β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test[1–3]

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ABSTRACT

Background: β-Carotene–rich orange-fleshed sweet potato (OFSP) is an excellent source of provitamin A. In many developing countries, sweet potato is a secondary staple food and may play a role in controlling vitamin A deficiency.

Objective: The objective was to determine the efficacy of daily consumption of boiled and mashed OFSP in improving the vitamin A status of primary school children.

Design: Children aged 5–10 y were randomly assigned to 2 groups. The treatment group ($n = 90$) consumed 125 g boiled and mashed OFSP (1031 retinol activity equivalents/d as β-carotene), and the control group ($n = 90$) consumed an equal amount of white-fleshed sweet potato devoid of β-carotene for 53 school days. All children were dewormed to exclude helminthic infection. The modified-relative-dose-response test for vitamin A status was conducted before and after intervention.

Results: The estimated intervention effect for the ratio of 3,4-didehydroretinol to retinol (DR:R) was −0.008 (95% CI: −0.015, −0.001; $P = 0.0203$), which indicated a greater improvement in vitamin A liver stores in the treatment group than in the control group. The proportions of children with normal vitamin A status (DR:R < 0.060) in the treatment group tended to increase from 78% to 87% ($P = 0.096$) and did not change significantly (from 86% to 82%) in the control group ($P = 0.267$). These proportions were not used to test the intervention effect or within-group changes because the study was powered to test the intervention effect on DR:R.

Conclusions: Consumption of OFSP improves vitamin A status and can play a significant role in developing countries as a viable long-term food-based strategy for controlling vitamin A deficiency in children. *Am J Clin Nutr* 2005;81:1080–7.

KEY WORDS Vitamin A status, modified relative dose response, β-carotene, orange-fleshed sweet potato, efficacy, school-feeding program, South Africa

INTRODUCTION

Vitamin A deficiency is of public health significance in the developing world. Globally, 140 million children aged <5 y, of whom nearly 100 million live in South Asia or sub-Saharan Africa, have low serum retinol concentrations (<0.7 μmol/L) (1). Countries of eastern and southern Africa have the highest prevalence (37%) of preschool children with low serum retinol concentrations, followed by South Asia (35%) and Western and Central Africa (33%) (1). In South Africa, 1 in 3 preschool children has a serum retinol concentration <0.7 μmol/L (2), and 55–68% of children aged 1–9 y consume <50% of the recommended dietary intake of vitamin A (700 μg retinol equivalents) (3); children living in rural areas are the most affected (2, 3).

Vitamin A deficiency is caused by a habitual diet that provides too little bioavailable vitamin A to meet physiologic needs. Rapid growth and frequent infections, which cause ineffective utilization of the vitamin, are also critical factors (4). The dietary sources of vitamin A are preformed vitamin A (commonly found in foods of animal origin) and provitamin A carotenoids (found in yellow and orange-fleshed fruit and vegetables and in dark-green leafy vegetables). Of the ≈600 carotenoids found in nature, only 3 are important precursors of vitamin A in humans, namely, β-carotene, α-carotene, and β-cryptoxanthin. β-Carotene is the major provitamin A component of most carotenoid-containing foods (5).

Strategies to control vitamin A deficiency include dietary diversification, food fortification, and vitamin A supplementation. Dietary diversification includes the production of β-carotene–rich crops, such as orange-fleshed sweet potato (OFSP). The consumption of OFSP increased the vitamin A intake of Kenyan women and children (6). A recent ex ante impact assessment indicated that OFSP could make a major contribution to controlling vitamin A malnutrition in sub-Saharan Africa (7). Replacing white-fleshed varieties with high β-carotene varieties that meet

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local preferences could benefit an estimated 50 million children aged <6 y who are currently at risk of diseases associated with vitamin A deficiency. The consumption of diets containing mostly plant sources of β-carotene, the primary source being red sweet potato, increased serum retinol concentrations in Indonesian children marginally deficient in vitamin A (8).

Under the International Potato Center's Vitamin A for Africa partnership, β-carotene–rich OFSP varieties are widely promoted. It is important to determine the efficacy of β-carotene–rich OFSP in improving vitamin A status in children. Because of its agronomic, technologic, and acceptability performance, the naturally high β-carotene–containing Resisto variety of sweet potato, which has a dark orange root, was chosen for the present study. This variety was originally developed in the United States through conventional breeding and was successfully introduced into a home-gardening project in South Africa (9) after being judged by members of the community to be tastier than the other varieties tested.

This study determined the efficacy of the daily consumption of boiled and mashed β-carotene–rich OFSP in improving vitamin A status, measured by the modified-relative-dose-response (MRDR) test, in primary school children participating in a school feeding program.

SUBJECTS AND METHODS

Study population

The study was conducted from March (end of the summer season) to June (during the winter season) 2002. The study subjects were 5–10-y-old Grades 1–3 primary school children from an area ≈40 km northwest of Durban, KwaZulu-Natal Province, and 26 km from an area with a known high prevalence of vitamin A deficiency (10) and a low dietary vitamin A intake (11). Most of the residents in the study area were of low socioeconomic status. Children received a cooked meal 4 d/wk, excluding Mondays, through a school feeding program for the first 3 wk of intervention. Thereafter, cooked meals were provided on Mondays until the end of the study. Meals were prepared by a member of the community and consisted primarily of rice with cabbage and potatoes or beans and, occasionally, *samp* (crushed maize kernels) with beans. The meals always contained sunflower seed oil, fried onions, and salt. Flavorings were also used and included curry powder, beef stock cubes, or *soya mince* (soybean protein with added spices and herbs). The community in which the study was conducted was unfamiliar with OFSP varieties; however, they were familiar with white-fleshed sweet potato (WFSP) varieties.

The Ethics Committee of the South African Medical Research Council approved the study. Permission and support for conducting the study was obtained from the school governing body, the school principal, and the educators. The purpose, procedures, nature, and potential benefits of the study were explained to the parents or legal guardians of children in Grades 1–3. Those parents who agreed that their children could participate in the study signed an informed consent form.

Study design

The study was a randomized controlled unmasked feeding trial. All biochemical measurements were, however, masked. Vitamin A status was measured using the MRDR test, in which the ratio of serum 3,4-didehydroretinol (DR) to retinol (R), DR:R (mol/mol), is calculated (12). Sample size calculations indicated that 86 children per group were needed to detect a 0.02 difference in serum DR:R between groups, assuming an SD of 0.04 in each group at a 5% significance level and 90% power. An additional 5% of children were included to compensate for potential loss to follow-up.

Children were excluded from the study if their parent or legal guardian did not sign the informed consent form, the child was unwilling to participate, or the hemoglobin concentration was <90 g/L. Eligible children were stratified by classroom and sex and were randomly assigned to 1 of the 2 groups within blocks of 8 children listed alphabetically. One group ($n = 90$; treatment group) received boiled and mashed β-carotene–rich OFSP [Resisto variety; 1031 retinol activity equivalents (RAE)/d] and the other ($n = 90$; control group) an equal amount of boiled and mashed WFSP (Bosbok variety; 0 RAE/d).

All the children in Grades 1–3 received one 500 mg mebendazole chewable anthelminthic tablet to exclude helminthic infection as a confounder. Treatment was given 1 mo before the baseline survey, 1 wk before the baseline survey, and 1 mo before the end measurements were taken.

Sweet potato cultivation and supply

To ensure that sweet potatoes of high quality were available, and to control and closely monitor the cultivation program, both the OFSP and WFSP were cultivated at the Vegetable and Ornamental Plant Institute of the Agricultural Research Council, Roodeplaat, Gauteng Province.

Cultivation was staggered so that specified amounts of sweet potatoes of similar age and maturity, especially with regard to the β-carotene content of the OFSP variety, could be harvested on predetermined dates. A 10-d supply of sweet potatoes was harvested biweekly, packed in 10-kg color-coded mesh bags, and transported 600 km overnight to the school. Once at the school, the sweet potatoes were kept at room temperature in a lockable room.

Intervention

Children were fed sweet potato 5 d/wk during the 1030 midmorning break. The usual school meal was served after the sweet potato serving was consumed. No intervention took place, ie, no sweet potato was fed, during the 8-d April school holiday, weekends, and public holidays. Sweet potato was provided to the children for 53 d over 10.6 wk.

Each child was issued with a color-coded identification label that showed the child's name, code, and school grade. Feeding bowls, files, sheets to record compliance, and kitchenware were also color-coded. Each classroom had a monitor recruited from the local community and trained to monitor and record daily compliance and the reasons for absence from school. Compliance was defined as the number of days that a child received and ate all of the sweet potato expressed as a percentage of the total number of days that sweet potato was provided.

Preparing and feeding the sweet potato

The sweet potatoes were cooked in the school kitchen, which was also used for preparing the school meal. Two cooks, one for cooking the OFSP and the other for cooking the WFSP, were recruited from the local community and trained to follow the

standardized procedure for preparing the sweet potato; ≈15 kg OFSP and 22 kg WFSP were cooked on each school day. The extra WFSP was fed to all nonparticipating children in each classroom.

Every school day, usually between 0740 and 0840, the sweet potatoes were washed and cooked unpeeled; small-sized sweet potatoes were kept intact, and medium to large-sized sweet potatoes were cut in half. The OFSP and WFSP were cooked separately in large pots. In general, only one-half of the depth of the sweet potatoes was covered with water and boiled in a closed pot until soft (≈55 min). The water was drained off before the sweet potatoes were peeled and mashed by hand. To promote the absorption of β-carotene, and given that oil was included in the school meal, sunflower seed oil was added to the mashed sweet potato: 140 mL to the OFSP and 200 mL to the WFSP, equivalent to 1.0–1.4 mL oil per portion of sweet potato served, depending on the amount of sweet potato left after peeling. After the oil was thoroughly mixed with the mashed sweet potato, the sweet potato was transferred to color-coded containers and taken to the classrooms. Classroom monitors observed the cooks serving the sweet potatoes. Ice cream scoops were used, which provided typical portion sizes of 125 g.

During feeding, the treatment and control groups were seated on opposite sides of the classroom to avoid the exchange or sharing of sweet potato. Consumption took place under close supervision by the classroom monitors, and the children were encouraged, but not forced, to eat the whole portion. A short questionnaire on the acceptability of the OFSP was completed for the treatment group at the end of the study.

Sampling and analysis of boiled and mashed sweet potato for β-carotene content

Samples of boiled and mashed OFSP and WFSP were collected 5 times during the intervention. Duplicate portions of ≈150 g each were collected from 2 different parts of the cooking pot on 3 consecutive days each time. Samples were put into plastic freezer bags and frozen within 3 h to −20 °C. To limit the degradation of β-carotene by oxygen during storage, air was squeezed from the sample bag before it was sealed. Once the 3-d sample collection was completed, the frozen samples were transported in a cool box containing ice packs to the laboratory of the Nutritional Intervention Research Unit, Cape Town, and stored at −80 °C until analyzed.

To determine the average sweet potato serving (in g) and the amount of β-carotene consumed, the weight of 33% of all servings was measured ($n = 30$ per group), on the same days that the sweet potato samples were collected, with an electronic microdigital computing scale (model MW-1200, Casbee Masskot scale; CAS Corp, Gangdong-Gu, Seoul, Republic of Korea).

The β-carotene content of the samples was determined within 3 mo after the last 3-d collection period. The 3 duplicate samples per collection period were combined, and 5 composite samples were analyzed in triplicate by HPLC (SpectraSERIES; Thermo Separation Products, Fremont, CA) on the same day by using a validated method established for this study. Three 2-g samples of each composite sample were extracted with tetrahydrofuran: methanol (1:1, by vol). A β-carotene standard (synthetic, crystalline, Type II, product C-4582; Sigma Chemical Co, St Louis, MO) was purified by HPLC, and an aliquot of the purified standard solution with a known concentration was used as the external standard for quantification of β-carotene in the sample extract.

Anthropometric measures

Anthropometric measurements were taken at baseline. Children were weighed while they were wearing light clothing and no shoes to the nearest 0.05 kg on a load-cell-operated digital scale (UC-300 Precision Health Scale; A&D Co Ltd, Tokyo, Japan). Children were measured without shoes to the nearest 0.1 cm with a vertically placed wooden board fitted with a measuring tape and a movable headpiece.

Assessment of vitamin A status

The MRDR test was used to determine the adequacy of liver vitamin A stores (12, 13). Between 0845 and 0900, 45–48 children per day were given a single oral dose of 2 mg (7.0 μmol) 3,4-didehydroretinyl acetate in 190 μL corn oil. To promote its absorption, each child was given a high-fat, low vitamin A–containing snack (30 g peanut butter on a 30-g slice of white bread) and a 250-mL cold drink (concentrated nonnutritive orange-flavored drink diluted with water) that was consumed under supervision.

During the baseline and follow-up surveys, the school meal was standardized (cabbage and rice) and devoid of vitamin A to exclude the effect that different meal compositions might have on the MRDR test. For the same reason, a 10-d washout period during which sweet potato was not served was implemented before the final blood samples were obtained.

The serum concentration of DR reaches its maximum ≈5 h after dosing in vitamin A–depleted children. Blood (6 mL that was also used for the biochemical measurements) was drawn from each child as close as possible to the child's 5 h ± 15 min time point after 3,4-didehydroretinyl acetate had been administered. Blood samples were taken between 1350 and 1430 h in the same sequence as the dosing. DR:R, or an MRDR value ≥0.060, indicated inadequate vitamin A liver stores.

Biochemical measurements

To ascertain the baseline nutritional status of participating children, iron and zinc status were determined. Blood was obtained by antecubital venipuncture in nonfasting subjects. Immediately after collection, 1.0 mL of the sample was removed to determine the hemoglobin concentration by the cyanmethemoglobin method with a portable photometer (Product no. 7316; Ames Minilab, Miles Inc, Elkhart, IN). A blood sample of known hemoglobin concentration was used as the reference. The remaining blood was transferred to a sterile Gel and Clot Activator tube (SST II Plus; Becton Dickinson and Co, Vacutainer Systems, Plymouth, United Kingdom). The latter was centrifuged (750 × *g* for 10 min at room temperature), and aliquots of serum were transferred to a series of containers that were put in a cool box containing ice packs. The serum was frozen later the same afternoon at −20 °C. All field procedures were done as quickly as possible, and care was taken throughout to protect the blood and serum samples from direct light. All tubes were trace element–free to avoid contamination with zinc and iron.

After completion of each of the baseline and follow-up surveys, which took 4 d each, the serum samples were transported to

the Nutritional Intervention Research Unit, Cape Town, and stored for 1–6 mo at −80 °C until analyzed. Serum concentrations of retinol and DR were measured in the same injected sample (13) by reversed-phased HPLC (SpectraSERIES). The baseline and postintervention serum of a subject were analyzed on the same day to minimize intraassay variation in retinol and DR concentrations. Serum ferritin was determined with an immunoradiometric assay (Ferritin MAb Solid Phase Component System; ICN Pharmaceuticals, Orangeburg, NY) with the use of an external control sample (Ligand 1, 2, 3, Chiron Diagnostics Ltd, Halstead, United Kingdom). Serum zinc was analyzed with a flame atomic absorption spectrophotometer (Philips Pye Unicam SP9, Cambridge, United Kingdom) (14) with a commercial control serum (Seronorm Trace Elements Serum; SERO AS, Billingstad, Norway) as a quality control.

Because hemoglobin, ferritin, retinol binding protein, and zinc are acute phase reactants, C-reactive protein (CRP) and α_1-acid glycoprotein (AGP) were measured as markers of infection. CRP was measured with an immunoturbidimetric method (Technicon method no. SM4-0183G89, Technicon RA-1000 auto-analyzer; Technicon Instruments, NY) with the use of Bayer TESTpoint Serum Protein Controls (Bayer Diagnostics, Fernwald, Germany). AGP was measured with an immunoturbidimetric technique and with nephelometry with an in vitro diagnostic reagent kit (Dade Behring, Marburg, Germany).

Statistical analysis

Data were entered in Microsoft EXCEL (Redmond, WA) and analyzed with the use of SPSS 10.0 for WINDOWS (SPSS Inc, Chicago, IL). Baseline means ± SDs for the biochemical measurements and anthropometric indexes were obtained for the treatment and control groups. Baseline differences for the anthropometric and biochemical measurements between groups were examined to verify the success of the randomization using *t* tests and chi-square tests. Data were analyzed on an intent-to-treat basis. Individual-level changes (postintervention value − baseline value) were calculated. Tests for normality of the changes within each group were done with the use of the Kolmogorov-Smirnov test. The null hypotheses of normality were not rejected, and *t* tests were carried out for differences between the treatment and control groups. The intervention effect and 95% CIs were estimated from the differences. Data were tested for equality of variances, and the appropriate value for significance (*P* value) was applied. The *t* test for paired data was used to compare pre- and postintervention values within each group. P values < 0.05 were considered statistically significant. The proportions of children with normal liver stores (DR:R $<$ 0.060) were not used to test the intervention effect and within-group changes because the study was powered to test the intervention effect on DR:R. The reported proportions of children with normal liver stores are only explanatory.

An analysis of covariance (ANCOVA) was carried out to investigate the effect of the number of days a child was absent from school (and hence did not receive sweet potato) after adjustment for the baseline value of the postintervention biochemical measurement. The number of days absent was not a significant factor in the ANCOVA model, and the estimated intervention effects concurred with the pre-post analysis reported in the text.

Height-for-age *z* scores (HAZ), weight-for-age *z* scores (WAZ), and weight-for-height *z* scores (WHZ) were obtained following World Health Organization procedures (15). The birth date of each child was obtained from the school register.

Anemia was defined as a hemoglobin concentration $<$ 115 g/L (16) and zinc deficiency as a serum zinc concentration $<$ 10.7 μmol/L (17). The acute phase response was defined as serum concentrations of CRP $>$ 10 mg/L and AGP $>$ 1.2 g/L (18). Three subsets of data were further examined with the following exclusions: *1*) only CRP was elevated, *2*) only AGP was elevated, and *3*) both CRP and AGP were elevated. Because the estimated intervention effects for DR:R, the DR dose response, and serum retinol in the above 3 subanalyses were not different from the estimates of the complete data set, only the latter are reported.

RESULTS

Two children had a hemoglobin concentration $<$90 g/L and were excluded from the study and referred to the local clinic for treatment. Of the 180 children enrolled, 178 completed the study; one child in each group was absent when blood was drawn at the end of the study. The birth date was not available for one child in the treatment group and for 3 children in the control group; thus, their anthropometric indexes could not be calculated.

Baseline characteristics of the treatment and control groups are presented in **Table 1**. There were no significant differences between the 2 groups in any of the characteristics shown. Of the 174 children with known birth dates, 92.5% were younger than 9 y and the rest were between 9 and 11 y old. Few children were stunted or overweight, and almost none was underweight. Twenty-two percent of children in the treatment group and 14% in the control group had a DR:R $\geq$ 0.060, which indicated inadequate vitamin A liver stores. Anemia was present in 27% and 37% of the treatment and control groups, respectively, but only ≈18% in both groups had both anemia and low serum ferritin concentrations. Slightly more than 50% of the children in both groups had low serum zinc concentrations. None of the children had clinical symptoms of infection on the days blood was drawn, but subclinical infection was present in a few children: between 7% and 9% of children in both groups had an elevated CRP concentration only or an elevated AGP concentration only, whereas 2–3% had both elevated CRP and AGP concentrations.

Sweet potato consumption and β-carotene intake

Mean compliance with sweet potato consumption was 90 ± 8% and 89 ± 9% in the treatment and control groups, respectively. Only 11 children (12%) and 9 children (10%) in the treatment and control groups, respectively, were $<$80% compliant; absence from school was the main reason for noncompliance. Five children in the treatment group and 2 in the control group said at some time during the intervention that they felt sick or did not want to eat the sweet potato.

The β-carotene contents of 5 composite samples of boiled and mashed OFSP taken at evenly spread intervals during the intervention are shown in **Table 2**. The Resisto variety contains β-carotene almost exclusively, whereas the Bosbok WFSP variety is devoid of provitamin A carotenoids. A few other unidentified carotenoids in the Resisto variety were noted, but these compounds existed in negligible amounts. Thus, it was not necessary to identify and quantify these other carotenoids. The servings sizes of sweet potato in both treatment groups are shown in **Table 3**. The average amount of OFSP and WFSP served per day was similar between the 2 groups. Children in the treatment

TABLE 1
Baseline characteristics of the study children[1]

Variable	Treatment group (OFSP) (n = 89)	Control group (WFSP) (n = 89)	P[2]
Sex (%)			
Male	49	48	
Female	51	52	
Age (y)[3]	7.4 ± 1.1[4]	7.3 ± 1.2	0.633[5]
Anthropometric index[3]			
Height (cm)	118.8 ± 8.1	118.4 ± 8.3	0.756[5]
Weight (kg)	23.4 ± 4.1	23.2 ± 4.2	0.679[5]
Stunted (%)[6]	3.4	5.8	0.449
Underweight (%)[6]	0	1.1	0.310
Overweight-for-height (%)[7]	4.5	2.3	0.422
Micronutrient indicator[8]			
Low serum retinol, < 0.70 μmol/L (%)	71	73	0.739
Vitamin A status			
Normal liver stores, DR:R < 0.060 (%)	78	86	0.177
Insufficient liver stores, DR:R ≥ 0.060 (%)	22	14	0.118
Anemia, hemoglobin < 115 g/L (%)	27	37	0.148
Low serum ferritin, < 15 μg/L (%)	60	52	0.291
Anemia and low serum ferritin (%)	17	19	0.696
Low serum zinc, < 10.7 μmol/L (%)	51	53	0.764
Elevated acute phase protein[8]			
CRP > 10 mg/L (%)	7	9	0.578
AGP > 1.2 g/L (%)	8	9	0.787
Elevated CRP and AGP (%)	2	3	0.650

[1] OFSP, orange-fleshed sweet potato (Resisto variety); WFSP, white-fleshed sweet potato (Bosbok variety); DR:R, ratio of 3,4-didehydroretinol to retinol; CRP, C-reactive protein; AGP, α_1-acid glycoprotein.
[2] Chi-square test unless indicated otherwise.
[3] The birth date was not available for 1 child in the treatment group and for 3 children in the control group; thus, their age and anthropometric index could not be calculated.
[4] $\bar{x}$ ± SD (all such values).
[5] t test.
[6] Proportion of children with height-for-age and weight-for-age z scores < −2 SDs of the median of the reference population (15).
[7] Proportion of children with weight-for-height z scores > 2 SDs of the median of the reference population (15).
[8] Proportion of children that satisfied the defined criteria; one child in each group was absent when blood was drawn at the end of the study.

group received an average of 12 375 μg β-carotene/d (1031 μg RAE/d). OFSP provided 2.5 times the recommended dietary allowance of vitamin A for 4–8–y-old children, which is 400 μg/d (19).

In the treatment group, 92% of children stated that they would like to eat OFSP every day of the week, 92% also found the taste acceptable, and 67% said that they would like to eat more than the serving size provided.

Changes in vitamin A status

Mean baseline and postintervention values; mean changes in DR:R, DR dose response, and serum retinol concentrations; and estimated intervention effects are presented in **Table 4**. Mean values at baseline were not significantly different between the 2 groups. A significant intervention effect was found for the main outcome of the study, DR:R, ie, an improvement in vitamin A

TABLE 2
β-Carotene content of boiled and mashed orange-fleshed sweet potato (Resisto variety) in the composite samples

Composite sample	β-Carotene content[1]	Vitamin A value[2]
	μg/100 g cooked root	*RAE/100 g cooked root*
A	10 699 ± 640	892 ± 53
B	10 013 ± 590	834 ± 49
C	9479 ± 821	790 ± 69
D	8329 ± 286	694 ± 24
E	11 378 ± 387	948 ± 32
Average	9980 ± 1167	832 ± 97

[1] All values are $\bar{x}$ ± SD of triplicate samples.
[2] All values are $\bar{x}$ ± SD. RAE, retinol activity equivalents [12 μg β-carotene = 1 μg retinol = 1 RAE (19)].

TABLE 3
Serving size and amount of β-carotene provided during the intervention in the treatment and control groups[1]

	Treatment group (OFSP)	Control group (WFSP)
Sweet potato (g/serving)[2]	123.5 ± 14.0	128.7 ± 16.4
β-Carotene (μg/serving)[3]	12 375	—
RAE per serving[4]	1031	—

[1] OFSP, orange-fleshed sweet potato (Resisto variety); WFSP, white-fleshed sweet potato (Bosbok variety); RAE, retinol activity equivalents [12 μg β-carotene = 1 μg retinol = 1 RAE (19)].
[2] Average weight; $\bar{x}$ ± SD of 360 servings.
[3] μg β-Carotene per serving: 124 g/100 g × 9980 μg β-carotene.
[4] RAE per serving: 12 375 μg β-carotene/12.

TABLE 4
Serum concentrations, changes, and intervention effects of vitamin A–status indicators in the treatment and control groups[1]

	Treatment group (OFSP) ($n = 89$)		Control group (WFSP) ($n = 89$)
DR:R (mol/mol)[2]			
Baseline	0.040 ± 0.028[3]		0.038 ± 0.024[4]
Postintervention	0.036 ± 0.019		0.042 ± 0.025[4]
Change	−0.004 (−0.009, 0.001)[5]		0.004 (−0.001, 0.009)
Intervention effect[6]		−0.008 (−0.015, −0.001)	
DR dose response (nmol/L)			
Baseline	22.7 ± 13.1		21.9 ± 13.7[4]
Postintervention	25.2 ± 11.1		27.7 ± 15.7[4]
Change	2.6 (−0.1, 5.2)		5.9 (2.5, 9.3)[7]
Intervention effect[8]		−3.3 (−7.6, 1.0)	
Retinol (μmol/L)			
Baseline	0.618 ± 0.184		0.603 ± 0.182
Postintervention	0.739 ± 0.199		0.690 ± 0.177
Change	0.121 (0.089, 0.153)[9]		0.087 (0.052, 0.122)[9]
Intervention effect[10]		0.034 (−0.013, 0.081)	

[1] One child in each group was absent when blood was drawn at the end of the study. OFSP, orange-fleshed sweet potato (Resisto variety); WFSP, white-fleshed sweet potato (Bosbok variety); DR:R, ratio of 3,4-didehydroretinol to retinol.
[2] Determined 5 h after a dose of 2 mg (7 μmol) 3,4-didehydroretinyl acetate.
[3] $\bar{x}$ ± SD (all such values).
[4] $n = 88$.
[5] $\bar{x}$; 95% CI in parentheses (all such values).
[6,8,10] Difference in mean change from baseline to postintervention between the treatment and control groups (*t* test): [6] $P = 0.0203$, [8] $P = 0.1310$, [10] $P = 0.1535$.
[7,9] Significantly different from baseline within group (*t* test for paired data): [7] $P < 0.001$, [9] $P < 0.0001$.

liver stores in the treatment group relative to the control group ($P = 0.0203$). The proportion of children with normal vitamin A status, or adequate vitamin A liver stores (DR:R < 0.060), in the treatment group tended to increase (from 78% to 87%; $P = 0.096$) after intervention, whereas that in the control group did not change significantly (from 86% to 82%; $P = 0.267$). The proportions of children with normal liver stores were not used to test the intervention effect and within-group changes because the study was powered to test the intervention effect on DR:R. The change in DR:R within each group was not significant, probably because the mean baseline DR:R of the children indicated adequate vitamin A stores. There were no significant intervention effects for serum retinol and DR dose response. Serum retinol concentrations increased significantly from baseline in both groups. The proportion of children with a low serum retinol concentration (<0.70 μmol/L) after intervention decreased from 71% to 50% ($P = 0.001$) in the treatment group and decreased from 73% to 49% ($P = 0.001$) in the control group. The serum DR dose response increased significantly only in the control group.

DISCUSSION

This randomized controlled study showed that feeding β-carotene–rich OFSP of the Resisto variety in a primary school feeding program in a rural community improved vitamin A liver stores as measured with the MRDR test.

The MRDR test (12) takes advantage of the vitamin A deficiency–dependent accumulation of apo-retinol binding protein (apo-RBP) in the liver (20) and is a better discriminator of intervention effects on vitamin A status than is serum retinol concentrations alone. The DR:R, which is not affected by subclinical infection (21, 22), showed an intervention effect, which indicated that β-carotene–rich OFSP improved vitamin A liver stores. The increase in the serum DR dose response in the control group indicated that apo-RBP accumulated in the liver as a result of inadequate endogenous liver retinol concentrations.

The MRDR test is more responsive to vitamin A intervention than are serum retinol concentrations when the supply of vitamin A is large enough to change overall vitamin A status (23). Serum retinol concentrations reflect recent vitamin A intakes (24). For the MRDR test to work correctly, ie, for it to pick up differences in vitamin A liver reserves, a washout period of ≥10 d is allowed before the postintervention MRDR test is conducted. The washout period allows for the re-accumulation of apo-RBP in the hepatocytes of children who may not have changed to a normal vitamin A status during intervention with OFSP. This washout period may explain the lack of an intervention effect on serum retinol concentrations observed in this study because the dietary component of the serum retinol concentration would have been removed for 10 d before resampling. In another study done in South Africa, a biscuit fortified with β-carotene, providing 50% of the recommended dietary allowance for vitamin A for 1 y, resulted in an improvement in serum retinol concentrations in primary school children, but reverted to preintervention concentrations after a 10-wk period during which the fortified biscuits were not available (25).

The reason why serum retinol concentrations increased from baseline in both groups is unknown. Deworming to exclude helminthic infection as a confounder may have played a role. A study in Indonesian children showed that serum retinol concentrations markedly improved when children consuming meals containing plant sources high in β-carotene were dewormed (8). Others, however, have found that deworming and supplementing

children with 210 μmol vitamin A did not affect serum retinol concentrations (21). The increase in serum retinol concentration is dependent on the degree of intestinal helminth infection (8) and likely on the type of parasite. The present study did not assess the prevalence or degree of helminthic infection.

A seasonal effect on serum retinol concentrations in response to dietary changes, eg, when provitamin A–rich mangoes are available as reported in rural Gambian women (26), cannot be excluded, although it is unlikely. The baseline survey was conducted at the end of the summer season, whereas the postintervention survey was conducted during the winter season when provitamin A–containing foods are less available. The habitual diet of the children with regard to vitamin A and provitamin A intakes during the intervention period was not controlled for. It was assumed that the habitual vitamin A intake was low, as was previously reported for rural areas in the same province (3, 11).

The bioavailability and bioconversion of provitamin A carotenoids depend on both the food matrix and host-related factors (27, 28). The β-carotene in orange-fleshed vegetables, such as the OFSP, does not play a role in photosynthesis and it is located in the cell chromoplasts (29), where it is found in lipid droplets or bound to a protein. The β-carotene in orange-fleshed vegetables is more readily released than that in dark-green leafy vegetables during cooking, thereby enhancing bioavailability. Between 3 g (8) and 5 g (30) fat per meal is required to ensure maximum carotenoid absorption. The sunflower seed oil added to the OFSP together with the fat content of the school meal provided ≥3 g fat.

The US Institute of Medicine recommendation that 12 μg β-carotene is equivalent to 1 μg retinol was used in this study (19). Bioefficacy, however, varies depending on vitamin A status and may be higher in vitamin A–deficient populations because such people are more efficient at converting provitamin A (31–34).

The low percentage of children with inadequate vitamin A liver stores (DR:R ≥ 0.060) at baseline was a limitation of the study. A bigger response may have occurred if a larger proportion of the study population had abnormal DR:R values from the onset. This finding shows that it is important to know the vitamin A status of the population before conducting intervention trials and to not rely solely on low serum retinol concentrations. If the study outcome had only relied on serum retinol concentrations, it would not have been possible to conclude anything regarding the efficacy of sweet potato in improving vitamin A status.

β-Carotene–rich foods are important for preventing vitamin A deficiency (31). A combination of orange fruit and squash was more effective in increasing serum retinol concentrations in anemic schoolchildren with marginal vitamin A status than was a combination of dark-green leafy vegetables and carrots (35). Consumption of meals containing β-carotene–rich red sweet potato also increased serum retinol concentrations in marginally vitamin A–deficient children (8). These findings may have been related to the type of β-carotene, because *cis*-isomers are less bioavailable and have less provitamin A activity than do the *trans* form (27, 28). The provitamin A in raw and boiled OFSP of the Resisto variety is almost exclusively β-carotene in the *trans* form (36).

OFSPs, which are naturally rich in β-carotene, are an excellent food source of provitamin A. These varieties can make a significant contribution to a viable long-term effective and sustainable food-based approach to prevent vitamin A deficiency in developing countries (6, 7). OFSP of the Resisto variety was successfully introduced into a home garden project that promoted the production and consumption of a variety of β-carotene–rich vegetables. It was shown that serum retinol concentrations in 2–5–y-old children improved within 20 mo of implementation (9).

Food diversification through the production of yellow-orange β-carotene–rich vegetables is seen as a viable long-term strategy to complement supplementation and fortification programs. High β-carotene–containing OFSP that provided ≈ 830 RAE/100 g cooked root was shown in this study to improve vitamin A status and to have the potential to control vitamin A deficiency in developing countries.

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PJvJ was the principal investigator responsible for all aspects of the study, with support from MF. All authors contributed to designing the study, interpreting the data, and writing the manuscript. SAT visited the laboratory of the Nutritional Intervention Research Unit and assisted in standardizing the HPLC method for analyzing serum DR and retinol. CJL was responsible for the statistical analysis. None of the authors had any conflict of interest with the funders of this study.

REFERENCES

1. Mason JB, Lotfi M, Dalmiya N, et al. The micronutrient report: current progress and trends in the control of vitamin A, iodine, and iron deficiencies. Ottawa, Canada: The Micronutrient Initiative/International Development Research Centre, 2001. Internet: http://www.micronutrient.org/frame_HTML/resource_text/publications/mn_report.pdf (accessed 15 June 2004).
2. South African Vitamin A Consultative Group. Anthropometric, vitamin A, iron, and immunisation coverage status in children aged 6–71 months in South Africa, 1994. S Afr Med J 1996;86:354–7.
3. Department of Health. The National Food Consumption Survey: children aged 1-9 years, South Africa, 1999. Pretoria, South Africa: Department of Health, Nutrition Directorate, 2000.
4. Underwood BA. Dietary approaches to the control of vitamin A deficiency: an introduction and overview. Food Nutr Bull 2000;21:117–23.
5. Parker RS. Absorption, metabolism, and transport of carotenoids. FASEB J 1996;10:542–51.
6. Hagenimana V, Oyunga MA, Low J, Njoroge SM, Gichuki ST, Kabira J. The effects of women farmer's adoption of orange-fleshed sweet potatoes: raising vitamin A intake in Kenya. Washington, DC: International Center for Research on Women, June 1999, Research Report Series 3.
7. Low J, Walker T, Hijmans R. The potential impact of orange-fleshed sweet potatoes on vitamin A intake in Sub-Saharan Africa. The VITAA Project, vitamin A and orange-fleshed sweetpotatoes in Sub-Saharan

Africa, 9–11 May, 2001, Nairobi, Kenya. Internet: http://www.cipotato.org/vitaa/Publications/Paper_vitaa.pdf (accessed 15 June 2004).
8. Jalal F, Nesheim MC, Agus Z, Sanjur D, Habicht JP. Serum retinol concentrations in children are affected by food sources of β-carotene, fat intake, and anthelmintic drug treatment. Am J Clin Nutr 1998;68:623–9.
9. Faber M, Phungula MAS, Venter SL, Dhansay MA, Benadé AJS. Home gardens focusing on the production of yellow and dark-green leafy vegetables increase the serum retinol concentrations of 2–5-y-old children in South Africa. Am J Clin Nutr 2002;76:1048–54.
10. Oelofse A, Faber M, Benadé JG, Benadé AJS, Kenoyer DG. The nutritional status of a rural community in KwaZulu-Natal, South Africa: the Ndunakazi project. Cent Afr J Med 1999;45:14–9.
11. Faber M, Smuts CM, Benadé AJS. Dietary intake of primary school children in relation to food production in a rural area in KwaZulu-Natal, South Africa. Int J Food Sci Nutr 1999;50:57–64.
12. Tanumihardjo SA, Cheng J-C, Permaesih D, et al. Refinement of the modified-relative-dose-response test as a method for assessing vitamin A status in a field setting: experience with Indonesian children. Am J Clin Nutr 1996;64:966–71.
13. Furr HC, Tanumihardjo SA, Olson JA. Training manual for assessing vitamin A status by use of the modified relative dose response and the relative dose response assays. Washington, DC: US Agency for International Development, 1992 (updated 1997).
14. Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1979;25:1487–91.
15. Dibley MJ, Goldsby JB, Staehling NW, Trowbridge FL. Development of normalized curves for the international growth reference: historical and technical considerations. Am J Clin Nutr 1987;46:736–48.
16. WHO/UNICEF/UNU consultation. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. WHO 2001, WHO/NHD/01.3. Internet: http://www.who.int/nut/documents/ida_assessment_prevention_control.pdf (accessed 15 June 2004).
17. Milne DB. Trace elements. In: Burtis CA, Ashwood ER, eds. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia, PA: WB Saunders Company, 1994:1317–53.
18. Paracha PI, Jamil A, Northrop-Clewes CA, Thurnham DI. Interpretation of vitamin A status in apparently healthy Pakistani children by using markers of subclinical infection. Am J Clin Nutr 2000;72:1164–9.
19. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001.
20. Muto Y, Smith JE, Milch PO, Goodman DeWitt S. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. J Biol Chem 1972;247:2542–50.
21. Tanumihardjo SA, Permaesih D, Muherdiyantiningsih, et al. Vitamin A status of Indonesian children infected with *Ascaris lumbricoides* after dosing with vitamin A supplements and albendazole. J Nutr 1996;126:451–7.
22. Wieringa FT, Dijkhuizen MA, West CE, Northrop-Clewes CA, Muhilal. Estimation of the effect of the acute phase response on indicators of micronutrient status in Indonesian infants. J Nutr 2002;132:3061–6.
23. Tanumihardjo SA. Can lack of improvement in vitamin A status indicators be explained by little or no overall change in vitamin A status of humans? J Nutr 2001;131:3316–8.
24. Tanumihardjo SA. Assessing vitamin A status: past, present and future. J Nutr 2004;134:290S–3S.
25. van Stuijvenberg ME, Dhansay MA, Smuts CM, Lombard CJ, Jogessar VB, Benadé AJS. Long-term evaluation of a micronutrient-fortified biscuit used for addressing micronutrient deficiencies in primary school children. Public Health Nutr 2001;4:1201–9.
26. Bates CJ, Villard L, Prentice AM, Paul AA, Whitehead RG. Seasonal variations in plasma retinol and carotenoid levels in rural Gambian women. Trans R Soc Trop Med Hyg 1984;78:814–7.
27. de Pee S, West CE. Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 1996;50:S38–53.
28. Castenmiller JJM, West CE. Bioavailability and bioconversion of carotenoids. Annu Rev Nutr 1998;18:19–38.
29. Czygan FZ. Pigments in plants. Stuttgart, Germany: Gustav Fischer, 1980.
30. Jayarajan P, Reddy V, Mohanram M. Effect of dietary fat on absorption of β-carotene from green leafy vegetables in children. Ind J Med Res 1980;71:53–6.
31. Nestel P, Trumbo P. The role of provitamin A carotenoids in the prevention and control of vitamin A deficiency. Arch Latinoam Nutr 1999;49:26S–33S.
32. West CE, Eilander A, van Lieshout M. Consequences of revised estimates of carotenoid bioefficacy for dietary control of vitamin A deficiency in developing countries. J Nutr 2002;132:2920S–6S.
33. Russell RM, Ross AC, Trumbo PR, West KP. Retinol equivalency ratio of β-carotene. J Nutr 2003;133:2915–6(letter).
34. West CE, Eilander A, van Lieshout M. Reply to Russell et al. J Nutr 2003;133:2917(letter).
35. de Pee S, West CE, Permaesih D, Martuti S, Muhilal, Hautvast JGAJ. Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. Am J Clin Nutr 1998;68:1058–67.
36. van Jaarsveld PJ, Marais De Wet, Harmse E, Nestel P, Rodriguez-Amaya DB. Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. J Food Comp Anal (in press).