The Xanthophyll Composition of Biofortified Maize (*Zea mays* Sp.) Does Not Influence the Bioefficacy of Provitamin A Carotenoids in Mongolian Gerbils (*Meriones unguiculatus*)

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Maize has been targeted for biofortification with provitamin A carotenoids through traditional breeding. Two studies were conducted in gerbils to evaluate factors that may affect provitamin A activity. Maize diets had equal theoretical concentrations of vitamin A (VA) assuming 100% bioefficacy. Study 1 (*n* = 57) varied the ratio of β-cryptoxanthin and β-carotene but maintained the same theoretical VA. Study 2 (*n* = 67) varied lutein and zeaxanthin. Other treatments were oil, VA, or β-carotene doses. Serum and livers were analyzed for VA and carotenoids. In study 1, total liver VA did not differ among the maize groups. In study 2, total liver VA of the VA and maize groups were higher than controls (*P* < 0.05). Conversion factors were 2.1−3.3 μg β-carotene equivalents to 1 μg retinol. Twice the molar amount of β-cryptoxanthin was as efficacious as β-carotene and the proportion of β-cryptoxanthin or xanthophylls did not appreciably change the VA value of biofortified maize.

KEYWORDS: *β-Cryptoxanthin; bioefficacy; biofortification; carotenoids; maize; vitamin A; xanthophylls*

INTRODUCTION

Vitamin A (VA) deficiency is a major health concern worldwide, and is especially prevalent in impoverished regions of Africa and South-East Asia. Vitamin A is required for the proper functioning of the visual and immune systems, as well as cellular differentiation. Ideally there would be sufficient VA from the diet to maintain adequate VA status. However, in regions most susceptible to deficiency, supplementation programs have been implemented (*1*) because sources of VA are scarce or unaffordable for those with the greatest need. Additionally, staple foods (e.g., rice, wheat, and maize) generally lack provitamin A carotenoids. In order to find a more sustainable and cost-effective solution to this problem, efforts to biofortify staple foods with provitamin A carotenoids have increased (*2*).

The primary carotenoids found in maize are lutein, zeaxanthin, β-carotene, α-carotene, and β-cryptoxanthin (*3, 4*). The xanthophylls lutein and zeaxanthin are naturally abundant in maize, but these cannot be converted to VA. Provitamin A carotenoids in most typical yellow maize are low and range

from 0.07 to 1.46, from 0 to 0.70, and from 0.07 to 1.05 μg β-carotene, α-carotene, and β-cryptoxanthin/g, respectively (*4*). Maize biofortification efforts have surveyed diverse germplasm sources, performed selections, created new hybrids, and enhanced the provitamin A carotenoid concentrations to 15 μg/g (*2*). For comparison, typical orange carrots have ~130 μg/g fresh weight (*5*). This level of maize biofortification may be adequate to maintain or increase liver VA reserves in humans when maize is consumed as a staple food (*6, 7*).

β-Cryptoxanthin is a xanthophyll that has provitamin A activity and contributes to the VA value of biofortified maize (*6, 8*). β-Cryptoxanthin supplements were more effective than β-carotene at improving total liver VA in Mongolian gerbils when provided on an equivalent theoretical VA basis and conversion rates based on mass were similar, i.e., 2.74 μg β-cryptoxanthin and 2.52 μg β-carotene were equivalent to 1 μg retinol (*8*). In order to effectively guide maize biofortification efforts, it is necessary to determine the relative contribution of β-cryptoxanthin to the VA value of maize and determine the interaction with other carotenoids.

Although lutein and zeaxanthin do not provide VA, they may be involved in maintaining human health. They are preferentially transported into the eye and accumulate in the macula, retina, and the iris, where they are thought to protect against oxidative damage from short wavelength blue light (*9*). The protection from free radicals may reduce the risk for age-related macular degeneration (*10, 11*); however, the impact of dietary intakes remains inconclusive (*12*). Carotenoid interactions during

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Table 1. Composition of Experimental Diets[a] Fed to Mongolian Gerbils Differing by Percent Maize

	g/kg feed	
	45% maize	50% maize
maize	450	500
vitamin-free casein	160	155
L-cystine	3	3
sucrose	110	110
maltodextrin	117	78
cottonseed oil	50	49
cellulose	65	60
mineral mix[b]	35	35
CaCO$_3$	2	2
MgO	0.8	0.7
vitamin mix[c]	5	5
vitamin D	0.0044	0.0044
vitamin E	0.2	0.2
choline bitartrate	2.5	2.5

[a] Provided by Harlan Teklad, Madison, WI. [b] Mineral mix (AIN-93M-MX). [c] Vitamin mix provided the following (mg/kg feed): biotin, 0.4; calcium panthothenate, 66.1; folic acid, 2; inositol, 110.1; menadione, 49.6; niacin, 99.1; *p*-aminobenzoic acid, 110.1; pyridoxine–HCl, 22; riboflavin, 22; thiamin–HCl, 22; vitamin B-12 (0.1% in mannitol), 29.7; ascorbic acid (97.5%), 1016.6.

absorption, particularly between xanthophylls and carotenes, may alter bioavailability (*13–15*). The primary goal of biofortifying maize with carotenoids is to increase the VA value. It is therefore important to determine how xanthophylls, which also could change during biofortification, affect the VA value of maize.

Mongolian gerbils are an appropriate model for studying β-carotene (*16, 17*), α-carotene (*18*), and β-cryptoxanthin (*8*). Two studies were conducted to evaluate factors that may affect the bioefficacy of the provitamin A carotenoids in biofortified maize using a gerbil model. In study 1, the objective was to assess the relative contribution of β-cryptoxanthin to VA reserves by feeding gerbils maize diets with theoretically equal VA (assuming 100% bioefficacy), but varying the ratio of β-cryptoxanthin to β-carotene. In study 2, the impact of lutein and zeaxanthin on the VA value of maize was determined by varying their concentrations.

MATERIALS AND METHODS

Maize and Diets. Eight different genetic stocks of maize were used to prepare the powdered maize diets for two studies. Upon receipt, the maize kernels were stored at −20 °C (white maize) or −80 °C (all other maize varieties). Prior to feed preparation, maize kernels were ground to pass a 1-mm screen (particles <0.7 mm) using a C&N hammer mill no. 8 (Christy-Norris, LTD, Ipswich, U.K.). For studies 1 and 2, the gerbils were fed diets consisting of 45 or 50% maize, respectively. The remainder was purified VA-free basal diet mix (**Table 1**; Harlan Teklad, Madison, WI), designed to provide a balanced diet using maize as the primary carbohydrate source. White maize diets were used during depletion, as well as for control groups. No carotenoids were detected in the white maize diets (*6*). Treatment diets in study 1 were mixed to contain either low, medium, or high relative concentrations of β-cryptoxanthin, but equal concentrations of total VA assuming 100% bioefficacy of the provitamin A carotenoids. This was achieved by changing the ratio of β-cryptoxanthin to β-carotene assuming that β-cryptoxanthin supplies 1 molecule of retinol and β-carotene provides 2 molecules. The diets for study 2 were also equalized for theoretical VA, but contained low, medium, or high relative concentrations of xanthophylls (combined lutein and zeaxanthin). Feeds were reanalyzed midway through the treatment periods to reevaluate the VA-value because of potential degradation of the provitamin A carotenoids.

Carotenoid Composition of Maize and Feeds. The carotenoid composition of the maize and diets were determined using a published

saponification method followed by HPLC analysis (*4*). The HPLC solvent gradient was slightly modified. Solvent A was methanol: water (92:8, v/v) with 10 mmol/L ammonium acetate and Solvent B was 100% methyl-tertiary-butyl ether. Samples were analyzed at 1 mL/min starting with 70% Solvent A and transitioning to 40% within 30 min. The column was equilibrated with 70% Solvent A for 10 min prior to the next injection. α-Carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin were identified using HPLC-purified standards. α-Carotene was isolated from high-carotenoid carrots and purified as previously published (*18*). β-Carotene (GNC Inc., Pittsburgh, PA), β-cryptoxanthin (CaroteNature, GmbH Lupsingen, Switzerland), lutein (Kemin Industries, Des Moines, IA), and zeaxanthin (GNC Inc., Pittsburgh, PA) were purchased and purified on a Waters Resolve 5-μm C18 (*3*). Concentrations were determined spectrophotometrically using their respective $E^{1\%}_{1cm}$ [i.e., 2800 for α-carotene, 2592 for β-carotene, 2386 for β-cryptoxanthin, 2550 for lutein, and 2348 for zeaxanthin (*19*)]. Chromatograms were generated at 450 nm.

Animals. Male 40-day-old Mongolian gerbils ($n = 124$) from Charles River Laboratories (Kingston, NY) were used. Gerbils were individually housed in plastic cages under controlled temperature, humidity, and 12 h light cycle. Food and water were given ad libitum. The gerbils were weighed daily to monitor health for the first 2 weeks and then three times/week until the study ended. Gerbils were acclimated to oral dosing by administering 40 μL cottonseed oil every 2 d, using a 100 μL Gilson positive displacement pipet (Rainin Instruments, Woburn, MA). In study 2, two gerbils died; one did not adapt to the feed initially and the other during the treatment period. Gerbils were killed by exsanguination under isoflurane anesthesia. Livers and blood were collected to analyze carotenoids and VA. Blood samples were centrifuged (2200 × *g*) at 4 °C for 15 min in BD Vacutainer Gel and Clot Activator tubes (Becton Dickinson, Franklin Lakes, NJ) for serum isolation. Serum and livers were stored at −70 °C until analysis. All animal handling procedures were approved by University of Wisconsin–Madison's Research Animal Resource Center.

Experimental Design. Gerbils were fed a 45% (study 1) or 50% (study 2) VA-free white maize diet during the 4-week depletion phases. After the depletion phase of study 1, seven gerbils were killed for baseline measurements and the remaining gerbils were divided into weight-matched groups. Three groups ($n = 10$/group) received 45% maize diet with low, medium, or high β-cryptoxanthin levels (**Table 2**), and twice daily doses of cottonseed oil. The remaining two groups ($n = 10$/group) were fed 45% white maize diets, and received doses of cottonseed oil (negative control) or VA in cottonseed oil. Gerbils received the treatment diets for 4 weeks.

After the depletion phase of study 2, six gerbils were killed for baseline measurements. The remaining gerbils were divided into weight-matched groups. Three groups ($n = 10$/group) received 50% low, medium, or high xanthophyll maize diets (**Table 2**) and cottonseed oil doses twice daily. The remaining three groups ($n = 10$/group) were fed 50% white maize diets with oil doses of retinyl acetate (VA group), β-carotene, or cottonseed oil (negative control) for 4 weeks.

Preparation of Vitamin A and β-Carotene Supplements. Vitamin A and β-carotene doses were prepared as previously described (*6, 15, 18*). The oil delivered 0.463 nmol VA/μL in study 1 and 0.735 nmol VA or 0.353 nmol β-carotene/μL oil in study 2. Oil supplements for both studies were divided in half and administered 5−7 h apart to expand the absorption period. The quantity of VA and β-carotene administered was determined by averaging the food intake of a subset of gerbils daily, and calculating the theoretical VA intake from the maize assuming 100% bioefficacy of the provitamin A carotenoids, i.e., 1 mol β-carotene equivalents provides 2 mol VA.

Analysis of Serum and Liver. Serum and livers were analyzed as previously described (*6, 15*). Chromatograms were generated at 325 nm for retinol and retinyl esters and 450 nm for carotenoids. Retinyl butyrate was used as an internal standard to determine extraction efficiency and also as an external standard for quantification of retinol and retinyl esters. Liver retinol, expressed as both a concentration (μmol/g) and total liver content (μmol/liver), was the sum of retinol and all identifiable retinyl esters. HPLC purified β-carotene was used for

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Table 2. Concentrations of Carotenoids and Theoretical Vitamin A in the Maize Treatment Diets[a]

	nmol/g feed				
diet	xanthophylls	β-cryptoxanthin	α-carotene	β-carotene	theoretical vitamin A[b]
study 1					
low βCX[c]	6.67 ± 0.69	0.33 ± 0.06	0.19 ± 0.01	2.75 ± 0.28	6.02 ± 0.55
medium βCX	6.94 ± 0.84	1.07 ± 0.08	0.17 ± 0.01	2.35 ± 0.49	5.98 ± 1.10
high βCX	8.12 ± 1.01	2.14 ± 0.12	0.16 ± 0.016	1.84 ± 0.18	6.06 ± 0.48
study 2					
low Xan	3.97 ± 0.43	ND	0.45 ± 0.02	4.38 ± 0.22	9.20 ± 0.46
medium Xan	12.9 ± 1.24	1.26 ± 0.12	0.33 ± 0.03	3.89 ± 0.29	9.36 ± 0.70
high Xan	21.7 ± 2.38	2.27 ± 0.11	0.10 ± 0.08	3.42 ± 0.13	9.21 ± 0.36

[a] Values are means ± SD, $n = 8$ analyses. Reported concentrations of β-carotene are for the combined *all-trans*, 9-*cis*, and 13-*cis* isomers For the βCX diets, *all-trans-β*-carotene was 66%, 13-*cis* was 19%, and 9-*cis* was 15%. The xanthophyll diets consisted of 78% *all-trans-β*-carotene, 10% 13-*cis*, and 12% 9-*cis*. White maize control diets did not contain detectable concentrations of carotenoids. [b] Theoretical vitamin A was determined assuming 100% bioefficacy of the provitamin A carotenoids, i.e., 1 mol β-carotene provides 2 mol retinol and 1 mol β-cryptoxanthin and α-carotene provide 1 mol retinol. [c] βCX, β-cryptoxanthin; ND, not detected; Xan, Xanthophyll. Xanthophylls indicate combined concentrations of lutein and zeaxanthin. Low, medium, and high are the relative concentrations of the carotenoids in the maize.

quantification of liver β-carotene concentrations. β-Carotene values were also expressed in terms of concentration (nmol/g) and total liver content (nmol/liver).

Statistical Analysis and Calculations. Values are means ± SD. Data were analyzed using Statistical Analysis System software (SAS Institute Inc., version 9.1, Cary, NC; 2003). Outcomes of interest (i.e., gerbil weights, liver weights, serum retinol concentration, and liver retinol reserves) among groups were analyzed by using one-way ANOVA at $\alpha < 0.05$. Differences between treatment groups were determined using least significant differences at $\alpha < 0.05$. Dixon's outlier criterion was applied where appropriate. Conversion factors for β-carotene and the provitamin A carotenoids in the biofortified maize were calculated by comparing total liver retinol of the VA group with the β-carotene supplement and maize groups after subtracting the mean total liver retinol of the control group from each treatment group. Because maize contains β-cryptoxanthin, β-carotene, and α-carotene, conversion factors were expressed as β-carotene equivalents and account for the fact that only half of each β-cryptoxanthin and α-carotene molecule supply VA.

RESULTS

Carotenoid Concentration of Feed. Feeds used in study 1 theoretically contained ~6 nmol VA/g diet assuming 100% bioefficacy of the provitamin A carotenoids, and 0.30, 1.05, or 2.13 nmol β-cryptoxanthin/g diet. Maize diets used for study 2 contained ~9.2 nmol VA/g diet and 3.76, 12.9, or 21.7 nmol lutein and zeaxanthin (**Table 2**).

Gerbil Weights. The baseline groups from both studies had significantly lower mean body weights than the other groups ($P < 0.05$). The final gerbil weights (75.2 ± 6.7 g) did not differ among the treatment groups.

Serum and Liver Retinol and Carotenoid Concentrations. Serum retinol concentrations varied among the groups in study 1 ($P = 0.025$). The medium β-cryptoxanthin and baseline groups had higher serum retinol concentrations than the high β-cryptoxanthin and negative control groups (**Figure 1A**, $P < 0.05$). In study 2, serum retinol concentrations did not differ between groups (**Figure 2A**, $P = 0.61$). Carotenoids were not detected in the serum.

One gerbil had hepatomegaly and was removed from the analysis using Dixon's outlier criterion. The main treatment effects for liver VA values were significant in both studies ($P < 0.0001$). In study 1, liver VA concentrations and total liver VA of the VA supplement group were significantly higher than all other groups (**Figure 1**, $P < 0.05$). The high β-cryptoxanthin group had significantly higher liver VA concentrations than the control group (**Figure 1B**, $P = 0.01$). Concentrations of VA did not differ among any other groups. However, total liver VA in the baseline, low β-cryptoxanthin diet, and high β-cryptox-

anthin diet groups were higher than the control (**Figure 1C**, $P < 0.05$). The medium β-cryptoxanthin diet did not differ from any group except the VA group. No carotenoids were detected in the livers from study 1.

The VA supplement group in study 2 had higher VA concentrations and total liver VA than any other group (**Figure 2**, $P < 0.05$). Hepatic VA concentrations only differed between the low xanthophyll diet and the control groups (**Figure 2B**, $P = 0.03$). However, all three xanthophyll groups had more total liver VA than the control group (**Figure 2C**, $P < 0.05$), and the high xanthophyll group had greater total hepatic VA than the baseline group ($P = 0.05$). β-Carotene was the only major dietary carotenoid that was quantifiable in the liver (3.5 ± 1.7 nmol/liver), and it did not differ between groups that consumed carotenoids ($P > 0.05$).

Conversion Factors. The conversion factors in study 1 were 2.5, 3.3, and 2.1 μg β-carotene equivalents to 1 μg retinol (1.3, 1.8, 1.1 mol β-carotene equivalents to 1 mol retinol) for the low, medium, and high β-cryptoxanthin groups, respectively. In study 2, after mitigating two high outliers from the control group, the conversion factors were 2.6, 3.2, and 2.4 μg β-carotene equivalents to 1 μg retinol (1.4, 1.7, 1.3 mol β-carotene equivalents to 1 mol retinol) for the low, medium, and high xanthophyll groups, respectively. The conversion factor was 4.6 μg β-carotene to 1 μg retinol (2.4 mol β-carotene to 1 mol retinol) for the β-carotene oil-based supplement.

DISCUSSION

Two studies were conducted to investigate factors that may affect the VA value of carotenoid biofortified maize. In study 1, Mongolian gerbils were fed diets containing theoretically equal concentrations of VA but different proportions of β-cryptoxanthin to β-carotene. The results indicate that β-cryptoxanthin is bioavailable from maize and contributes to liver VA reserves of VA-depleted Mongolian gerbils. In study 2, the large variation in xanthophyll concentrations in maize diet had negligible effect on the bioefficacy of the provitamin A carotenoids.

These results are important for maize breeders and geneticists who are developing allele specific molecular marker selection methods to breed for levels of specific carotenoids. These recent advances enhance the ability to select more specifically for increased levels of β-carotene and β-cryptoxanthin, as well as to select for higher total carotenoids (*20–22*). Both studies in this report support previous findings (*6, 8*) for the potential of carotenoid biofortified maize to decrease the prevalence of VA deficiency in countries where maize is eaten as a staple food (*7*). At this stage of biofortification efforts, maize breeders
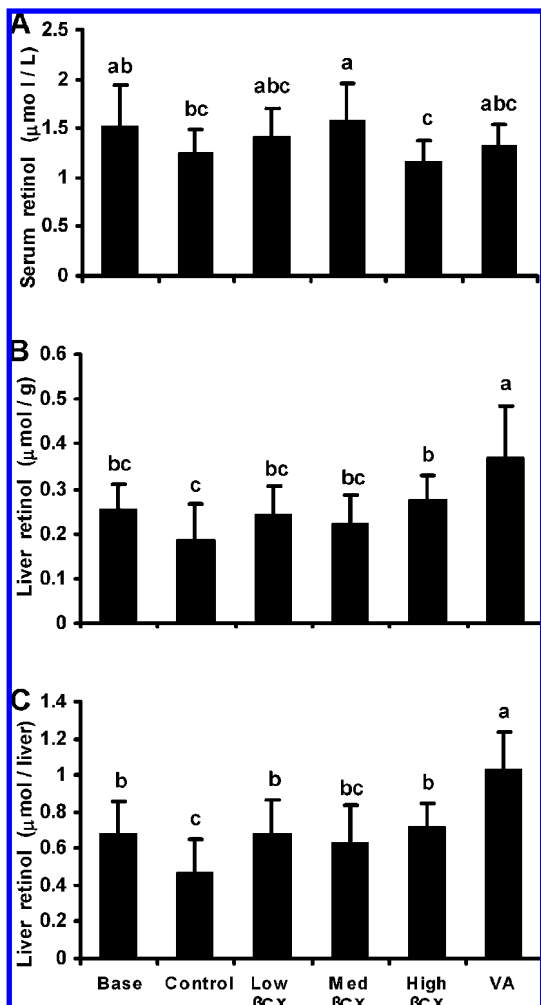
Figure 1. Study 1: Serum retinol concentrations (μmol/L) (**A**), liver vitamin A concentrations (μmol/g) in retinol (**B**), and total liver retinol reserves (μmol/liver) (**C**) in Mongolian gerbils. Measurements were taken at baseline (Base), or after 4-week treatment in which the gerbils were fed 45% low, medium (Med), or high β-cryptoxanthin (βCX) maize diets and dosed with cottonseed oil, or fed 45% carotenoid-free maize diets with oil doses of vitamin A (VA) or cottonseed oil (Control). VA in oil was equalized to the maize treatment diets based on intake of provitamin A carotenoids on the prior day assuming 100% bioefficacy. Treatment effects were observed for serum retinol ($P = 0.025$), liver VA concentrations ($P < 0.0001$), and total liver VA ($P < 0.0001$). Values are means $\pm$ SD; $n = 10$/group except for baseline ($n = 7$). Means with different letters are statistically different, $P < 0.05$.

should be encouraged to breed for higher carotenoids in general and not be overly concerned about the ratio of xanthophylls to carotenes. This is fortuitous as inexpensive visual selection for total carotenoids, deeper yellow and orange color intensities, can be performed alone or in conjunction with molecular marker-based selection.

Many factors affect the bioefficacy of provitamin A carotenoids (*23*). Interactions between carotenoids may occur at several points during the processing of provitamin A carotenoids and their conversion to VA. Study 1 was designed to focus on the implications of the species of provitamin A carotenoid in the maize. No differences were observed between the liver VA reserves of gerbils fed diets with varying proportions of VA from β-cryptoxanthin and β-carotene. This shows that twice the molar amount of β-cryptoxanthin is as efficacious as β-carotene from maize, because the proportions of β-carotene and β-cryp-
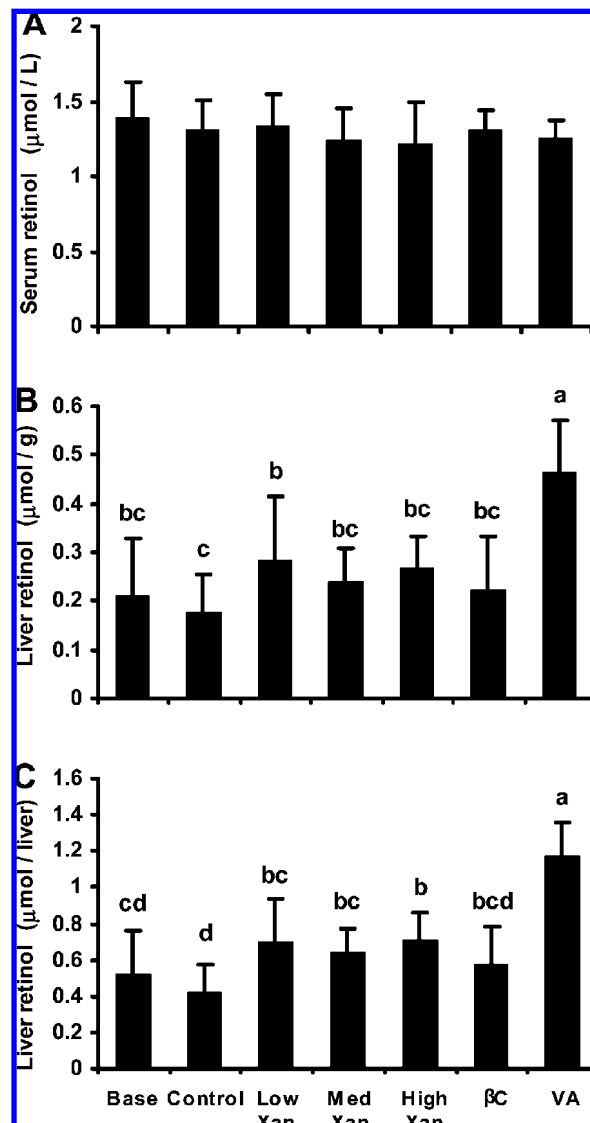

Figure 2. Study 2: Serum retinol concentrations (μmol/L) (**A**), liver vitamin A concentrations (μmol retinol/g) (**B**), and total liver retinol reserves (μmol/liver) (**C**) in Mongolian gerbils. Measurements were taken at baseline (Base) or after 4-week treatment in which the gerbils were fed 50% low, medium (Med), or high xanthophyll (Xan) maize diets and dosed with cottonseed oil, or fed 50% carotenoid-free maize diets with oil doses of β-carotene (βC), vitamin A (VA), or cottonseed oil (Control). β-Carotene and VA in oil were equalized to the treatment maize diets based on intake of provitamin A carotenoids on the prior day assuming 100% bioefficacy. Values are means $\pm$ SD; $n = 10$/group except for the low xanthophylls ($n = 8$), control ($n = 8$), and baseline ($n = 6$) groups. Treatment effects were observed for liver VA concentrations ($P < 0.0001$) and total liver VA ($P < 0.0001$), but not for serum ($P = 0.61$). Means with different letters are statistically different, $P < 0.05$.

toxanthin were the only changes in the VA component of the maize diets. Furthermore, the high β-cryptoxanthin group was the only treatment group to have both significantly higher liver VA concentrations and total liver VA than the control group. This would indicate that β-cryptoxanthin may be more efficacious than β-carotene, which is supported by a supplement study (*8*). Compared with neutral hydrocarbon carotenoids, polar carotenoids may be more easily incorporated into micelles allowing more efficient transfer into enterocytes from the lumen, leading to increased bioavailability (*24*). The xanthophylls and carotenes compete for incorporation into micelles in the small

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intestine (*25*). The gerbil is an appropriate model for this point of interaction between different species of carotenoids.

Another point of interaction is the enzymatic central cleavage by β-carotene 15,15′-oxygenase of provitamin A carotenoids to retinol, which occurs largely in the intestine through a monooxygenase mechanism (*26*). In humans, bioefficacy of β-carotene to retinol is dependent on dose size (*23, 25*) and vitamin A status (*7*). Therefore, β-carotene 15,15′-oxygenase activity may be more efficient for diets with lower concentrations of provitamin A carotenoids, as would be the case for the low β-cryptoxanthin group. Because this group had the lowest number of provitamin A molecules, the β-carotene that moved into the enterocytes was mostly converted to VA. This is supported by lack of quantifiable β-carotene in the gerbil livers from study 1. Incorporation of retinyl esters and provitamin A carotenoids into chylomicra (*27*) are potential sites of interaction as well. The gerbil has proven to be a good model for investigating the interaction and bioefficacy of the provitamin A carotenoids.

Carotenoid transporters on the apical surface of enterocytes (*28*) compete for carotenoids and therefore carotenoid species would interact at this point. We hypothesize that the gerbil does not have a transporter for the dihydroxycarotenoids, lutein and zeaxanthin. This is supported by two studies which found that lutein is not appreciably stored in Mongolian gerbils (*14, 29*), and is a recognized limitation of study 2. The provitamin A carotenoids are most likely facilitated by a different transporter than the dihydroxycarotenoids (*28*). We have proposed that presence of quantifiable β-carotene in the liver is a marker of adequate VA status (*6*). β-Cryptoxanthin was not quantifiable in the gerbil livers in these studies. Therefore, this would limit the applicability of this hypothesis to the carotenes. Most likely, gerbils have a binding protein in the liver (*30*) that allows uptake and storage of α- and β-carotene similar to that found in the ferret (*31*), but more specific to the hydrocarbon carotenoids. A study feeding VA-adequate gerbils higher doses of β-cryptoxanthin could confirm or refute this observation.

Conversion factors ranged from 2.1 to 3.3 μg and 2.4 to 3.2 μg β-carotene equivalents to 1 μg retinol in the maize treatment groups in studies 1 and 2, respectively. These conversion factors are similar to other published data in VA-depleted gerbils, i.e., 2.3−3.5 μg β-carotene to 1 μg retinol from supplements and food (*6, 15*). The β-carotene group had a similar but slightly higher conversion factor than the treatment diets; therefore, the maize matrix did not reduce the bioavailability of the provitamin A carotenoids. Additionally, large oil doses of β-carotene quickly transit through the intestine of gerbils with 45 ± 19% of the recovered dose reaching the cecum at 3 h escaping absorption (*14*). Carotenoids consumed in the maize may have been more efficacious because they were consumed throughout the day, in contrast to the large doses of β-carotene administered.

Serum retinol concentrations in healthy animals and humans are under homeostatic control until liver reserves fall below 0.07 μmol/g liver. Some differences in serum retinol concentrations were observed between groups in study 1, but all values were in the normal range, i.e., >0.7 μmol/L. The medium β-cryptoxanthin and the baseline groups were significantly higher than the high β-cryptoxanthin group. The medium β-cryptoxanthin group was also higher than the control. None of these treatment groups had liver VA near the deficiency cutoff, and their statistical relationships do not reflect the relative intakes of each group. While extremely low liver reserves will cause a decrease in serum retinol, factors that affect subtle changes or differences in serum retinol concentration during normal status are largely unknown. Serum retinol concentrations in study 2 were not different between any of the groups.

Both of these studies provide additional evidence that maize biofortified with provitamin A carotenoids maintains VA status in depleted Mongolian gerbils (*6, 8*). The factors evaluated are important for maize biofortification efforts. Study 1 demonstrated that maize with differing proportions of VA from β-cryptoxanthin and β-carotene contributes equally to liver VA stores of depleted gerbils, which would suggest that β-cryptoxanthin is as efficacious as β-carotene at providing VA on a theoretical basis. Additionally, there was some evidence that maize with high proportions of theoretical VA from β-cryptoxanthin could potentially increase VA stores more effectively. In study 2, it was found that varying concentrations of xanthophylls in maize diets did not reduce the bioefficacy of the provitamin A carotenoids over a 4-week treatment period. Maize breeders are encouraged to develop varieties and hybrids of maize containing substantial quantities of β-cryptoxanthin. Although dihydroxycarotenoids, particularly lutein, may alter the bioavailability of β-carotene (*13*), varying concentrations of lutein and zeaxanthin in the maize diets did not have a significant effect on the bioefficacy of the provitamin A carotenoids when consumed as a staple food by gerbils. Additional studies should investigate the relationship between xanthophylls and the bioefficacy of provitamin A carotenoids in humans, and the bioaccessibility of β-cryptoxanthin from additional food matrices.

SAFETY

Use of a fume hood for volatile organic solvents is recommended.

ABBREVIATIONS USED

βC, β-carotene; βCX, β-cryptoxanthin; Med, medium; VA, vitamin A; Xan, xanthophylls.

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