet data. Tukey's adjustments were used to make comparisons between groups for piglets and sows. Treatment effects and interaction terms were considered significant at $P \leq 0.05$. Slopes were determined for liver retinol accrual over time and considered significant if different from zero.

Results

Carotenoid content of feed and total retinol intake. The following carotenoids were quantified in the OM feed (μg/g):

TABLE 1 Serum retinol concentrations in piglets killed 0, 10, 20, and 28 d after birth from sows that were fed either OM or WM with a retinyl palmitate supplement at the beginning of gestation[1]

Time after birth	*n*	All	*n*	OM	WM + retinyl palmitate
		μmol/L		*μmol/L*	*μmol/L*
0 d	26	0.25 ± 0.08[c]	12–14	0.23 ± 0.07	0.26 ± 0.08
10 d	28	0.69 ± 0.19[a]	12–16	0.60 ± 0.16	0.76 ± 0.19
20 d	28	0.62 ± 0.15[a, b]	14	0.57 ± 0.13	0.66 ± 0.16
28 d	20	0.55 ± 0.17[b]	10	0.53 ± 0.16	0.56 ± 0.17

[1] Values are means ± SDs. A 3-way ANOVA showed a difference by time ($P < 0.0001$) and piglets by treatment group where serum retinol of WM piglets (0.57 ± 0.25 μmol/L, $n = 54$) was higher than OM (0.48 ± 0.20 μmol/L, $n = 48$) ($P = 0.0098$) and a trend existed for sex difference ($P < 0.053$), where females (0.56 ± 0.24 μmol/L) had a higher mean value than the males (0.50 ± 0.21 μmol/L). Interactions were not significant. Individual time points without a common superscript letter differ. OM, orange maize; WM, white maize.

lutein and zeaxanthin (11.7 ± 2.35); all-*trans*, 9-*cis*, and 13-*cis* β-carotene (10.6 ± 1.6); β-cryptoxanthin (0.34 ± 0.09); and α-carotene (0.58 ± 0.19). Only trace amounts of carotenoids were found in the WM feed. The weekly OM theoretical retinol concentration was 41.8 ± 2.3 and 41.9 ± 2.7 nmol/g feed (12 μg/g feed) for the OM from Indiana and Wisconsin, respectively. The overall WM feed theoretical retinol concentration was 0.35 ± 0.24 nmol/g (0.14 μg/g feed). Using the IOM bioconversion factor of 12 μg β-carotene equivalents to 1 μg retinol activity equivalents (RAEs) (2), the total RAE in the feed for sows fed OM was 2530 μg RAE/d during gestation and 5060 μg RAE/d during lactation, whereas sows fed WM received 28.6 and 57.2 μg RAE/d, respectively. The total RAE throughout gestation and lactation was 433 and 4.9 mg for OM and WM, respectively. Thus, in theory, OM provided more vitamin A to the sows than the retinyl palmitate supplement (i.e., 300 mg retinol equivalents) during the entire study duration.

Piglet weights. Piglet weights did not differ between treatment groups or sexes. Piglet weights at 0, 10, 20, and 28 d were (pooled means ± SDs) 1.52 ± 0.29, 3.58 ± 0.74, 5.40 ± 0.72, and 5.71 ± 1.37 kg, respectively.

Serum retinol. Serum retinol concentrations were higher in piglets from mothers fed WM than those fed OM ($P = 0.0098$) and differed by time ($P < 0.0001$). Across treatments, serum retinol increased from d 0 to 10 and then remained unchanged or decreased between d 10 and 28 for piglets in both treatment groups (**Table 1**). Piglets at d 0 had significantly lower serum retinol values than at later time points, regardless of diet. The WHO defines serum retinol concentrations <0.7 μmol/L to be indicative of VAD in humans (28). Using this indicator, only the mean value for the 10-d-old piglets from the WM-fed sows was an adequate serum retinol concentration, i.e., >0.7 μmol/L (Table 1).

Liver retinol and carotenoid reserves. Piglet liver weights were higher in piglets from the OM group ($P = 0.033$) and increased with time ($P < 0.0001$), but no interaction between treatment and time was detected. Piglet liver weights were 46 ± 12, 111 ± 15, 131 ± 15, and 148 ± 48 g for the OM group and 42 ± 12, 96 ± 20, 121 ± 16, and 139 ± 23 g for the WM group at 0, 10, 20, and 28 d, respectively. OM resulted in higher ($P < 0.0001$) hepatic retinol concentrations (μmol/g) in piglets than in those from sows fed WM (**Fig. 1**), which were calculated by summing retinol and all identifiable retinyl esters with photodiode array detection. Hepatic retinol concentrations differed with time ($P < 0.0001$) but not between sexes. The interaction between treatment and time was significant ($P = 0.0013$), whereas there was no interaction for treatment and sex or sex and time. Although piglets from sows fed WM had the same liver vitamin A concentration at 0 d as piglets from sows fed OM, the liver retinol concentrations of piglets from mothers fed OM were higher at d 10, 20, and 28 (Fig. 1*A*). In piglets from sows fed OM, the liver retinol concentration increased from 0 to 20 d, although d 10 through 28 did not differ. In both groups, the liver retinol concentration increased to above the current human deficiency cutoff of 0.07 μmol retinol/g liver on d 10 (24), emphasizing the importance of colostrum; however, this was not maintained in the piglets whose mothers were fed WM. Using

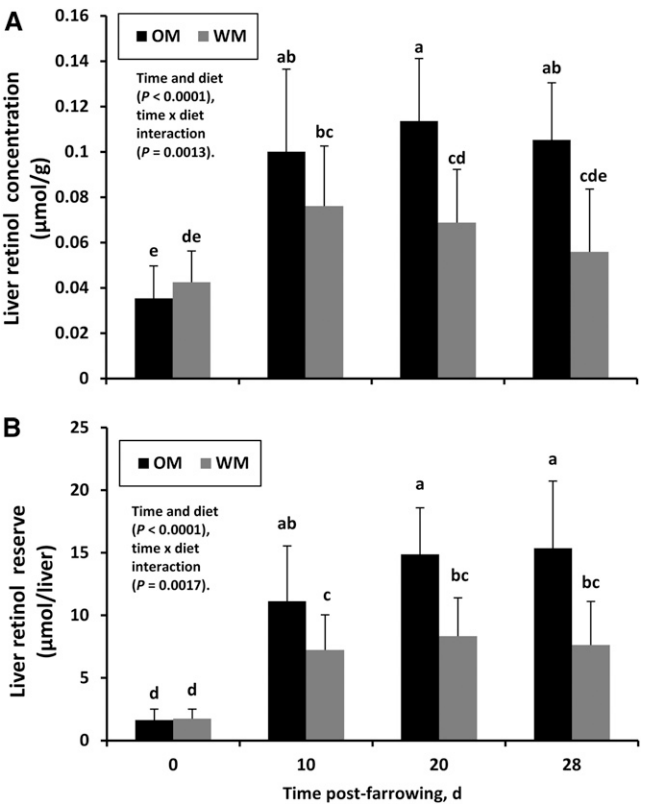

FIGURE 1 Liver retinol concentrations (*A*) and total liver retinol (*B*) in piglets from sows fed OM or WM + retinyl palmitate dose. Values are means ± SDs, $n = 10$–16. Means without a common letter differ, $P < 0.05$. A 3-way ANOVA showed that time and diet were significant ($P < 0.0001$) for both concentration and total retinol and an interaction existed between time and diet ($P \leq 0.0017$). Sex did not influence the results. OM, orange maize; WM, white maize.

the more conservative cutoff of 0.1 μmol/g (24), only the piglets whose mothers were fed OM reached and maintained that concentration.

We also calculated total liver reserves (μmol/liver) (Fig. 1*B*). Similar to liver retinol concentrations, there were main effects of treatment ($P < 0.0001$) and time ($P < 0.0001$) as well as an interaction between treatment and time ($P = 0.0017$), but sex had no effect. No interaction existed between treatment and sex or sex and time. Total liver reserves were significantly higher at 10, 20, and 28 d in piglets from sows fed OM than in piglets from sows fed WM. However, within the respective treatment groups, the later time points were only significantly higher than d 0 piglets and did not differ between 10, 20, and 28 d. Nonetheless, after evaluating the slope over this time period (Fig. 1*B*), the total hepatic vitamin A for piglets whose mothers were fed WM remained constant between d 10 and 28, whereas the OM piglet concentrations indicated continued accrual of total liver vitamin A reserves ($P = 0.007$).

Sow liver vitamin A concentrations were determined at kill (28 d after giving birth) and were 0.22 ± 0.05 μmol/g liver for sows fed OM and 0.22 ± 0.06 μmol/g liver for sows fed WM. This value is >100% higher than the conservative cutoff for adequate liver reserves, i.e., >0.1 μmol/g liver. Thus, OM during gestation and lactation performed as well as a single high-dose supplement in rescuing the mothers from their prior vitamin A-depleted status. Sow liver β-carotene concentrations (all isomers) were 0.25 ± 0.07 nmol/g liver in the OM group and undetectable in the WM group.

Sow milk. Sows had significantly higher colostrum retinol concentrations at birth than milk at any other time point, regardless of diet (**Fig. 2**). The milk retinol concentration for sows fed OM was higher than for those fed WM ($P = 0.038$). Time was a variable ($P < 0.0001$), but no time × diet interaction existed. Similar levels of significance were achieved when corrected for fat content (results not shown). AUC analysis on 4 sows/group with complete data revealed that sows fed OM had a higher milk retinol concentration throughout the lactation period studied [28.9 ± 5.7 (μmol × d)/L] than sows fed WM [17.0 ± 4.5 (μmol × d)/L ($P = 0.024$). Swine efficiently cleave β-carotene or do not absorb much intact (25,29) and we confirmed a lack of provitamin A carotenoid transfer to the milk by analyzing it for carotenoids, which were undetectable.

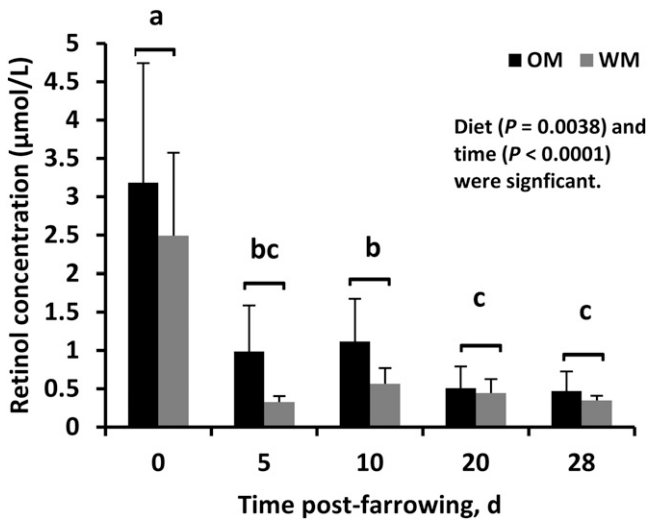

FIGURE 2 Milk retinol concentrations from sows fed OM or WM + retinyl palmitate (vitamin A) dose. Milk was collected at 0, 5, 10, 20, and 28 d post-farrowing. Values are means ± SDs, $n = 3$–5. A repeated-measures ANOVA showed that treatment ($P = 0.038$) and time were significant ($P < 0.0001$). There was no interaction between time and treatment. OM, orange maize; WM, white maize.

Discussion

This study used piglets born to vitamin A-depleted sows that had been "rescued" from VAD after being fed vitamin A-free diets for at least 2 prior parities. Although retinyl palmitate doses are usually given to postpartum women, the sows were given a high dose at the beginning of gestation to compare the maternal-fetal transfer of retinol from retinol binding protein (RBP) during gestation and lactation with that from continuous transfer as retinol and retinyl esters from small daily intakes of β-carotene from high-provitamin A OM. Prior studies have predicted and confirmed the influence of high-dose supplements to lactating sows on nursing piglet vitamin A status (16,18). Although swine do not absorb and store appreciable amounts of β-carotene intact (29), they are recommended as a model for lactation (25) and vitamin A studies for translational studies in humans (25,29). Sows continued being fed their respective diets throughout lactation, allowing for comparison between the 2 treatment groups on retinol transfer through maternal milk and nursing piglet and sow vitamin A status. Piglets weigh approximately the same as human infants at <6 mo of age (30,31), which made them a better model than rodents for this time-sensitive study.

The xanthophyll profile (i.e., lutein and zeaxanthin) of the OM used in this study and typical yellow maize used in swine feed are similar (13). Biofortified maize is bred to contain higher amounts of provitamin A carotenoids (i.e., β-carotene and β-cryptoxanthin) compared with conventional yellow maize varieties, which have 0.25–2.5 μg provitamin A/g (10). The OM provitamin A concentrations before and after mixing with the diet were 14 and 12 μg/g, respectively. As a total comparison of RAEs in the feed, sows fed the OM received ~40% more than the sows from the WM and retinyl ester dose. Considering the identical liver reserves in the sows at the end of the study, the extra vitamin A from the β-carotene cleaved in the intestine from OM was shunted to the milk and into the livers of the nursing piglets, demonstrating the importance of continuous dietary vitamin A or provitamin A consumption during lactation. Milk from sows fed the WM diet was not able to prevent VAD as measured by hepatic retinol concentrations, even though these sows had a better vitamin A status during gestation and lactation considering the timing of the supplement. The sows fed WM would have been in negative vitamin A balance during the study, whereas those fed OM were in positive vitamin A balance. One could assume that at any one time in the study, the sows fed WM had a better vitamin A status based on liver retinol reserves, because the groups had identical final retinol concentrations.

The serum retinol concentrations of piglets from mothers fed WM were higher than those in piglets from mothers fed OM, although total liver reserves after baseline were consistently lower for the WM than the OM group. Piglets from both diet treatments had an increase in serum retinol between d 0 and 10, but only the value at 10 d for piglets on the WM diet reached an "adequate" mean retinol concentration (>0.7 μmol/L). Determining vitamin A status by serum retinol concentration is common but not ideal, because it is homeostatically controlled and may not change in response to an intervention (24). The piglets from sows fed OM had adequate liver reserves (i.e., ≥0.1 μmol/g liver) but did not maintain adequate serum retinol

concentrations considering the widely used standard cutoff. This cutoff has utility as a population indicator but does not always reflect differences in liver retinol reserves (24), which is one reason why WHO recommends that 2 indicators be used to best define vitamin A status (28). Furthermore, the modified relative dose response test, which reliably indicates liver reserves <0.1 μmol/g liver (24), is in good agreement with serum retinol concentrations <0.5 and >1.6 μmol/L (32). Thus, serum retinol concentrations between 0.5 and 1.6 μmol/L are inconclusive. The higher serum retinol concentrations in the piglets from the WM fed sows may be due to a decrease in degradative utilization in an effort to maintain function (33), which could result in a higher concentration due to enhanced recycling (34). In a prior study, piglet serum retinol concentrations decreased with time after birth but did not differ between vitamin A treatments (18). In a recent study in Senegalese infants, serum retinol concentrations predicted only 15% VAD, whereas liver reserves measured by the modified relative dose response test indicated that 73.5% were VAD and identified those infants whose mothers had received postpartum supplementation (35). Liver reserves, which were measured in both the piglets and sows, are the gold standard for determining vitamin A status, because they reflect vitamin A storage that can be drawn upon during times of low intake. The piglets from mothers fed WM had critically low liver retinol reserves, even though their mothers had more than double the adequate liver concentration. This reinforces the importance of continued vitamin A dietary sources during lactation to support milk retinol concentrations (27,31,36,37).

The piglet liver results at d 0 indicated that fetal transfer of vitamin A during gestation was similar for treatments; however, the OM treatment was clearly more efficacious as a source of retinol during lactation. OM feeding during gestation led to a biologically important enhancement of colostrum values leading to a rapid increase in retinol stores of the nursing piglets, which was maintained and much higher than the liver stores of the piglets whose mothers were fed WM. The retinol concentration in the liver during this time was well above the deficiency cutoff of 0.07 μmol/g liver and met the 0.1-μmol/g cutoff for adequacy (24), whereas the mean liver concentration in piglets from sows fed the WM + retinyl ester dose was >0.07 μmol/g liver only at d 10. The smaller, consistent intake of provitamin A carotenoids provided additional vitamin A directly to the milk during lactation via retinyl esters in the chylomicra, whereas piglets from sows fed the WM diet were still relying on mobilization of stored liver reserves through plasma retinol delivered to the milk from RBP as their sole source of vitamin A.

The frequent intake of provitamin A carotenoids from biofortified maize may sustain adequate vitamin A status in deficient populations if widely adopted as their staple food. A study in India found a 54% reduction in childhood mortality in children who were given small weekly doses of preformed vitamin A, which represented achievable daily consumption amounts from foods (38). This is a much higher reduction in mortality than a meta-analysis performed on routine supplementation trials, i.e., 24% (5). This may be due to the fact that lung and spleen, two organs essential for immune function, take up vitamin A mainly from chylomicra, which has a shorter residence time in the serum than retinol bound to RBP (39). More frequent doses of vitamin A or daily provitamin A-containing food would consistently maintain vitamin A concentrations in these key organs through chylomicron delivery.

In this study, the bioconversion of provitamin A carotenoids to retinol was estimated using the Institute of Medicine bioconversion factors of 12 μg β-carotene and 24 μg β-cryptoxanthin to 1 μg retinol (2). Point values of bioconversion factors from single test meals made with biofortified maize were calculated as 6.5 ± 3.5 in young U.S. women (14) and 3.2 ± 1.5 μg β-carotene to 1 μg retinol in Zimbabwean men (15). Bioconversion factors are influenced by several factors (13) and vitamin A status plays a major role in how much retinol is made from provitamin A carotenoids (8). Larger, long-term feeding studies are needed in target populations to tease out the appropriate bioconversion factors to use for biofortified crops.

Although biofortified maize has many advantages relative to supplementation strategies, including potential agricultural and economic growth (40,41), questions still remain about how effective it will be in reducing VAD prevalence (42). One of the biggest challenges regarding the future of biofortified maize is getting the producers and consumers to accept and demand the biofortified crops so that consumption is sufficient for VAD populations to reach adequate vitamin A status (8,43). WM is generally preferred over yellow maize for food in most African countries (10). However, in a Zambian feeding study of preschool-age children using high-provitamin A maize, children adapted to consuming OM meals (i.e., porridge and *nshima*) within the first week of the study and intakes of OM were the same as WM throughout the study (44). Several studies have reported willingness to consume OM by African consumers traditionally accustomed to eating WM (45–48) and Low et al. (49) demonstrated the effectiveness of an appropriate nutrition education strategy in creating demand for orange sweet potato by consumers traditionally accustomed to eating white sweet potatoes. Using biofortification as a tool to combat VAD may require 2 or more generations to achieve its potential impact on improved population vitamin A status (8,13). Further, ongoing work is needed to extend these findings from the swine model to gain a better understanding of the efficacy and effectiveness of high-provitamin A carotenoid maize consumption to improve vitamin A status at the population level.

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