ajfand SCHOLARLY, PEER REVIEWED
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
Volume 17 No. 2 April 2017
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2017; **17(2):** 11865-11878 **DOI: 10.18697/ajfand.78.HarvestPlus02**

CHAPTER 2

EFFECT OF REGULAR CONSUMPTION OF PROVITAMIN A BIOFORTIFIED STAPLE CROPS ON VITAMIN A STATUS IN POPULATIONS IN LOW-INCOME COUNTRIES

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Volume 17 No. 2
April 2017
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ABSTRACT

Biofortification of staple crops with provitamin A (PVA) carotenoids is an innovative strategy for controlling vitamin A (VA) deficiency in low-income countries (LIC). Plant breeding programs have been successful in developing biofortified varieties of cassava, maize, and orange-fleshed sweet potatoes that contain amounts of PVA-carotenoids that have the potential to impact VA status in human populations. Nutrition studies indicate that beta-carotene in biofortified staple crops is converted efficiently to VA in the body. Randomized, controlled, community-based efficacy and effectiveness trials have been conducted to assess the effect of regular consumption of PVA-carotenoid biofortified staple crops on VA status. Results indicate that regular consumption of biofortified staple crops increases plasma beta-carotene concentrations consistently, but has a moderate effect, or no effect, on VA status, when assessed by serum retinol concentration, breast milk retinol concentration, or total body VA stores. Studies are currently underway to further investigate whether consumption of biofortified staple crops improves VA status in population subgroups at risk of VA deficiency, and to better understand how to optimize the biological impact of these interventions in resource-poor settings.

Key words: Beta-carotene, Biofortification, Bioavailability, Cassava, Maize, Provitamin A, Sweet potatoes, Vitamin A

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INTRODUCTION

Increasing the provitamin A (PVA)-carotenoid content of staple crops is an innovative strategy for controlling vitamin A (VA) deficiency among high-risk populations in the developing world. The primary focus of breeding programs to date has been to identify varieties that are rich in beta-carotene, which has roughly twice the VA activity of other PVA-carotenoids based on its chemical structure. Orange-flesh sweet potatoes (OSP) were the first of the biofortified crops to be released. Initial carotenoid-rich cultivars were released in 2002 and OSP are currently available through agricultural extension workers or community-based programs in 21 countries. Current varieties provide 30-100 µg beta-carotene/g OSP. Even a small piece of OSP — such as what a child might consume as a snack — can therefore contribute substantially to dietary VA intakes. Biofortified cassava and biofortified maize were released in 2011-12 and are currently available in five countries [1]. While breeding programs have met their targets of 15 µg beta-carotene equivalents/g crop, released varieties currently provide only ~5-10 µg/g. As breeders have sought to improve carotenoid profiles and agronomic properties of the PVA-carotenoid biofortified crops, nutrition research has proceeded to investigate the bioavailability and bioconversion of beta-carotene from these staple foods. Furthermore, human studies are accumulating evidence on the potential impact of regular biofortified crop consumption on VA status among groups at risk of deficiency.

VA Equivalency of PVA-carotenoids in Biofortified Crops

To assess whether PVA-biofortified crops are likely to have an impact on the VA status of human populations, it is important to know whether the beta-carotene in biofortified crops is bioavailable and converted to VA in the body. A VA equivalency factor of 12:1 (12 µg beta-carotene=1 µg retinol) has been established for beta-carotene from fruits and vegetables in mixed diets [2]. Several small studies have been carried out to assess the VA equivalency of beta-carotene in PVA-biofortified cassava and biofortified maize, by measuring beta-carotene and retinyl ester concentrations in the triglyceride-rich lipoprotein fraction of plasma (TRL-plasma) after consumption of biofortified foods. Because TRL-plasma is the fraction of the plasma that contains newly absorbed carotenoids and newly formed retinyl palmitate, analysis of postprandial TRL-plasma allows the effects of a single dose of beta-carotene to be assessed.

The bioavailability and VA equivalency of beta-carotene in biofortified cassava was assessed in two studies in US women (Table 1) [3, 4]. Women received single meals containing three different portions of cassava porridge, known as *gari*, which is an important fermented staple food in Sub-Saharan Africa. Because the bioavailability of PVA is influenced by fat content, the meals were prepared with varying amounts of fat to determine its influence on bioavailability in the different porridge preparations. The cassava meals were provided in random order, separated by 2 weeks. In the first study, women received: 1) biofortified cassava porridge with 20 g fat, 2) biofortified cassava porridge with 6 g fat, and 3) non-biofortified cassava porridge with a reference dose of preformed VA [3]. In the second study, women received: 1) biofortified cassava gari, 2) non-biofortified cassava gari blended with red palm oil (containing beta-carotene), and 3) non-biofortified cassava gari with a reference dose of preformed VA [4]. TRL-plasma concentrations of retinyl esters increased in response to consumption of both biofortified

cassava porridge and gari, and the VA equivalencies of beta-carotene in porridge and gari were estimated as ~4.4:1 and 4.2:1, respectively. Additional dietary fat did not affect estimates of VA equivalency, suggesting that 6 g fat/meal is sufficient for optimal absorption of beta-carotene [4].

The bioavailability and VA equivalency of beta-carotene in biofortified maize was assessed in US women and in Zimbabwean men (Table 1) [5, 6]. Women received single meals containing three different maize porridges, in random order, separated by two weeks: 1) biofortified maize porridge, 2) non-biofortified maize porridge with a reference dose of beta-carotene, and 3) non-biofortified maize porridge with a reference dose of preformed VA [5]. TRL-plasma concentrations of retinyl esters increased in response to consumption of biofortified maize porridge, and the VA equivalence of beta-carotene was estimated as 6.8:1. In Zimbabwe, men received a single meal of [^{2}H]-labeled biofortified maize with 20 g butter, followed by a reference dose of $^{13}C_{10}$-labeled retinyl acetate 8 days later [6]. Serum enrichments of [^{2}H]-retinol increased, and the VA equivalency of beta-carotene was estimated as 3.2:1. Collectively, these results indicate that beta-carotene from biofortified cassava and maize is converted efficiently to VA in the body, and suggest that regular consumption of these biofortified foods could have a positive impact on VA status in human populations.

In a community setting in Zambia, the VA equivalency of PVA-carotenoids from biofortified maize in a 90-day feeding trial was estimated as 10.4:1, by comparing the mean change in total body VA stores assessed by the retinol isotope dilution test [7] in children who consumed either biofortified maize with a placebo dose, or non-biofortified maize with a VA dose [8]. Stable isotope dilution techniques allow for an estimation of the body's exchangeable pool of VA to be calculated from the ratio of labeled to non-labeled retinol in plasma or serum following the administration of a stable isotope-labeled VA dose. Because stable isotope dilution enables an estimation of total body VA stores, it is considered to be a more sensitive method for determining VA status in groups of subjects [7].

Variability in VA equivalency among studies may be related to differences in diet- and host-related factors that can potentially affect bioavailability and/or bioconversion of PVA-carotenoids, such as dietary fat and fiber in mixed-meals; amount of beta-carotene in a meal; gastrointestinal illnesses; VA, iron, and/or zinc status; and/or genetic polymorphisms of the gene that encodes β-carotene dioxygenase, which can reduce bioconversion of beta-carotene to VA in the body [9].

Efficacy and Effectiveness of PVA-biofortified Crops for Improving VA Status

The efficacy of regular consumption of PVA-biofortified cassava and maize for increasing VA status has been assessed in community settings in LIC. Kenyan schoolchildren were randomly allocated to receive daily, 6 days/week, for 18.5 weeks either: 1) non-biofortified cassava with a placebo supplement, 2) biofortified cassava with a placebo supplement, or, 3) non-biofortified cassava with a beta-carotene supplement [10]. As a primary outcome, serum retinol concentrations were assessed, while hemoglobin and serum beta-carotene concentrations were assessed as secondary outcomes. Consumption of biofortified cassava resulted in a large increase in serum beta-

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carotene concentration, and a significant, modest gain in serum retinol concentration, but had no effect on hemoglobin concentration or the prevalence of low serum retinol concentrations (<0.70 µmol/L). These results indicate that while beta-carotene was well-absorbed, bioconversion of beta-carotene to retinol was less than optimal. In Nigeria, a randomized, community-based trial is currently underway to assess the efficacy of biofortified cassava for improving the VA status of preschool-age children. As a primary outcome, change in total body VA stores, which is considered to be a more sensitive indicator of VA status than serum retinol concentrations, will be assessed using retinol isotope dilution; results are anticipated in 2017 [11].

In 2010 [12] and 2012 [8], two randomized, controlled efficacy studies were performed in the Eastern Province of Zambia. The first study fed biofortified maize compared with non-biofortified maize to three to five year old children for 70 days, and evaluated changes in VA status with the modified relative dose response (MRDR) test [12]. The MRDR test requires one blood draw after a dose of 3,4-didehydroretinyl acetate (DRA) to human subjects. The MRDR test can be used as a qualitative indicator of liver stores of VA because the test operates on the fact that as VA liver reserves become low, retinol binding protein accumulates; therefore, after a single oral dose of DRA, the 3,4-didehydroretinol from hydrolysis of DRA binds to accumulated RBP and is rapidly released into the serum after which it can be measured by HPLC. On the background of the high-dose VA supplement program, both groups lost VA stores during the intervention. This study was followed by a placebo-controlled trial that included biofortified maize with much higher PVA carotenoid content, a longer feeding period (90 days), and total body VA stores assessed using a retinol isotope dilution technique as the primary VA status outcome in five to seven year old children who were no longer eligible for the high-dose VA supplementation program [8]. Non-parametric analysis showed that consumption of biofortified maize improved total body VA stores as effectively as a VA supplement, and VA stores were significantly greater in both the biofortified maize and VA groups than in the group that did not receive any VA during the intervention.

A series of trials were carried out in Zambia to assess the public health impact of biofortified maize consumption, incorporating both multiple target groups and indicators of VA status. The first of these, conducted from 2012-13, was a cluster-randomized controlled trial of preschool-aged children [13]. It was designed to test whether regular consumption of biofortified maize, compared to conventional white maize, could shift the population distribution of serum retinol as has been shown previously with industrial food fortification [14]. While this six-month intervention did significantly improve children's serum beta-carotene concentrations, it failed to show an effect on serum retinol. The lack of effect has been attributed primarily to the relative adequacy of VA status in children in this age group (4-8 y), potentially as a result of exposure to high-dose VA supplements every six months. A 2013 trial at this same research site considered the impact of a short-term feeding intervention with either 1) biofortified maize with a placebo capsule, 2) non-biofortified maize with a VA capsule, or 3) non-biofortified maize with a placebo capsule, targeting lactating mothers [15]. As infants are born with low stores of VA, adequate VA concentrations in breast milk are crucial for infant health

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and nutrition [16]. Breast milk retinol is therefore a highly relevant indicator for public health. This three week intervention increased plasma beta-carotene concentrations, but had no effect on plasma or milk retinol concentrations. However, there was a significant downward trend in the prevalence of low milk retinol concentrations in the VA and biofortified maize groups compared to the control group. The final trial in this series is a mother-baby feeding trial, where a cohort of infants is being followed from receipt of their six-month high-dose VA capsule through their first birthday. Starting at 9 months of age, infants and their mothers received twice-daily meals of either 1) non-biofortified maize, 2) biofortified maize, or 3) preformed VA fortified maize [17]. As a primary outcome, this trial will measure the change in infants' total body VA stores, which are dependent on VA intake both from breast milk and from complementary foods; results are anticipated in 2017.

The effectiveness of two large-scale OSP programs for improving VA status was assessed in Mozambique and Uganda [18, 19]. The programs promoted household production and consumption of OSP, and included nutrition education. In Mozambique, after two years of intervention, mean serum retinol concentrations measured in dried blood spots increased significantly, and the prevalence of low serum retinol concentrations (<0.70 µmol/L) decreased in children in OSP households compared to control households [18]. In Uganda, the intervention had no impact on serum retinol concentration, or the prevalence of low serum retinol concentrations, in children three to five years of age, after two years of intervention. However, when the analysis was restricted to children with complete data on important covariates, the prevalence of serum retinol concentrations <1.05 µmol/L decreased significantly in children in OSP households compared to children in control households. The intervention had no impact on maternal serum retinol concentration, or the prevalence of serum retinol concentrations <1.05 µmol/L, possibly because their VA status was adequate at baseline [19].

In an OSP feeding study in South Africa, school-age children were fed a morning OSP snack or an equal amount of white-fleshed sweet potato (WSP) before lunch in a controlled environment for 53 school days [20]. The OSP group had a significantly greater improvement in liver VA stores compared with the control group in response to the intervention, which was evaluated with the MRDR test.

The efficacy of consumption of biofortified OSP for improving VA status in women of reproductive age was assessed in a community-setting in Bangladesh [21]. Women with marginal VA status were randomly assigned to receive, six days/week for ten weeks, either 1) non-biofortified WSP with a placebo capsule, 2) boiled biofortified OSP with a placebo capsule, 3) fried biofortified OSP with a placebo capsule, or 4) boiled non-biofortified WSP with a VA capsule. Regular consumption of OSP increased plasma beta-carotene concentration significantly, and the increase tended to be greater with added dietary fat (fried OSP vs. boiled OSP). Despite the increase in plasma beta-carotene, the intervention had no effect on VA status, assessed by serum retinol concentration and total body VA stores, suggesting the conversion of beta-carotene to VA is limited in this population.

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A similar study assessed the efficacy of consumption of biofortified OSP for improving VA status in lactating women in Bangladesh [22]. Women were randomly assigned to receive six days/week for three weeks, either 1) non-biofortified WSP with a placebo capsule, 2) biofortified OSP with a placebo capsule, or 3) non-biofortified WSP with a VA capsule. Short-term consumption of biofortified OSP increased plasma beta-carotene concentration significantly, but had no impact on serum or milk retinol concentrations, as was observed for non-lactating women.

In summary, regular consumption of PVA-carotenoid biofortified crops has the potential to improve VA status in populations in LIC; however, the impact of interventions on VA status has been inconsistent. Although consumption of biofortified staple crops increased plasma beta-carotene concentration consistently, the interventions had a moderate effect, or no effect on serum retinol concentrations. Serum retinol is currently considered by the WHO to be appropriate for assessing population VA status and evaluating the effects of interventions when the population has a marginal or low VA status [23, 24]. However, serum retinol concentrations are homeostatically controlled, and therefore may not always change in response to an intervention. Lack of conversion of beta-carotene to VA may be related to VA status, and other host- or diet-related factors. Studies are underway to further investigate the potential for these interventions to improve VA status in populations at risk of VA deficiency, using multiple biochemical and functional indicators of VA status, and to better understand how to optimize the biological impact of interventions with PVA-carotenoid biofortified staple crops.

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Table 2.1: Estimates of VA Equivalency of beta-carotene from Biofortified Cassava and Biofortified Maize

Study population, n, reference	VA status at baseline	Biofortified crop, duration of supplementation	Method for estimating VA equivalency	VA equivalency ratio (µg beta-carotene:µg retinol)
US women, 21-44 y, n=10, [3]	NR, assumed adequate	100 g of cassava porridge (2 mg beta-carotene) as a single meal containing 20 g fat	Comparison of retinyl esters in TRL plasma in response to biofortified cassava vs reference dose of VA (0.3 mg)	4.2:1 (SD 3.1)
US women, 21-44 y, n=10, [3]	NR, assumed adequate	100 g of cassava porridge (2 mg beta-carotene) as a single meal containing 6 g fat	Comparison of retinyl esters in TRL plasma in response to biofortified cassava vs a reference dose of VA (0.3 mg)	4.5:1 (SD 3.1)
US women, 19-43 y, n=8, [4]	NR, assumed adequate	200-226 g of cassava gari (1 mg beta-carotene) as a single meal containing 9 g fat	Comparison of area under the curve for retinyl palmitate in TRL plasma in response to biofortified cassava vs a reference dose of VA (0.3 mg)	4.2:1 (SD 1.5)
US women, 18-30 y, n=6, [5]	NR, assumed adequate	250 g maize porridge (527 µg beta-carotene), as a single meal containing 8 g fat	Comparison of retinyl esters in TRL plasma in response to biofortified maize vs reference dose of VA (286 µg RAE)	6.5:1 (SD 3.5)
Zimbabwean men, 40-69 y, n=8, [6]	Adequate SR:2.07±0.60 µmol/L[1]	300 g maize porridge,intrinsically labeled with ^{2}H (1.2 mg [^{2}H]-beta-carotene) containing 20g fat	Comparison of retinol formed from [^{2}H]beta-carotene vs reference dose of [$^{13}C_{10}$]-retinyl acetate	3.2:1 (SD 1.5)
Zambian children, 5-7 y, n = 133, [8]	Adequate SR:0.98 ± 0.27 µmol/L[1]	200 g maize/d for 90 d (18 µg β-carotene equivalents/g maize)	Comparison of total body VA stores among children fed orange maize and placebo, white maize and a placebo, or white maize and a VA supplement (0.4 mg)	10.4:1

VA=vitamin A; NR=not reported; TRL=triglyceride rich lipoprotein; SD=standard deviation; SR=serum retinol
[1]mean ± SD

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Table 2.2: Effect of Consumption of PVA-biofortified Crops on VA status in Community-based Studies

Study population, age, n, reference	VA status at baseline	Biofortified crop, duration of supplementation	Method for estimating intervention effect	Intervention Effect
Kenyan children, 5-13 y of age, n=335, [10]	Marginal SR:0.82±0.17 µmol/L (mean±SD)	375 g/d cassava (1,460 µg beta-carotene/d), 6 d/wk for 18.5 wk	Change in serum retinol and serum beta-carotene concentrations in biofortified cassava group vs. non-biofortified cassava group	Significant intervention effect on serum retinol +0.04 µmol/L (95% CI: 0.00, 0.07 µmol/L) and on serum beta-carotene (524% increase (448, 608%)); No effect on prevalence of low serum retinol concentrations
Zambian children, 3–5 y of age, n=178, [12]	Adequate to Marginal, 11.8% with low liver VA stores[1]	~150 g maize as 2 meals/d, 6 d/wk for a total of 70 d	Change in MRDR response (liver VA stores[1]) in biofortified vs. non-biofortified maize groups	No effect on liver VA stores[2]; No effect on serum retinol concentration
Zambian children, 5-7 y of age, n=133, [8]	Adequate All with adequate total body VA stores[2]	200 g maize as 3 meals/d (18 µg β-carotene equivalents/g maize), 6 d/wk for 90 d	Change in total body VA stores[2] in biofortified maize vs. non-biofortified maize	Significant effect on total body VA stores; No effect on serum retinol concentration
Zambian children, 4-8 y of age; n=1024, [13]	Marginal to adequate SR:0.98 ± 0.27 µmol/L (mean±SD)	200 g maize as 2 meals/d, 6 d/wk for 6 mo; orange maize provided 3 mg beta-carotene/d	Endline serum beta-carotene and retinol concentrations and endline deficiency prevalence (serum retinol <0.70 µmol/L) in biofortified maize group vs. non-biofortified maize group	Significant effect on serum beta-carotene; No effect on serum retinol, or prevalence of low serum retinol concentrations
Zambian lactating women, 18-35 y of age, n=149, [15]	Marginal to adequate PR: 1.32 µmol/L	287 g maize as 2 meals/d, 6 d/wk for 3 wk; orange maize	Change in plasma and milk retinol concentrations in biofortified maize group vs. non-biofortified maize group	Significant effect on plasma beta-carotene; No effect on plasma or milk retinol; significant downward trend in prevalence of low milk retinol concentration in the

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	(geometric mean); 31.1% with PR <1.05 µmol/L	provided ~5.2 mg beta-carotene/d		biofortified maize and VA capsule groups compared to the non-biofortified maize group
Mozambican children, mean age: 17.4 months, n=733, [18]	Marginal SR: 0.57 µmol/L	2 y, large-scale intervention to promote production and consumption of OSP	Change in prevalence of low serum retinol concentration (<0.70 µmol/L) in children in OSP program area vs. in children in control area without OSP program	Significant reduction in prevalence of low serum retinol concentration
Ugandan children, 3-5 y of age, n=396, [19]	Marginal SR:0.92±0.03 µmol/L (mean±SD)	2 y, large-scale intervention to promote production and consumption of OSP	Change in prevalence of low serum retinol concentration (<1.05 µmol/L) in children in OSP program area vs. in children in control area without OSP program	Significant reduction (9.5 percentage points) in prevalence of low serum retinol concentration, in a subset of children with complete data on confounding factors
Ugandan women 34.1 ± 0.78 y of age, n=95, [19]	Adequate SR:1.75±0.05 µmol/L (mean±SD)	2 y, large-scale intervention to promote production and consumption of OSP	Change in prevalence of low serum retinol concentration (<1.05 µmol/L) in women in OSP program area vs. the control area	No effect on prevalence of low serum retinol concentration
South African children, 5-10 y of age, n=180, [20]	Marginal, 14-22% with low liver VA stores[1]	125 g OSP for 53 school days	Change in MRDR response (liver VA stores[1]) OSP vs. WSP group.	Significant effect on liver VA stores[2]

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Bangladeshi women, 27 ± 1.3 y of age, n=120, [21]	Marginal SR: >0.70 to ≤1.05 µmol/L, by design	~128 g OSP (~7200 µg/d beta-carotene), 6 d/wk for 10 wk	Change in plasma beta-carotene and retinol concentrations, and total body VA stores[1], in women in OSP groups (with and without added dietary fat) vs. WSP group	Significant effect on plasma beta-carotene concentration; No effect on plasma retinol concentration; No effect on total body VA stores
Bangladeshi lactating women, 18-37 y of age, n=135, [22]	Marginal SR:>0.70 to <1.05 µmol/L, by design	~ 200 g/d OSP (12 mg/d beta-carotene), 6 d/wk for 3 wk	Change in serum beta-carotene and retinol concentrations, and breast milk beta-carotene and retinol concentrations in OSP group vs WSP group	Significant effect on plasma beta-carotene concentration; No effect on serum retinol concentration; No effect on milk retinol concentration); No effect on milk beta-carotene

VA=vitamin A; SR=serum retinol; PR=plasma retinol; SD=standard deviation; OSP=orange-fleshed sweet potato; WSP=white-fleshed sweet potato; MRDR=modified relative dose response.

[1]Liver VA stores assessed by the modified relative dose response; [2]Total body VA stores assessed by retinol isotope dilution test.

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