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BRIEF COMMUNICATION

Impact of biofortified maize consumption on serum carotenoid concentrations in Zambian children

Amanda C. Palmer[1] · Neal E. Craft[2] · Kerry J. Schulze[1] · Maxwell Barffour[1] · Justin Chileshe[3] · Ward Siamusantu[4] · Keith P. West Jr[1]

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Abstract

Biofortified maize, designed as an intervention strategy to prevent vitamin A deficiency, can provide upwards of 15 µg β-carotene per g dry weight. Some varieties also have elevated concentrations of other carotenoids. We conducted a cluster randomized, controlled feeding trial in rural Zambia to test the impact of daily consumption of biofortified maize over a 6-month period on vitamin A status. Serum concentrations of retinol and carotenoids were assessed by high-performance liquid chromatography. Data on circulating carotenoids by intervention group in 679 children are reported here. As previously shown, consumption of this β-carotene-rich maize significantly improved serum β-carotene concentrations (0.273 vs. 0.147 µmol/L, $p < 0.001$, in this subset of children). Here we show significant increases in α-carotene, β-cryptoxanthin, and zeaxanthin ($p < 0.001$). There was no impact on lutein or lycopene concentrations. Consumption of biofortified maize can have broader implications beyond the control of vitamin A deficiency (Trial registration: NCT01695148).

Introduction

Increasing dietary intakes of vitamin A (VA), either as retinyl esters or proVA carotenoids, is essential for improving status and preventing VA deficiency. One dietary strategy focuses on biofortification of staple crops by breeding or genetically engineering varieties with a higher carotenoid content. Conventionally bred "orange" maize was designed to provide 50% of the Estimated Average Requirement for reproductive-aged women, with a breeding target of 15 µg β-carotene per g dry weight [1]. Studies have shown that orange maize consumption can improve VA stores and increase breast milk retinol [2]. In a population of children in rural Zambia, we have reported significant improvements in serum β-carotene concentration with a 6-month biofortified maize intervention, although no impact was seen on serum retinol in this marginally deficient population [3]. With its enhanced carotenoid profile, biofortified maize consumption may have nutritional and health benefits beyond effects on VA status alone. We report here on the response of other serum carotenoids to the biofortified maize intervention.

Materials and methods

Detailed methods for this trial (NCT01695148) have been published previously [3]. Briefly, we identified children not yet enrolled in school who were living in population-dense areas of Mkushi District in Zambia's Central Province. Clusters of ~15–25 children were randomly assigned to orange or conventional white maize intervention groups, or a non-intervened group. Consent was obtained from parents. The protocol was approved by the Ethics Review Committee of the Tropical Diseases Research Centre and the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health.

Orange maize was provided by HarvestPlus. After cooking, orange maize provided 10.8 µg β-carotene per g dry weight, as well as elevated concentrations of lutein, zeaxanthin, β-cryptoxanthin, and α-carotene (2.0, 2.5, 0.8, and 0.3 µg/g, respectively), based on high-performance

✉ AmandaC. Palmer
acpalmer@jhu.edu

[1] Center for Human Nutrition, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

[2] Craft Technologies Inc., Wilson, ND, USA

[3] Tropical Diseases Research Centre, Ndola, Zambia

[4] National Food and Nutrition Commission, Lusaka, Zambia

Table 1 Baseline characteristics of households and children enrolled in the provitamin A biofortified maize trial, by intervention group

	White (n = 321)	Orange (n = 358)	P value[a]
Household socio-economic status (%)			
Literate household head	82.8	84.4	0.59
Family asset ownership			
Land	45.0	42.5	0.52
Livestock	39.1	41.7	0.49
Bicycle	53.0	54.9	0.63
Cellular telephone	75.5	70.0	0.11
Low quality housing[b]	36.9	44.5	0.04
Safe drinking water	58.4	52.7	0.13
Electricity	5.0	6.0	0.58
Child characteristics			
Age (years)[c]	5.7 ± 1.2	5.7 ± 1.3	
Male sex (%)	48.3	48.6	0.93
Nutritional status			
Stunting (%)	21.9	30.1	0.02
Underweight (%)	10.0	15.2	0.04
Retinol (μmol/L)	0.99 ± 0.27	1.01 ± 0.27	0.30
< 0.70 μmol/L (%)	11.3	9.8	0.53
< 1.05 μmol/L (%)	60.9	60.6	0.93
Hemoglobin (g/dL)	11.6 ± 1.3	11.8 ± 1.2	0.03
Anemic (%)[d]	36.1	28.8	0.04
Recent morbidity (%)			
Malaria parasitemia	19.1	19.2	0.98
Fever in past 2 weeks	29.2	27.5	0.62
Cough in past 2 weeks	59.1	53.1	0.12

[a]Based on chi-squared test or t test

[b]Mud floor and walls, improvised or thatched roof, no windows, or open to outside

[c]Mean ± standard deviation, all such values

[d]Using age-specific cut-offs: < 11.0 g/dL for children < 5 years; < 11.5 g/dL for children > 5 years

liquid chromatography (HPLC) analysis. Conventional maize contained ≤ 0.1 μg/g of these carotenoids. We delivered 200 g dry weight per day as boiled *nshima*, twice daily, with low-VA relish, 6 days per week for 6 months. Leftovers were weighed and recorded. As reported previously [3], median orange maize consumption was 141.6 g per day (interquartile range: 105.9–168.9).

Data on socioeconomic status were collected at baseline. At baseline and endline, we measured height (Shorrboard portable stadiometer) and weight (Seca 874 digital scale), collected 7.0 mL of blood by antecubital venipuncture in dim light, and tested hemoglobin (HemoCue Hb 201$^+$; HemoCue AB). Samples were maintained in coolers to clot before they were centrifuged at 3000 rpm for 10 min and serum aliquoted for storage in liquid nitrogen. Samples were stored at −70 °C at the Tropical Diseases Research Centre in Ndola prior to shipment on dry ice to Craft Technologies Inc. (North Carolina, USA) for analysis of retinol and carotenoids by HPLC [3].

We used Stata version 13 (StataCorp LP) and considered $p < 0.05$ as statistically significant. Analysis was restricted to children in the trial's two intervention arms with serum carotenoids measured at both timepoints. Non-normal distributions were natural log-transformed. Between-group differences at baseline were tested by chi-squared or t test. We used multiple linear regression with generalized estimating equations, accounting for the cluster randomized design, to test the intervention's effect on serum carotenoids. We controlled for baseline status and between-group differences that may have occurred by chance ($p < 0.1$). All analysis was based on intent to treat.

Results

Data were available from 679 children, who were comparable to those in the larger trial. There were minor differences between intervention groups at baseline (Table 1): those receiving orange maize had poorer housing quality and a higher prevalence of stunting and underweight; anemia was more prevalent in the white maize group. There were no differences in serum carotenoids at baseline (Table 2). After intervention, children receiving orange maize had significantly higher concentrations of serum β-carotene, α-carotene, β-cryptoxanthin, and zeaxanthin (all $p < 0.001$). There was no difference in lutein or lycopene. Adjustment for baseline differences in socio-economic or nutritional status had no influence on our results (data not shown).

Discussion

Maize varies widely in its carotenoid profile, with lutein as the predominant carotenoid in most brightly colored varieties [4]. Lutein—along with α-carotene—lies in the β,ε branch of the carotenoid biosynthetic pathway. Breeding programs for VA biofortification have used marker-assisted selection to identify varieties with low expression of lycopene epsilon cyclase (*lcyE*), alternatively directing carotenoid biosynthesis towards the β,β branch [5]. Breeders further screen for polymorphisms in *crtRB1*, which encodes β-carotene hydroxylase, favoring a higher β-carotene versus β-cryptoxanthin content [5]. This is evident in the serum response to orange maize consumption, where we show a marked increase in β-carotene [3].

Here we also show increases in serum α-carotene and β-cryptoxanthin. The impact on serum β-cryptoxanthin is consistent with the high bioavailability of this xanthophyll

Table 2 Serum carotenoid concentrations at baseline and endline among children enrolled in the provitamin A biofortified maize trial, by intervention group

	White ($n = 321$)	Orange ($n = 358$)	P value[a]
β-carotene (μmol/L)			
Baseline	0.168 (0.155, 0.183)[b]	0.166 (0.154, 0.180)	0.84
Endline	0.147 (0.135, 0.160)	0.273 (0.254, 0.292)	< 0.001
α-carotene (μmol/L)			
Baseline	0.009 (0.009, 0.010)	0.009 (0.009, 0.010)	0.60
Endline	0.011 (0.010, 0.011)	0.020 (0.018, 0.021)	< 0.001
β-cryptoxanthin (μmol/L)			
Baseline	0.013 (0.012, 0.014)	0.014 (0.013, 0.015)	0.72
Endline	0.013 (0.012, 0.013)	0.036 (0.034, 0.039)	< 0.001
Lycopene (μmol/L)			
Baseline	0.036 (0.032, 0.042)	0.035 (0.031, 0.040)	0.76
Endline	0.045 (0.039, 0.052)	0.039 (0.035, 0.045)	0.30
Lutein (μmol/L)			
Baseline	0.370 (0.344, 0.399)	0.373 (0.347, 0.400)	0.91
Endline	0.440 (0.407, 0.475)	0.445 (0.411, 0.482)	0.96
Zeaxanthin (μmol/L)			
Baseline	0.068 (0.063, 0.073)	0.069 (0.064, 0.073)	0.81
Endline	0.081 (0.075, 0.088)	0.127 (0.119, 0.135)	< 0.001

[a]Natural log-transformed concentrations were compared by linear regression with generalized estimating equations; final concentrations were adjusted for initial concentrations and differences in baseline characteristics that were significant at $p < 0.1$

[b]Values are geometric means (95% CI)

[6]. Recent data suggest that efficient absorption of β-cryptoxanthin may translate into a greater VA equivalency than previously assumed [6]. That we demonstrate changes in all three major proVA carotenoids with no impact on serum retinol is most likely due to marginally adequate VA status in this population [3]. Serum retinol responses to carotenoid interventions would likely be detectable only in more deficient settings. This orange maize variety was rich in zeaxanthin, significantly increasing serum concentrations. While lutein was also elevated in this orange variety, we suspect that high intake of other lutein-rich vegetables in this setting may have lessened our ability to detect any intervention effect on serum concentrations of this carotenoid. Indeed, serum lutein concentrations in both groups were ~2.5 times higher than seen in the United States [7]. The lack of impact on lycopene was unsurprising given its low content in maize.

Consistent with previous reports [8], we show that biofortified maize consumption can increase concentrations of carotenoids key for human health. In addition to their proVA activity, α-carotene, β-carotene, and β-cryptoxanthin are efficient antioxidants and higher dietary intakes are associated with protection from oxidative stress-related cancers and other chronic diseases [9]. Lutein and zeaxanthin—the constituents of macular pigment in the retina—may confer protection against age-related macular degeneration, cataracts, and retinitis pigmentosa [10]. As breeding programs and impact modeling continue, scientists need to factor in the other health benefits of orange maize consumption in sub-Saharan African settings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Pillay K, Siwela M, Derera J, Veldman FJ, Provitamin A carotenoids in biofortified maize and their retention during processing and preparation of South African maize foods. J Food Sci Technol. 2014;51:634–44. https://doi.org/10.1007/s13197-011-0559-x.
2. Haskell MJ, Tanumihardjo SA, Palmer AC, Melse-Boonstra A, Talsma EF, Burri BJ. Effect of regular consumption of provitamin A biofortified staple crops on vitamin A status in populations in low-income countries. Afr J Food Agr Nutr Dev. 2017;17:11865–78.
3. Palmer AC, Siamusantu W, Chileshe J, Schulze KJ, Barffour M, Craft NE, et al. Provitamin A-biofortified maize increases serum beta-carotene, but not retinol, in marginally nourished children: a cluster-randomized trial in rural Zambia. Am J Clin Nutr. 2016;104:181–90. https://doi.org/10.3945/ajcn.116.132571.
4. Harjes CE, Rocheford TR, Bai L, Brutnell TP, Kandianis CB, Sowinski SG, et al. Natural genetic variation in lycopene epsilon cyclase tapped for maize biofortification. Science. 2008;319:330–3. https://doi.org/10.1126/science.1150255.
5. Chandler K, Lipka AE, Owens BF, Li HH, Buckler ES, Rocheford T, et al. Genetic analysis of visually scored orange kernel color in maize. Crop Sci. 2013;53:189–200. https://doi.org/10.2135/cropsci2012.02.0129.
6. Burri BJ, Beta-cryptoxanthin as a source of vitamin A. J Sci Food Agric. 2015;95:1786–94. https://doi.org/10.1002/jsfa.6942.
7. Ford ES, Gillespie C, Ballew C, Sowell A, Mannino DM. Serum carotenoid concentrations in US children and adolescents. Am J Clin Nutr. 2002;76:818–27.
8. Sheftel J, Gannon BM, Davis CR, Tanumihardjo SA, Provitamin A-biofortified maize consumption increases serum xanthophylls and ^{13}C-natural abundance of retinol in Zambian children. Exp Biol Med (Maywood). 2017;242:1508–14. https://doi.org/10.1177/1535370217728500.
9. Miao B, Wang XD. Provitamin A carotenoids and cancer prevention. In: Tanumihardjo S, editor. Carotenoids and human health. New York, NY: Humana Press; 2012.
10. Lima VC, Rosen RB, Farah M, Macular pigment in retinal health and disease. Int J Retin Vitr. 2016;2:19. https://doi.org/10.1186/s40942-016-0044-9.