Biofortified yellow cassava and vitamin A status of Kenyan children: a randomized controlled trial[1,2]

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ABSTRACT

Background: Whereas conventional white cassava roots are devoid of provitamin A, biofortified yellow varieties are naturally rich in β-carotene, the primary provitamin A carotenoid.

Objective: We assessed the effect of consuming yellow cassava on serum retinol concentration in Kenyan schoolchildren with marginal vitamin A status.

Design: We randomly allocated 342 children aged 5–13 y to receive daily, 6 d/wk, for 18.5 wk *1*) white cassava and placebo supplement (control group), *2*) provitamin A–rich cassava (mean content: 1460 μg β-carotene/d) and placebo supplement (yellow cassava group), and *3*) white cassava and β-carotene supplement (1053 μg/d; β-carotene supplement group). The primary outcome was serum retinol concentration; prespecified secondary outcomes were hemoglobin concentration and serum concentrations of β-carotene, retinol-binding protein, and prealbumin. Groups were compared by using ANCOVA, adjusting for inflammation, baseline serum concentrations of retinol and β-carotene, and stratified design.

Results: The baseline prevalence of serum retinol concentration <0.7 μmol/L and inflammation was 27% and 24%, respectively. For children in the control, yellow cassava, and β-carotene supplement groups, the mean daily intake of cassava was 378, 371, and 378 g, respectively, and the total daily supply of provitamin A and vitamin A from diet and supplements was equivalent to 22, 220, and 175 μg retinol, respectively. Both yellow cassava and β-carotene supplementation increased serum retinol concentration by 0.04 μmol/L (95% CI: 0.00, 0.07 μmol/L); correspondingly, serum β-carotene concentration increased by 524% (448%, 608%) and 166% (134%, 202%). We found no effect on hemoglobin concentration or serum concentrations of retinol-binding protein and prealbumin.

Conclusions: In our study population, consumption of yellow cassava led to modest gains in serum retinol concentration and a large increase in β-carotene concentration. It can be an efficacious, new approach to improve vitamin A status. This study was registered with clinicaltrials.gov as NCT01614483. *Am J Clin Nutr* 2016;103:258–67.

Keywords: biofortification, efficacy, food-based approach, nutrition-sensitive intervention, vitamin A

INTRODUCTION

Biofortified yellow cassava has great potential to alleviate vitamin A deficiency complementary to other interventions such as vitamin A supplementation and fortification (1). Vitamin A deficiency prevails in sub-Saharan Africa despite national supplementation and food fortification programs, and 30% of preschool children in developing countries have vitamin A deficiency (2). Vitamin A supplementation in preschool children reduces all-cause mortality by 24% (3). Considering the high prevalence of vitamin A deficiency, even small increases in the supply of vitamin A through biofortified crops are likely to result in major public health gains.

Cassava is an important staple food for many people in developing countries, particularly in sub-Saharan Africa, large parts of Latin America, and Asia. The crop is well suited to arid and semiarid areas and is grown even in remote areas by poor subsistence farming families with the highest burden of vitamin A deficiency. In many areas, children from such families are difficult to reach through supplementation or fortification (1). Because cassava is multiplied through vegetative propagation, farmers can grow improved varieties indefinitely with marginal

[1] Supported by the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement 211484, conducted within the framework of INSTAPA Project. HarvestPlus provided financial support for biochemical analyses and supplies. Capsugel (Bornem, Belgium), Laboratory&Allied (Nairobi, Kenya), DSM Nutritional Products/Sight and Life (Basel, Switzerland), and Laboratorium Medisan (Heerenveen, Netherlands) provided financial and technical support in producing supplements.

[2] Online Supplemental Material is available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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Received September 25, 2014. Accepted for publication November 6, 2015. First published online December 16, 2015; doi: 10.3945/ajcn.114.100164.

Supplemental Material can be found at: http://ajcn.nutrition.org/content/suppl/2015/12/16/ajcn.114.100164.DCSupplemental.html

inputs (4). Most cassava varieties grown in Africa have white roots with virtually no provitamin A. There are clones originating in the Amazon basin with yellow roots due to the natural presence of provitamin A. These yellow varieties have been crossbred with African cassava varieties, by using conventional techniques, to increase provitamin A content (5).

Yellow cassava contains provitamin A carotenoids primarily as β-carotene, which humans absorb and convert to retinol (vitamin A). Because of differences in the food matrix, this bioefficacy varies greatly between crops but is generally higher for roots and tubers than for dark-green leafy vegetables (6). In addition, conversion of β-carotene may depend on mutations in the gene encoding for β-carotene 15,15′-monooxygenase (*BCMO1*), the enzyme that converts β-carotene to retinol (7, 8). This conversion is thought to depend on vitamin A status and to be more efficient in deficiency (9, 10). Zinc deficiency can theoretically impair provitamin A bioefficacy because it is essential for transport and metabolism of vitamin A (11).

Serum retinol concentration indicates population vitamin A status and is a suitable marker to evaluate the impact of interventions (12). Its distribution shifts only transiently (<2 mo) by supplementation, but it is sustainably increased by regular intake of vitamin A (13).

We aimed to assess the effect of consuming biofortified yellow cassava on serum retinol concentration in Kenyan children with mild to moderate vitamin A deficiency. In preplanned subgroup analyses, we also explored effect modification by *BCMO1* genotype and for vitamin A and zinc status.

METHODS

Study design

The study was designed as a randomized controlled trial with 3 parallel arms with a daily intake, 6 d/wk, of *1*) white cassava and placebo supplement (control group), *2*) provitamin A–rich cassava and placebo supplement (yellow cassava group), or *3*) white cassava and β-carotene supplement (β-carotene supplement group). The latter group was included as a positive control.

The study was registered (clinicaltrials.gov: NCT01614483) and approved by ethical committees in Kenya and the Netherlands, with oversight by a data safety monitoring board. Written consent was obtained from parents and children.

Subjects and screening

The study was conducted from May until November 2012 in 3 primary schools in Kibwezi District, Kenya. All children aged 5–13 y with parental consent were invited for screening. Parents were asked to not give foods to their child on the morning of the screening visit. At this visit (**Figure 1**), we collected capillary blood samples in a tube containing EDTA. We assessed hemoglobin concentration and C-reactive protein concentration in whole blood by point-of-care tests and *Plasmodium* infection in whole blood by using dipstick tests. We stored plasma samples in liquid nitrogen for subsequent measurement of plasma retinol-binding protein concentration as a proxy for vitamin A status. This measurement was done in the field, within 10 d after blood collection. Children were excluded from further study when having a 14-d parent-reported history of infectious or systemic disease, *Plasmodium* infection, hemoglobin concentration <70 g/L, or inflammation (C-reactive protein concentration >8 mg/L, the minimum detection level for the point-of-care test). Of those remaining, we selected 360 children with the lowest retinol-binding protein concentrations for further study, considering that intervention efficacy was likely to be inversely associated with vitamin A status.

Run-in period

At the start of a 2-wk run-in period, these 360 children received praziquantel (40 mg/kg body weight) and albendazole (100 mg, single dose) against helminth infections. During the run-in period, they were daily offered servings of boiled white cassava and capsules with placebo supplements. At the end of the run-in period (baseline), children were excluded when having missed >20% of the feeding sessions or when having been unable to consume ≥80% of their target portion of cassava (325 g and 375 g for children 5–8 y and 9–13 y, respectively).

At the end of the run-in period, children were invited for a second visit (baseline). Parents were asked to not give foods to their child on the morning of this visit. Weight and height were measured according to WHO guidelines (14) to the nearest 0.1 kg and 0.1 cm by using a mechanical floor scale and a portable stadiometer (Seca). We collected venous blood samples in a tube without anticoagulant and suitable for trace element analysis (Becton Dickinson) and in a tube with K_2EDTA (Becton Dickinson). We assessed hemoglobin concentration and C-reactive protein concentration in whole blood by point-of-care tests and excluded those with hemoglobin concentration <70 g/L or inflammation (C-reactive protein concentration >8 mg/L).

Randomization and blinding

Children were allocated by stratified block randomization, after baseline data collection. Randomization was done by one of the authors (HV) not involved in the field work, based on a list with child names and corresponding plasma retinol-binding protein concentrations measured before the run-in period. Tables with random numbers were used to generate the allocation sequence consisting of random permuted blocks with size 6 or 9 within each of 3 strata, corresponding to tertiles of retinol-binding protein concentration. The final allocation list contained each child's name and a group code letter A–C. Both the field team and participants were unblinded to the type of cassava (white or yellow) but were blinded to supplementation with β-carotene or placebo supplements, which were formulated as opaque capsules.

Interventions and follow-up

White cassava and 7 different varieties of yellow cassava were grown at a location close to the study site, with staggered planting to allow roots to be harvested at 7–10 mo after planting. Yellow cassava varieties had been screened and preselected based on low cyanide concentration and suitability for freshly boiled consumption and contained 4.7–6.9 μg/g total β-carotene (fresh weight). Cyanide content was rechecked for each variety before consumption as described elsewhere (15). The roots were harvested daily in the afternoon and prepared the subsequent morning at each school; roots were peeled, chopped, rinsed, and boiled for 1 h or until done. Cassava was drained, mashed with

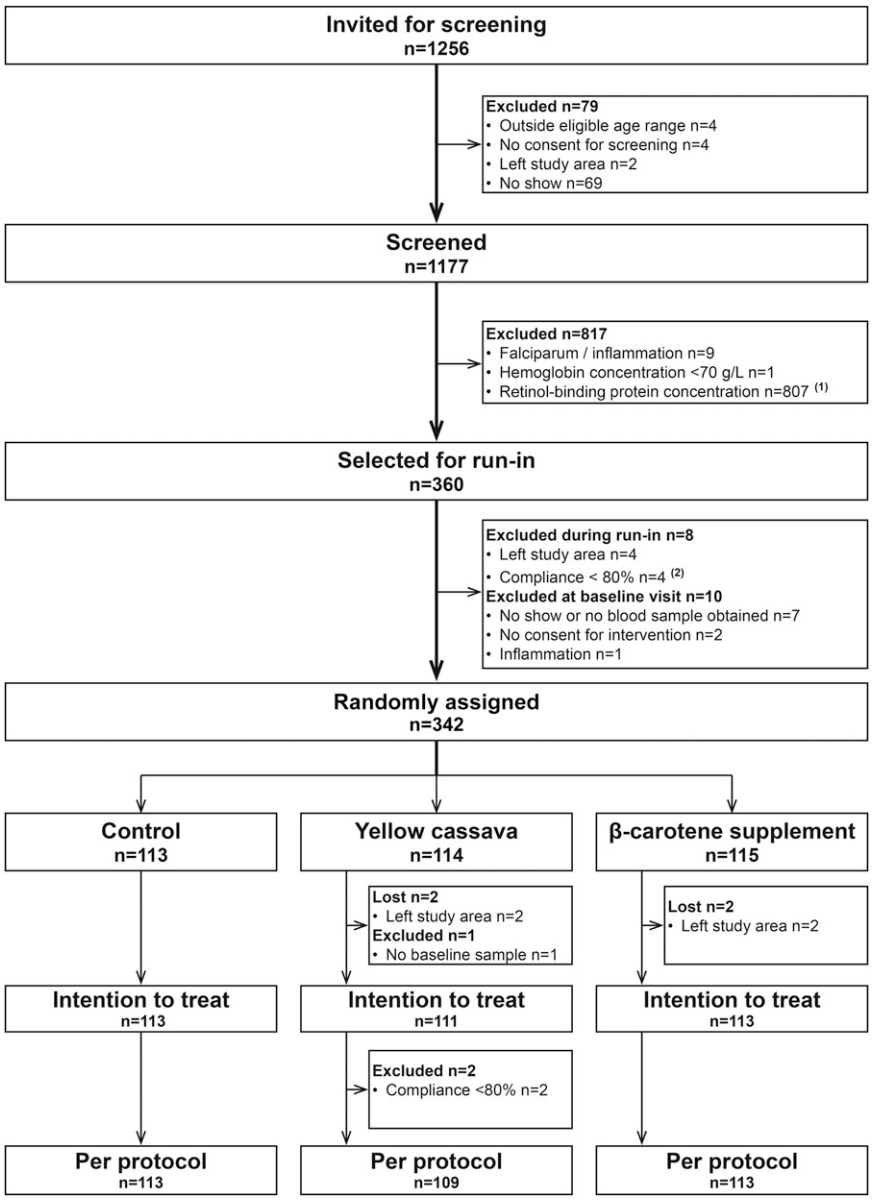

FIGURE 1 Flow of participants. [1]The upper segment of retinol-binding protein concentrations were not eligible for run-in and were excluded. [2]Children who could not consume 80% of their respective portion size were excluded.

oil and salt as per standardized recipe, and served warm as a midmorning snack, 6 d/wk for 18.5 wk. During feeding sessions, intervention groups were physically separated at each school and monitored to avoid food sharing and spilling. Target portions were ≥325 g and ≥375 g for children aged 5–8 y and 9–13 y, respectively. The amount of cassava eaten was recorded daily for each child as the difference in weight of the serving before and after eating. At each school, interventions started for all children simultaneously, 2 d after the run-in period.

Supplemental capsules contained either 1053 μg β-carotene (Betatap 20%S; DSM Nutritional Products) or no active ingredient (placebo), with starch as filler. Capsules were administered directly after feeding and were swallowed with water under supervision. Portions (375 g) of cooked white and yellow cassava were sampled daily at each school and mixed with a food processor after the addition of the preservative antioxidant *tert*-butylhydroquinone (2.5 mL/kg) dissolved in methanol (20 g/100 mL). Duplicate samples (15 g/d) were pooled by week per school and stored at −15°C in Kenya, transported, and stored at −80°C in the Netherlands.

On completion of the intervention period, we repeated blood and data collection by using the same procedures as at baseline, except that the trace element tube was replaced by a serum separation tube (Becton Dickinson). Whole blood was stored in DNA-stabilizing buffer (AS1; Qiagen) and kept at 4°C during transport to the Netherlands until DNA analysis. Serum samples were shielded from light, processed under subdued light conditions, and kept in amber cryovials at −196°C in the field and −80°C during transport and storage until assessment of retinol concentrations in the Netherlands. For ethical reasons, all children received a supplement with vitamin A (100,000 IU) on completion of the intervention. In addition to the experimental cassava, we

provided daily voluntary lunches with cooked maize and beans for all schoolchildren.

24-h recall data

At 13–16 wk after randomization, mean daily intake of energy, fat, and vitamin A was measured in 334 subjects by quantitative 24-h recall and repeated in a subsample ($n = 101$) on nonconsecutive days (see **Online Supplemental Material**).

Biochemical analyses

For screening, we assessed the indicators in the field as follows: *1*) hemoglobin concentration by photometer (Hemocue 201+, HemoCue), *2*) whole-blood C-reactive protein concentration by immunoturbidimetric assay (QuikRead; Orion Diagnostica), *3*) plasma retinol-binding protein concentrations in duplicate by ELISA (K-assay KT-504; Kamiya Biomedical Company) with an iMark microplate absorbance reader (Bio-Rad Laboratories; intra-assay CV: 2.4%, interplate CV: 1.5% in 32 plates), and *4*) *Plasmodium* infection in whole blood by using dipstick tests specific for *P. falciparum* histidine-rich protein-2 and lactate dehydrogenase specific to either *Plasmodium falciparum* or non-*falciparum* human *Plasmodium* species (CareStart G0121 and G0171; AccessBio).

The remainder of this section describes measurements at baseline and at the end of intervention.

Hemoglobin concentration

This was measured within 6 h after blood collection by using a hematology analyzer (Celltac-α, MEK-6410K; Nihon Kohden).

Serum concentrations of retinol and β-carotene

Serum collected at baseline and end of intervention was analyzed (May 2013) in pairs to reduce analytic variation. Concentrations of retinol and carotenoids were measured by HPLC (Thermo Scientific Accela LC system; Thermo Fisher Scientific) and analyzed by using EZChrom Elite version 3.2.2 SP2 software (Agilent Technologies). In total, 500 μL serum, 500 μL NaCl (0.9 w/v% in water), and 1000 μL ethanol (with added retinyl acetate as internal standard) were mixed and extracted twice with 30 mL hexane. The hexane layers were pooled and evaporated to dryness in a vacuum concentrator at 35°C (RVC 2.25 CD plus; MartinChrist). The residue was dissolved in a 250-μL mixture of methanol and butanol (60:40 vol:vol %); 15 μL was injected per HPLC analysis. Sample preparations were done under subdued yellow light. Retinol, retinyl acetate, lutein, zeaxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene were separated on a C18 reversed phase column (Vydac 201TP52, Grace, Columbia, Maryland, USA) by using gradient elution and monitored at 325 nm (retinol and retinyl acetate) and 450 nm (carotenoids) on a photodiode array detector. Runtime was 25 min per sample. Within- and between-run CVs for high and low controls were 3.1% and 2.7% for retinol (concentration ~60 μg/100 mL), and 9.4% and 11.1% for β-carotene (concentration ~10 μg/100 mL).

Serum retinol-binding protein concentration

For serum samples collected at the end of intervention, retinol-binding protein concentrations were determined by ELISA (Quantikine DRB400; R&D Systems). Results were read in duplicate for 10% of samples. Interplate CV for 5 plates was 5.4%, and intra-assay CV was 2.9%.

Iron and inflammation markers in serum

Iron markers (concentrations of ferritin, soluble transferrin receptor) and inflammation markers (concentrations of C-reactive protein and α_1-acid glycoprotein), prealbumin concentration, and zinc concentration were measured at the Meander Medical Hospital (Amersfoort, Netherlands) on a Beckman Coulter UniCel DxC 880i analyzer according to manufacturer's instructions, with external reagents and standards to allow measurement of zinc concentrations (DiaLab GmBH, catalog number 507240 Zinc, 5-Br-PAPS, and 507263SV Zinc Standard).

DNA sequence variants

By using a candidate gene approach, we assessed allele variants on 5 loci in the *BCMO1* gene that had previously been shown to be associated with a decreased conversion of β-carotene to retinol (7, 8)—namely, rs12934922 (R267S), rs7501331 (A379V), rs11645428, rs6564851, and rs6420424. DNA was isolated from whole-blood samples (FADWE002, Favorgen Biotech Corporation Ping Tung, Taiwan), and the designated allele variants of the *BCMO1* gene were scored from 0 to 1 by using the Assay Design Tool (Illumina Technical Support) based on compatibility to successful GoldenGate genotyping. Sequence variants with a score >0.4 were genotyped by Illumina's VeraCode GoldenGate Genotyping Assay on a BeadXpressplatform by using the VeraCode technology. Sequence variants clustering was also assessed visually to determine success of genotyping. Sequence variants with a GenCall score >0.5 and a call rate ≥0.90 were included in the final analysis. Of 5 sequence variants analyzed, one (rs12934922) yielded no detectable signal and was excluded for analysis. For several children, DNA qualities were low, resulting in 56–88 children being excluded depending on the sequence variant investigated.

Total β-carotene concentration in cassava

Carotenoids from either raw or cooked cassava were analyzed by using the same HPLC system as described above for serum samples. To extract carotenoids cassava, we mixed 2 g homogenized cassava, 0.2 g magnesium carbonate, 5 mL deionized water, and 1000 μL ethanol (with added retinyl acetate as internal standard) and extracted 3 times with 20 mL methanol-tetrahydrofuran (1:1 vol:vol%) by using a rod mixer (Polytron PT 20 OD; Kriens/Luzern) until the residue was colorless. Extracts were filtered on a glass funnel with filter paper (Whatman grade 1, GE healthcare life Sciences); the combined filtrates were transferred to a 50-mL volumetric flask and made up to volume with methanoltetrahydrofuran (1:1 vol:vol%). Then, 4 mL filtrate with 1 mL 10% NaCl solution was transferred to a 10-mL glass stoppered centrifuge tubes (Kimax, Kimble Chase), and carotenoids were extracted 3 times with 1.5 mL petroleum-ether containing 0.01% butylated hydroxytoluene. The combined ether fractions were evaporated under nitrogen at 35°C. The residue was dissolved in 2 mL methanol/butanol (60/40 vol:vol%), and 1 μL was injected into the HPLC

system. Carotenoids were separated on a Vydac 201TP52 column by using gradient elution and monitored at 450 nm on a photodiode array detector. Runtime was 20 min per sample. We measured the summed content of all forms of β-carotene, the predominant provitamin A carotenoid in yellow cassava (16). Within- and between-run CVs for high and low control for β-carotene were 3.9% and 7.7% (concentration ~470 μg/100 g).

Statistics

Data were analyzed by using SPSS software, version 20 (SPSS Inc.). For data analysis, we used markers measured in samples collected at baseline and at the end of intervention. For C-reactive protein, this resulted in different cutoff levels (>5 mg/L) to define inflammation than used at screening. Inflammation was also defined by serum α_1-acid glycoprotein protein

TABLE 1
Baseline characteristics, by intervention group (per protocol analysis)[1]

Characteristic	Control (n = 113)	Yellow cassava (n = 109)	β-carotene supplement (n = 113)
Vital and personal characteristics			
Age, y	8.9 ± 2.1[2]	8.8 ± 2.4	8.9 ± 2.4
Sex, F, n (%)	52 (46)	55 (50)	58 (51)
BMI-for-age z score, SD	−1.4 ± 0.9	−1.5 ± 0.9	−1.5 ± 0.9
Height-for-age z score, SD	−1.2 ± 1.2	−1.1 ± 1.1	−1.3 ± 1.0
Children being stunted, n (%)	24 (21)	22 (20)	22 (20)
Serum inflammation markers			
C-reactive protein concentration, mg/L	0 (0, 0)[3]	0 (0, 0)	0 (0, 0)
α_1-acid glycoprotein protein concentration, g/L	0.87 ± 0.26	0.87 ± 0.23	0.86 ± 0.20
C-reactive protein concentration >5 mg/L, n (%)	3 (2.7)	4 (3.7)	1 (0.9)
C-reactive protein concentration >5 mg/L or α_1-acid glycoprotein concentration >1 g/L, n (%)	29 (26)	25 (23)	24 (21)
Serum vitamin A markers, all children			
β-carotene concentration, μmol/L	0.34 (0.24, 0.48)	0.35 (0.24, 0.56)	0.35 (0.24, 0.48)
Retinol concentration, μmol/L	0.80 ± 0.17	0.83 ± 0.17	0.82 ± 0.18
Prealbumin concentration, g/L	0.18 ± 0.03	0.19 ± 0.03	0.18 ± 0.03
Vitamin A status			
Deficient (retinol concentration <0.70 μmol/L), n (%)	31 (27)	29 (27)	30 (27)
Marginal (retinol concentration 0.70–1.05 μmol/L), n (%)	72 (64)	71 (65)	75 (66)
Serum vitamin A markers, children without inflammation			
Retinol concentration, μmol/L	0.82 ± 0.17	0.84 ± 0.17	0.83 ± 0.17
Vitamin A status			
Deficient (retinol concentration <0.70 μmol/L), n (%)	20 (24)	20 (24)	20 (22)
Marginal (retinol concentration 0.70–1.05 μmol/L), n (%)	54 (64)	57 (68)	63 (72)
Serum zinc markers, all children			
Zinc concentration, μmol/L	13.5 ± 2.3	14.3 ± 2.0	14.0 ± 2.3
Deficient (zinc concentration <9.9 μmol/L), n (%)	4 (4)	1 (1)	3 (3)
Serum zinc markers, children without inflammation			
Zinc concentration, μmol/L	13.1 ± 2.3	14.2 ± 1.8	14.0 ± 2.2
Deficient (zinc concentration <9.9 μmol/L), n (%)	4 (5)	1 (1)	3 (4)
Iron markers, all children			
Hemoglobin concentration, g/L	131 ± 10	130 ± 12	132 ± 12
Serum ferritin concentration, μg/L	18.4 (11.4, 24.2)	17.8 (11.2, 25.6)	15.6 (10.4, 24.4)
Serum soluble transferrin receptor concentration, mg/L	1.72 (1.51, 2.05)	1.83 (1.60, 2.12)	1.86 (1.62, 2.17)
Anemia, n (%)	8 (7)	8 (7)	7 (6)
Iron deficiency, n (%)	33 (29)	44 (40)	48 (43)
Iron deficiency anemia, n (%)	6 (5)	7 (6)	5 (4)
Iron markers, without inflammation			
Serum ferritin concentration, μg/L	17.1 (10.8, 22.3)	17.5 (11.4, 24.5)	15.4 (10.1, 23.0)
Iron deficiency, n (%)	26 (31)	33 (39)	37 (42)
Iron deficiency anemia, n (%)	5 (6)	4 (5)	4 (5)

[1]Inflammation was defined as serum C-reactive protein concentrations >5 mg/L and/or serum α_1-acid glycoprotein concentration >1 g/L. Anemia was defined as a hemoglobin concentration <115 g/L or <120 g/L for children aged 5–11 y and >12 y, respectively. Iron deficiency was defined as a serum ferritin concentration <15 μg/L and soluble transferrin receptor concentration >1.55 mg/L. Iron deficiency anemia denotes iron deficiency with concurrent anemia.

[2]Mean ± SD (all such values).

[3]Median; 25th, 75th percentiles in parentheses (all such values).

concentration >1 g/L (17). Other cutoff levels were as follows: vitamin A deficiency, serum retinol concentration <0.7 μmol/L (18); zinc deficiency, serum zinc concentration <9.9 μmol/L (19); anemia, hemoglobin concentration <115 g/L and <120 g/L for children aged 5–11 y and 12–13 y, respectively (20); and iron deficiency, serum ferritin concentration <15 μg/L (20) and serum soluble transferrin receptor concentration >1.55 mg/L (21). Compliance with treatment was computed as the percentage of the age-specific target amount of cassava consumed for the total duration of the intervention.

Our preplanned primary outcome was serum retinol concentration at the end of intervention; secondary outcomes were serum concentrations of total β-carotene, retinol-binding protein, prealbumin, and hemoglobin concentration. Distributions of dependent variables were checked for normality and log-transformed for serum β-carotene concentration. As preplanned, the primary analysis was per protocol and restricted to children who attended >80% of intervention days and consumed >80% of the target amount of cassava over the total intervention period. For primary and secondary analyses, we used ANCOVA to compare intervention groups, adjusting for the stratified design, serum concentrations at baseline of C-reactive protein, α_1-acid glycoprotein, retinol, and zinc; sex; and anemia. In the crude analysis, we adjusted only for the stratified design; for the adjusted analysis, covariates (serum concentrations at baseline of C-reactive protein, α_1-acid glycoprotein, and zinc; sex; and anemia) were eliminated by using a backward elimination procedure (22) from the model when their exclusion affected the magnitude of the intervention effect by <10%. In the adjusted analysis of each outcome, we also adjusted for the same marker measured at baseline (e.g., in the analysis of serum retinol concentration, we adjusted for serum retinol concentration at baseline) except for serum retinol-binding protein concentration (not measured at baseline). In the analysis of serum concentrations of retinol and retinol binding protein, we also adjusted for serum concentrations of C-reactive protein and α_1-acid glycoprotein at the end survey to eliminate possible effects of inflammation. Serum β-carotene concentration was log-transformed, both as outcome and as covariate measured at baseline. To express results for serum β-carotene concentration in natural units, we exponentiated log-transformed estimates of intervention effects and expressed them as percentages.

We assessed effect modification by baseline vitamin A and zinc status by using stratified analyses and directly by evaluating product terms of intervention with zinc deficiency, vitamin A status, and sequence variants of DNA in multiple linear regression analyses. When analyzing effect modification by sequence variants of DNA, we combined the groups receiving yellow cassava and β-carotene supplements to increase the sample size for each genotype.

We based the statistical power of this trial on a mean ± SD expected treatment effect of 0.09 ± 0.19 μmol/L (23); with 80% power and a 2-sided significance of 0.05, we needed 105 children per group, allowing a 10% dropout rate.

RESULTS

Of 1256 screened children, 342 (27%) were randomly allocated. Exclusions were mostly because we selected for children with low plasma concentrations of retinol-binding protein. Four children were lost to follow-up, 1 child did not have a baseline sample, and 2 children did not meet compliance criteria, resulting in 335 children in the per protocol analyses (Figure 1).

Baseline serum retinol concentrations indicated a study population with marginal vitamin A status (**Table 1**). One-fourth of children had inflammation as indicated by elevated concentrations of C-reactive protein, α_1-acid glycoprotein, or both. When these children were excluded from the analysis, one-fourth were vitamin A deficient.

Compliance with the cassava feeding was 100% with mean portion sizes of 336 g and 382 g for children aged 5–8 y and 9–13 y, respectively (**Table 2**). The mean daily intake of vitamin A

TABLE 2
Daily intake of energy, fat, vitamin A, and β-carotene from diet and intervention

	Control (n = 113)	Yellow cassava (n = 109)	β-carotene supplement (n = 113)
General diet (excluding experimental cassava)[1]			
Energy, kJ	5739 (4175, 8049)[2]	5673 (4605, 6988)	5863 (4391, 7929)
Fat, % energy	13.1 (11.3, 16.6)	14.1 (12.3, 16.9)	14.0 (12.0, 15.9)
Vitamin A intake,[3] μg RAE[4]	16.9 (4.6, 38.6)	13.8 (4.0, 46.1)	21.1 (9.5, 40.5)
Children without any preformed retinol, n (%)	86 (77)	83 (76)	74 (65)
Supplied by intervention			
Amount of cassava eaten daily,[5] g	378 (339, 383)	371 (333, 381)	378 (340, 384)
β-carotene from cassava,[6] μg	38 (34, 39)	1463 (1313, 1501)	38 (34, 39)
β-carotene from supplement, μg	0	0	1053 (1044, 1053)
Vitamin A supplied by general diet and intervention, μg RAE[6,7]	22 (10, 44)	220 (205, 241)	175 (164, 195)

[1]Based on 24-h recall data with n = 111 in the control group, n = 108 in the yellow cassava group, and n = 113 in the β-carotene supplement group.
[2]Median; 25th, 75th percentiles in parentheses (all such values).
[3]Assuming that 12 μg β-carotene from a mixed diet is absorbed and converted to 1 μg retinol.
[4]RAE, retinol activity equivalent.
[5]Cassava was cooked in water for 1 h, any leftover water was discarded, and the cassava was mashed with 12 g oil and 8 g salt/kg cooked cassava.
[6]Based on mean ± SD β-carotene concentrations of 3.94 ± 0.75 μg/g (range: 3.04–5.44 μg/g) (fresh weight) and 0.10 ± 0.03 μg/g (range: 0.04–0.15 μg/g) for boiled yellow and white cassava, respectively.
[7]Assuming that 7 μg β-carotene supplement or cassava is absorbed and converted to 1 μg retinol.

from the general diet was low at ~20 μg retinol activity equivalents in all groups. The mean ± SD of weekly pooled samples of total β-carotene content in boiled yellow cassava was 3.94 ± 0.75 μg/g (range: 3.04–5.44 μg/g). The total retinol activity equivalent intakes were 22 μg, 220 μg, and 175 μg in the groups receiving control, yellow cassava, and β-carotene supplements, respectively. We found no *Plasmodium* infection at the end of intervention.

In the primary analysis, both consumption of yellow cassava and supplementation with β-carotene increased serum retinol concentration by 0.04 μmol/L (**Table 3**). Intention-to-treat analyses showed similar effects (not shown). Correspondingly, consumption of yellow cassava and supplementation with β-carotene increased serum β-carotene concentration by 524% and 166%, respectively. There were no marked effects of yellow cassava and β-carotene supplementation on the prevalence of vitamin A deficiency. We found no evidence that interventions changed serum concentrations of retinol-binding protein or prealbumin or hemoglobin concentration. Intervention estimates with and without adjustment for inflammation markers at the end survey were similar (not shown).

There was no evidence that intervention effects varied by initial vitamin A status or initial zinc status (**Figure 2**A) or *BCMO1* genotype (Figure 2B). For rs7501331, all children except for one were wild type. Minor allele frequencies were 0.09 for rs11645428, 0.60 for rs6564851, and 0.56 for rs6420424, and there was no evidence that any allele variants except rs7501331 were in Hardy-Weinberg disequilibrium, which indicates that these variants do not pose strong selective pressure on the study population.

DISCUSSION

Daily consumption of yellow cassava resulted in modest increases in serum retinol concentration and large increases in β-carotene concentration compared with control, showing that β-carotene was well absorbed. There was no evidence that the effect on serum retinol concentration depended on initial vitamin

TABLE 3
Effect of consumption of yellow cassava and supplementation with β-carotene on various outcomes[1]

			Intervention effect	
Outcome/intervention group	*n*	Estimate	Crude[2] (95% CI)	Adjusted[3] (95% CI)
Serum retinol concentration, μmol/L				
Control	113	0.77 ± 0.01[4]	Reference	Reference
Yellow cassava	109	0.81 ± 0.01	0.05 (0.01, 0.10)	0.04 (0.00, 0.07)
β-carotene supplement	113	0.81 ± 0.01	0.05 (0.00, 0.09)	0.04 (0.00, 0.07)
Prevalence of vitamin A deficiency,[5] *n* (%)				
Control	113	34 (30.1)	Reference	NA
Yellow cassava	109	29 (26.6)	−3.5% (−15.1%, 8.3%)	NA
β-carotene supplement	113	33 (29.2)	−0.9% (−14.9%, 9.1%)	NA
Serum β-carotene concentration, μmol/L				
Control	113	0.42[6]	Reference	Reference
Yellow cassava	109	0.94[6]	537% (453%, 633%)	524% (448%, 608%)
β-carotene supplement	112	0.65[6]	164% (130%, 203%)	166% (134%, 202%)
Serum retinol-binding protein concentration, μmol/L				
Control	112	0.62 ± 0.01	Reference	Reference
Yellow cassava	108	0.62 ± 0.01	0 (−0.04, 0.04)	0 (−0.04, 0.04)
β-carotene supplement	113	0.65 ± 0.01	0.03 (−0.01, 0.07)	0.03 (−0.01, 0.07)
Serum prealbumin concentration, g/L				
Control	113	0.18 ± 0.00	Reference	Reference
Yellow cassava	108	0.18 ± 0.00	0 (−0.01, 0.01)	0 (−0.01, 0.01)
β-carotene supplement	111	0.18 ± 0.00	0 (−0.01, 0.01)	0 (−0.01, 0.01)
Hemoglobin concentration, g/L				
Control	112	130.6 ± 0.7	Reference	Reference
Yellow cassava	109	130.4 ± 0.7	−0.3 (−3.1, 2.5)	0.0 (−2.8, 2.7)
β-carotene supplement	112	129.9 ± 0.7	−0.2 (−2.1, 1.8)	−0.7 (−2.6, 1.2)

[1]Intervention groups were compared by using ANCOVA (see text). NA, not applicable.
[2]Adjusted for stratified design only.
[3]In the analysis of each outcome, we also adjusted for the same marker measured at baseline (e.g., in the analysis of serum retinol concentration, we adjusted for serum retinol concentration at baseline) except for serum retinol-binding protein concentration (not measured at baseline). In the analysis of serum retinol concentration and serum retinol binding protein concentration, we also adjusted for serum concentrations of C-reactive protein and α_1-acid glycoprotein measured at the end of intervention. Serum β-carotene concentration was log-transformed, both as outcome and as covariate measured at baseline. To express results for serum β-carotene concentration in natural units, we exponentiated log-transformed estimates of intervention effects and expressed them as percentages.
[4]Mean ± SE (all such values).
[5]Serum retinol concentration <0.7 μmol/L.
[6]Geometric mean.

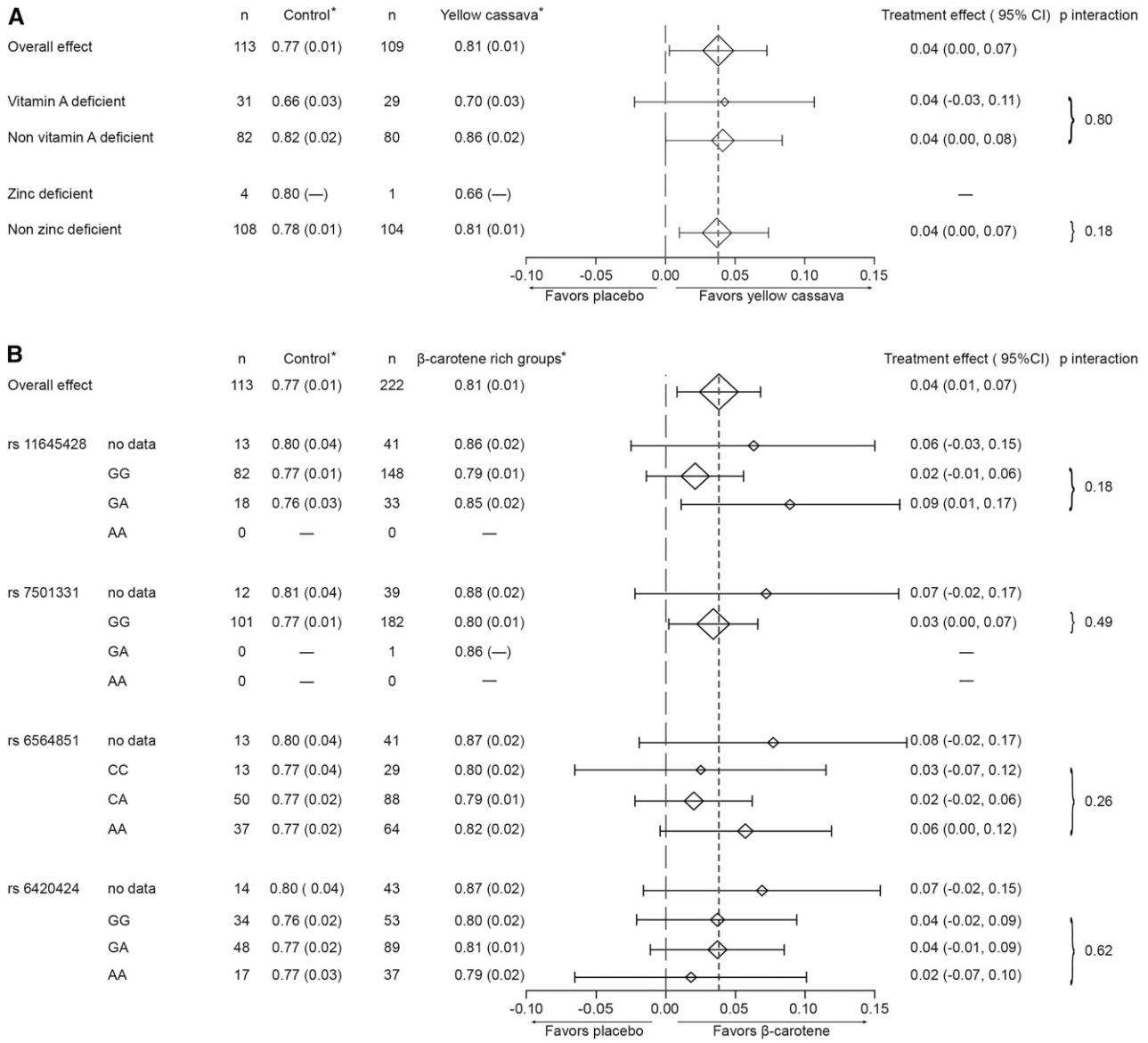

FIGURE 2 Effect of yellow cassava (A) and increased intake of β-carotene (B) on serum retinol concentration, by subgroups. *Adjusted means ± SEs. Normal genotypes are displayed on top. In the analyses of serum retinol concentration, we adjusted for the stratified design, serum retinol concentration at baseline, and concentrations of serum C-reactive protein and α_1-acid glycoprotein at the end of intervention.

A status, zinc status, or *BCMO1* genotype. Similar effects were obtained with β-carotene supplementation.

To our knowledge, this is the first randomized trial with yellow cassava in a target population where vitamin A deficiency is prevalent. Attrition was low, with only 4 children lost to follow-up, and compliance with feeding was high, with almost all children consuming more than their assigned portions sizes and only 2 children failing to finish their rations on a daily basis.

Serum retinol concentration, our primary outcome, is recommended by the WHO to assess population vitamin A status and to evaluate the effect of interventions (12). Serum retinol concentration reflects liver vitamin A stores in the range $<$0.07 μmol/g liver (20 μg/g), which corresponds to serum retinol concentration $<$1.75 μmol/L (50 μg/dL) (24). At baseline, all children in our study were within this range. Approximately one-fourth had serum retinol concentrations $<$0.70 μmol/L (20 μg/dL), indicating deficiency, and only 8% had serum retinol concentrations $>$1.05 μmol/L (30 μg/dL) (Table 1), the cutoff that corresponds to mean concentrations in most well-nourished populations with "adequate" stores (12).

We expected to find a larger intervention effect of yellow cassava or β-carotene supplementation on serum retinol concentration, particularly in the subgroup of children with vitamin A deficiency. The large increase in serum β-carotene concentration and modest increase in serum retinol concentration in our study reflect poor bioconversion of β-carotene to retinol. Zinc deficiency has been suggested to reduce enzymatic cleavage of β-carotene (25), but we could not draw conclusions on its influence on the effect of interventions because it occurred in only 4 children in our study.

Similar to our study, daily consumption of biofortified sweet potato in South African children and Bangladeshi women resulted

in large increases in serum β-carotene concentration but no evident effect on serum retinol concentration (26, 27). In other trials, provitamin A–rich vegetables and fruits resulted in larger effects on serum retinol concentration than in our study, but vitamin A deficiency persisted in a substantial proportion of study participants despite large effects on β-carotene concentrations (23, 28).

Could poor bioconversion be due to genetic factors? A specific allelic variant (rs7501331; A379V) within the *BCMO1* coding region is common in Caucasians and was found to reduce β-carotene conversion by 32% (8). Our study participants, however, were nearly all homozygous wild type for this allelic variant. We found no evidence for effect modification by any of the other allelic variants investigated. It should be noted, however, that subgroup analysis may be limited by sample size, which is often inadequate to provide adequate statistical support for effect modification (29), which might have been the reason we did not find evidence. Subjects with wild type for rs11645428 seemed to have a lower response to intervention than heterozygous subjects. This finding is in agreement with a reduction of 51% in β-carotene conversion reported by others (7). Whether this variant or as yet unidentified variants in the *BCMO1* gene may explain the low β-carotene conversion found in this African study population needs further elucidation.

The question arises whether the sharp increase in serum β-carotene concentration in our trial and similar studies has functional significance. Circulating β-carotene is taken up and concentrated in various tissues (30) such as liver and adipose tissues, whereas kidney and lung tissues have minor roles for storage (31). In steady state, only 1% of the total body content of carotenoids is contained in serum (6). Studies have shown that *BCMO1* is expressed not only in enterocytes but also in various cell types in a broad variety of human tissues, including liver tissue (32, 33). This could indicate that these tissues have the capacity to convert circulating or locally stored carotenoids on need. The hypothesis that conversion into vitamin A takes place in tissues and not only in the intestine (31) would explain the efficacy of orally dosed β-carotene in reducing manifestations of vitamin A deficiency as shown in several trials (34–36). If so, β-carotene in tissues may function as a store. This would need a reconsideration of the definition of vitamin A deficiency, which is currently based on liver retinol stores only. Thus, evaluation by serum retinol concentration [or its proxy markers serum concentration of prealbumin and retinol-binding protein (37)] may well overestimate the prevalence of deficiency in surveys and underestimate the true impact of interventions based on plant foods.

Our study was conducted in primary schoolchildren who are at lower risk of vitamin A deficiency than preschool children (2, 12). We selected older children to reduce possible interference with the national vitamin A supplementation program for children 6–59 mo of age; to achieve higher intake of cassava and β-carotene, because older children are able to consume more food; and to simplify logistics of the trial because the school setting enabled us to optimize supervision and compliance. Interventions with yellow cassava may have similar or even a larger impact in younger children because they are bound to be more vitamin A deficient, especially in areas where vitamin A supplementation programs are poorly implemented.

We used yellow varieties that were still below the breeding target concentrations of 15 μg/g for cassava (38). Cassava breeding is an ongoing, rapidly progressing process, and new varieties with higher concentrations will become available in the near future and will have a larger impact on vitamin A status than we have shown here. Acceptance of the crop does not seem to pose any difficulties as evidenced by a consumer acceptability study in our research area (39).

Thus, in our population of children with marginal vitamin A status without concurrent infection, consumption of yellow cassava led to modest gains in serum retinol concentration and a large increase in β-carotene concentration.

We thank Brahim Tabali for analyzing retinol-binding protein samples and our data safety and monitoring board members (Richard Hurrell, Saskia de Pee, and Phelgona Otieno).

The authors' responsibilities were as follows—EFT: assisted in the study design, coordinated the field work, conducted the statistical analyses, and prepared the first draft of the manuscript; IDB and AM-B: designed and supervised the study and assisted in the statistical analyses; HV: conceived and designed the study, provided technical and statistical support, and assisted in preparation of the first draft of the manuscript; GNKM: assisted in the field work; AMM: provided general management and logistic support; AYD: conducted the biochemical analyses; BM-D: supervised cassava production; EB and MBZ: assisted in the study design; and all authors: contributed to the final manuscript. None of the authors reported a conflict of interest. The funders had no role in the design, implementation, analysis, or interpretation of the data.

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