Yellow maize with high β-carotene is an effective source of vitamin A in healthy Zimbabwean men1–4

Tawanda Muzhingi, Tendekayi H Gadaga, Andrew H Siwela, Michael A Grusak, Robert M Russell, and Guangwen Tang

ABSTRACT

Background: The bioconversion efficiency of yellow maize β-carotene to retinol in humans is unknown.

Objective: The objective of this study was to determine the vitamin A value of yellow maize β-carotene in humans.

Design: High β-carotene–containing yellow maize was grown in a hydroponic medium with 23 atom% 2H2O during grain development. Yellow maize β-carotene showed the highest abundance of enrichment as [13H4]β-carotene. Eight healthy Zimbabwean men volunteered for the study. On day 1 after a fasting blood draw, subjects consumed 300 g yellow maize porridge containing 1.2 mg β-carotene, 20 g butter, and a 0.5-g corn oil capsule. On day 8, fasting blood was drawn, and subjects consumed 1 mg [13C10]retinyl acetate in a 0.5-g corn oil capsule and 300 g white maize porridge with 20 g butter. Thirty-six blood samples were collected from each subject over 36 d. Concentrations and enrichments of retinol and β-carotene were determined.

Results: The area under the curve (AUC) of retinol from 1.2 mg yellow maize 1,5 to 1.2 mg β-carotene was 72.9 nmol ⋅ d, and the AUC of retinol from 1 mg retinyl acetate 13C10 was 161.1 nmol ⋅ d. The conversion factor of yellow maize β-carotene to retinol by weight was 3.2 ± 1.5 to 1.

Conclusion: In 8 healthy Zimbabwean men, 300 g cooked yellow maize containing 1.2 mg β-carotene that was consumed with 20.5 g fat showed the same vitamin A activity as 0.38 mg retinol and provided 40–50% of the adult vitamin A Recommended Dietary Allowance. This trial was registered at clinicaltrials.gov as NCT00636038. Am J Clin Nutr 2011;94:510–9.

INTRODUCTION

Vitamin A deficiency (VAD) is a major public health problem in Zimbabwe (1). It is well known that adequate vitamin A intake is important for vision, growth, cellular differentiation and proliferation, reproduction, and the integrity of the immune system (2). The consequences of VAD include night blindness, reduced growth in children, and increased morbidity and mortality (3). The main risk factors for VAD are low dietary intake of vitamin A–rich foods, low dietary intake of fats and oils in meals, and a high prevalence of diseases (4). Infectious diseases exacerbate VAD by a variety of mechanisms, including reduced food intake, reduced intestinal absorption, and urinary loss of vitamin A (4). In addition, poverty in developing countries leads to insufficient intakes of foods of animal origin, which are rich in preformed vitamin A (5).

Plant foods are rich in provitamin A carotenoids and are a major vitamin A source for a vast population of the poor.

In Zimbabwe, like most sub-Saharan African countries, the levels of fruit and vegetable consumption are far below the World Health Organization/Food and Agriculture Organization minimum recommendation for fruit and vegetables of 146 kg per person per year (6, 7). In contrast, maize per capita consumption in Zimbabwe is high, and averaged >100 kg per year in 1996 (8). In 2000, maize consumption in Zimbabwe was ~330 g per person per day (9). Comparably, in eastern Zambia, adjacent to Zimbabwe, maize consumption was 483 g per capita per day in 1997 (10). Maize is an important food crop in southern Africa, where it provides more than two-thirds of the daily energy intake (11).

Yellow maize has been recognized for decades as the main source of provitamin A for hogs and other farm animals that rely on winter-feed rations (12). This practice of yellow maize use as livestock feed has been identified as a barrier to yellow maize consumption in Zimbabwe and parts of southern Africa, where white maize (which lacks carotenoids) is preferred for human consumption (13, 14). However, studies in Kenya, Mozambique, and Zimbabwe showed that barriers to yellow maize consumption...
could be overcome by education campaigns and by pricing yellow maize lower than white maize (13–15).

Currently, there are efforts to increase the provitamin A concentrations of yellow maize as a VAD intervention strategy in maize-diet–based countries with a high prevalence of VAD. However, no human studies have evaluated the bioavailability and bioconversion of intrinsic deuterium-labeled yellow maize β-carotene. This study was aimed at evaluating the vitamin A value of stable-isotope–labeled yellow maize [2H]β-carotene in healthy Zimbabwean adult men. To evaluate the vitamin A equivalence of the labeled yellow maize β-carotene, a known amount of [13C10]retinyl acetate in corn oil was used as a reference dose, and the β-carotene absorption and the conversion of β-carotene to retinol in vivo were determined.

SUBJECTS AND METHODS

Production of intrinsically labeled yellow maize

Seeds of a high–β-carotene maize line (DEexp × CI7 − F1) developed by Torbert Rocheford (Purdue University, West Lafayette, IN) were kindly provided for these studies (16). Seeds were imbibed (soaked in water) and germinated on filter paper for 4 d, until primary roots were ≥3 cm in length. Seedlings were then planted in plastic canisters (5-cm diameter), which had their bottoms removed and replaced with a black plastic mesh (4 mm openings); each seedling’s root was inserted through the mesh. Seedling canisters were positioned in lids that were suspended over 20-L tubs of continuously aerated nutrient solution that contained the following micronutrients in mmol/L: KNO3, 1; KH2PO4, 1; Ca(NO3)2, 0.5; MgSO4, 1; and K2SiO4, 0.1; and the following micronutrients in mmol/L: CaCl2, 25; H3BO3, 25; MnSO4, 0.5; H2MoO4, 0.5; and NiSO4, 0.1; and Fe(III)HEDTA at 20 nmol/mL and MES buffer at 2 mmol/L. The 23 atom% 2H2O allowed us to achieve a target peak enrichment of M + 9 [original mass of β-carotene (M) plus 9 atoms of 2H] for the maize β-carotene (determined empirically in pilot studies). Plants were maintained on this media, with the solution topped off as needed (with the same 2H2O solution), until the ears had matured (≈45 d later). The 2H2O nutrient solution was aerated with an air stream that was purged of water vapor. During this labeling period, the temperature within the labeling system was maintained at 25°C (day) and 15°C (night); the relative humidity was maintained at between 35% and 75%. The clear plastic-walled labeling system was used to maintain an elevated 2H2O concentration in the gas atmosphere surrounding the plants and developing ears, thereby achieving better enrichment. This closed system also required that carbon dioxide was added to the gas atmosphere within the chamber during the daytime hours to support plant growth; the carbon dioxide concentration with the chamber was monitored with an infrared carbon dioxide gas analyzer (model 225-MK3; Analytic Development Co, Hertfordshire, United Kingdom) and was maintained at 400 ± 50 ppm. At maturity, the ears were collected and dried at room temperature (in low light) for 10 d. Seeds were then removed from the ears and stored in sealed plastic bags at −20°C until shipment to Boston for further processing. The seeds used in this study were collected from 2 separate harvests, in June and October 2006.

Production of intrinsically labeled yellow maize dose

The first batch of 612 g of deuterium-labeled yellow maize was received in June 2006 from the USDA–Agricultural Research Service Children’s Nutrition Research Center, Houston, TX, soon after harvest. The second batch of 262 g of deuterium-labeled yellow maize was received from the same laboratory in October 2006. On receipt from Houston, the yellow maize was analyzed by HPLC and liquid chromatography (LC)–mass spectrometry (MS) in the Carotenoids and Health Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. This was done to determine whether the levels of provitamin A carotenoids and deuterium enrichment were sufficient for a human study (Table 1). The yellow maize kernels were vacuum-packed and stored at −80°C until further analysis. In January 2008, the yellow maize kernels from both the June 2006 and the October 2006 harvests were pooled, homogenized, and ground into flour with a grinder (Braun KSM 4B; Braun Inc, Woburn, MA). On 16 January 2008, the yellow maize thick porridge (sadza, the traditional staple food in Zimbabwe) was prepared by cooks and dietitians in the kitchen of the Metabolic Research Unit at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University according to Zimbabwean recipes (17). The sadza was prepared as follows: 4 cups of water were boiled in a pot; one-half cup of yellow maize flour was mixed with one cup of cold water in a separate bowl, and the mixture was added to the boiling water in a pot and stirred for ≈2 min with a wooden spoon; the mixture was left to boil into a paste/gel for ≈10 min, and more yellow maize flour was then added slowly while the mixture was stirred continuously, until a thick gel
Maize doses were stored at an unspecified location in 2006 and then transported under ice packs for 20 h to Bulawayo, Zimbabwe. On arrival at the study site, the yellow maize doses were divided into 8 doses, each of which weighed 300 g. After cooking, the sadza was formed. The sadza was left to simmer for 5 min and then allowed to cool. In total, 729 g of yellow maize flour was used to make the sadza. The total weight of the yellow maize sadza was 2401 g. (Traditionally, only yellow maize flour and water are used to make sadza and in this study nothing else was added to the sadza during cooking.) The sadza was divided into 8 doses, each of which weighed 300 g. After cooking, the all-trans-β-carotene equivalence of the yellow maize sadza was 4.1 μg/g (wet weight). Each dose contained 1.2 mg all-trans-β-carotene equivalents and was individually vacuum packed and stored at −80°C until consumption on 4 February 2008.

### Volunteers and study design

To recruit 8 volunteers for the study, 12 healthy men (aged >40 y and <70 y), nonsmoking, and not having taken vitamin A or β-carotene supplements within the past month, were screened as volunteers from the city of Bulawayo. Eight subjects were finally enrolled and completed the study. No specific racial or ethnic background was required. Of the 8 volunteers, one was white, one was Indian (south Asian origin), and 6 were black Zimbabwean men. Volunteers attended a screening session during which they were instructed on how to follow a diet containing low amounts of vitamin A and carotenoid-containing foods. They were provided with lists of foods to select and to avoid while living at home. The subjects were provided with food record books and instructed to list all food and drink consumed. No alcohol was allowed during the study. The following situations excluded potential volunteers from the study: severe and symptomatic cardiac disease or hypertension; history of bleeding disorders; chronic history of gastric, intestinal, liver, pancreatic, or renal disease; any portion of the stomach or the intestine removed (aside from the appendix); history of intestinal obstruction or malabsorption; active smoking (smoking was stopped ≥1 mo before the beginning of the study and during the study); history of chronic alcoholism; a convulsive disorder; or an abnormality in screening blood or urine samples. HIV status was not used in the inclusion or exclusion criteria and was not asked or assessed. Informed consent was obtained from all volunteers under the guidelines established by the Institutional Review Board of Tufts University, the Tufts Medical Center, and the Medical Research Council of Zimbabwe.

### Study design and procedures

For the 2 wk before the first dose, and on days 16–21, 23–28, and days 30–35, subjects consumed their normal diets, but without vitamin supplements or foods that contained large amounts of β-carotene or vitamin A. A diagrammatic summary of the study design and procedures is shown in Figure 1. Participants received all their meals at the study site from day 1 to day 15, and on day 22, day 29, and day 36.

The subjects were admitted at 0700 on day 1 of the study after an overnight fast. At 0730, a fasting 10 mL blood sample (time = 0 h) was withdrawn into a no-additive evacuated tube from a forearm vein by registered nurses. At that time the yellow maize sadza dose was taken out of the freezer and left to stand until equilibrated to room temperature. Subjects then spread 20 g butter on their yellow maize dose and consumed it, together with a capsule containing 0.5 g corn oil, under the supervision of coinvestigators, nurses, and research assistants to ensure compliance. In this study the 20 g butter was added to the sadza only to ensure intestinal absorption of maize β-carotene; this is not how sadza is traditionally consumed in Zimbabwe (17). Note that maize seeds also contain endogenous oil. Based on previous analyses (18), we estimate that the sadza made from this hybrid corn would have introduced ≈1.2 g of endogenous fat per 100 g sadza, or 3.6 g fat per our 300-g serving size. Thus, each serving of sadza, with butter and corn oil added, would have contained ≈24.1 g fat. Ten milliliters of blood were drawn at 3, 6, 9, 11, and 13 h after the dose (an intravenous line was inserted for drawing these samples). A low–vitamin A, low–β-carotene breakfast, lunch, and dinner were offered at 0900, 1200, and 1900, respectively. Fasting blood samples were collected at 0730 from day 2 to day 7, and a low–vitamin A, low–β-carotene breakfast, lunch, and dinner were provided at the same times as on day 1.

On day 8 the subjects were admitted at 0750; a fasting 10 mL of blood (time = 0 h) was withdrawn from a forearm vein into a no-additive evacuated tube. The subjects took 1 mg [13C10]retinyl acetate (synthesized by the Cambridge Isotope Laboratory, Andover, MA) in a 0.5-g corn oil capsule, with 300 g white

### Table 1

<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Form</th>
<th>Lutein (μg/g)</th>
<th>Zeaxanthin (μg/g)</th>
<th>β-Cryptoxanthin (μg/g)</th>
<th>13 cis-β-C (μg/g)</th>
<th>all-trans-β-C (μg/g)</th>
<th>9 cis-β-C (μg/g)</th>
<th>Calculated β-C equivalence (μg/g)</th>
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<tr>
<td>06/2006</td>
<td>Dry wt</td>
<td>13.16 ± 2.33</td>
<td>8.44 ± 1.49</td>
<td>1.13 ± 0.19</td>
<td>2.93 ± 1.13</td>
<td>11.41 ± 3.56</td>
<td>2.58 ± 0.85</td>
<td>14.74</td>
</tr>
<tr>
<td>10/2006</td>
<td>Dry wt</td>
<td>14.29 ± 1.26</td>
<td>4.38 ± 0.30</td>
<td>0.65 ± 0.05</td>
<td>1.46 ± 0.17</td>
<td>13.63 ± 1.12</td>
<td>1.80 ± 0.17</td>
<td>15.58</td>
</tr>
<tr>
<td>01/2008</td>
<td>Dry wt</td>
<td>9.22 ± 0.43</td>
<td>2.19 ± 0.17</td>
<td>0.53 ± 0.02</td>
<td>3.73 ± 0.38</td>
<td>10.66 ± 0.4</td>
<td>0.64 ± 0.11</td>
<td>13.05</td>
</tr>
<tr>
<td>01/2008</td>
<td>Wet wt</td>
<td>3.11 ± 0.13</td>
<td>0.68 ± 0.06</td>
<td>0.16 ± 0.01</td>
<td>1.17 ± 0.12</td>
<td>3.33 ± 0.12</td>
<td>0.20 ± 0.03</td>
<td>4.10</td>
</tr>
</tbody>
</table>
maize sadza and 20 g butter. The $[^{13}C_{10}]$retinyl acetate, which converts to $[^{13}C_{10}]$retinol once in circulation, was used as a reference dose to assess the conversion efficiency of the maize $\beta$-carotene to retinol. Ten milliliters of blood were withdrawn at 3, 6, 9, 11, and 13 h after the dose (an intravenous line was inserted for drawing these samples). A low–vitamin A, low–$\beta$-carotene breakfast, lunch, and dinner were provided at the same times as on day 1. White maize sadza was prepared at the National University of Science and Technology from locally grown noncommercial white maize. White maize is white because it has little or no carotenoid content (19).

Fasting blood samples were collected on days 9, 10, 11, 13, 15, 19, 22, 29, and 36, after the subjects had fasted for 12 h overnight. A low–vitamin A, low–$\beta$-carotene breakfast, lunch, and dinner were provided at the same times as on day 1. Throughout the 36-d study period, all the blood samples were kept at room temperature for 0.5 h after drawing and were then centrifuged at 4°C and 800 $\times$ g for 15 min. Serum was processed and stored at $-80^\circ$C until shipment to the United States. After the study was completed, ice packs frozen at $-80^\circ$C were packed with the chloroform layer and evaporated under N2 gas on an N-EVAP (Organamation Associates Inc, South Berlin, MA). The residue was dissolved in 100 $\mu$L ethanol, which formed a clear solution, and 20 $\mu$L was injected into an HPLC system. Concentrations of carotenoids and retinol in a 100-$\mu$L aliquot of serum were measured with an HPLC system equipped with a C18 column (SC-150; Bischoff Chromatography, Leonberg, Germany) and a Waters 2996 programmable photodiode array detector (Waters Corp, Milford, MA), and data were retrieved with the wavelength set at 450 nm for carotenoids and at 340 nm for retinoids for quantification (20). The total amounts of retinoids and carotenoids in serum (endogenous and labeled) were determined with the use of external calibration curves obtained from pure standards (from Sigma-Aldrich, St Louis, MO). Serum samples from each subject were analyzed for concentration of serum carotenoids, vitamin A, and vitamin E. Pooled spare-serum from previous human studies were used as quality control samples and were included in each batch of analysis to determine within-batch, interbatch, and day-to-day variations. Internal standards of retinyl acetate and echinenone were used to calculate recoveries of retinoids and carotenoids, respectively. Together with the percentage enrichment (see below), the amount of labeled retinol formed from the labeled $\beta$-carotene dose was determined.

**HPLC analysis of serum samples**

Under red light, serum samples stored at $-80^\circ$C were taken out and thawed until equilibration at room temperature before aliquots were prepared for analysis. Three milliliters of chloroform: methanol (2:1, vol:vol) was added to a 100-$\mu$L serum sample. The mixture was vortexed and then centrifuged for 10 min at 4°C and at 3000 rpm. The chloroform layer was collected. Hexane (2 mL) was added to the aqueous layer to re-extract the fat-soluble compounds. The hexane layer was combined with the chloroform layer and evaporated under N2 gas on an N-EVAP (Organamation Associates Inc, South Berlin, MA). The residue was dissolved in 100 $\mu$L ethanol, which formed a clear solution, and 20 $\mu$L was injected into an HPLC system. Concentrations of carotenoids and retinol in a 100-$\mu$L aliquot of serum were measured with an HPLC system equipped with a C18 column (SC-150; Bischoff Chromatography, Leonberg, Germany) and a Waters 2996 programmable photodiode array detector (Waters Corp, Milford, MA), and data were retrieved with the wavelength set at 450 nm for carotenoids and at 340 nm for retinoids for quantification (20). The total amounts of retinoids and carotenoids in serum (endogenous and labeled) were determined with the use of external calibration curves obtained from pure standards (from Sigma-Aldrich, St Louis, MO). Serum samples from each subject were analyzed for concentration of serum carotenoids, vitamin A, and vitamin E. Pooled spare-serum from previous human studies were used as quality control samples and were included in each batch of analysis to determine within-batch, interbatch, and day-to-day variations. Internal standards of retinyl acetate and echinenone were used to calculate recoveries of retinoids and carotenoids, respectively. Together with the percentage enrichment (see below), the amount of labeled retinol formed from the labeled $\beta$-carotene dose was determined.

**Gas chromatography–electron capture negative chemical ionization–MS and LC–atmospheric pressure chemical ionization–MS analysis**

To determine the percentage enrichment of labeled retinol, 400 $\mu$L of the serum sample was extracted by following the procedure described in the section “HPLC analysis of serum samples” (21). The extract was injected into an HPLC apparatus equipped with a C18 column (Perkin-Elmer Inc, Norwalk, CT) (21). The retinol collected from the HPLC system was dried under nitrogen gas, and the residue was derivatized with $\sim$10 $\mu$L N,O-bis(trimethylsilyl) trifluoroacetamide that...
contained 10% trimethylchlorosilane (Pierce, Rockford, IL) for 30 min at 70°C to form retinyl trimethylsilyl ether (22). After cooling, 2–3 μL was injected into the gas chromatography (GC–MS) system. The GC–MS instrument was an Agilent 6890 with a 5973 Network Mass Selective Detector, and the GC column was a Zebron ZB-1MS capillary (Phenomenex Inc, Torrance, CA). Helium and methane were used as the carrier gas and reaction gas, respectively. The GC oven temperature was increased from 50°C to 220°C at a speed of 15°C/min, to 230°C at 5°C/min, and to 310°C at 20°C/min; then it remained at 310°C for 5 min before it was cooled down to 50°C. This method produced a trans retinol peak at 12 min. The mass spectrometer was set at a mass-to-charge ratio (m/z) of 260–280. The linearity of the GC–MS response and the detection limit of the GC–electron capture negative chemical ionization–MS system have been addressed previously (22).

To determine the percentage enrichment of labeled β-carotene in yellow maize and in human serum, an LC–atmospheric pressure chemical ionization–MS analysis of m/z at (M+H)+ of 537, 538 ([13C]), 539 ([13C]2), 540 ([13C]3), 541 ([13C]4), 542 ([13C]5), 543 ([13C]6), 544 ([13C]7), and 545 ([13C]8) was conducted (23). The molecular mass of unlabeled β-carotene in positive-mode LC–MS analysis is 537 (M+H)+, 538 (M+H+, [13C]1–β-C), 539 (M+H+, [13C]2–β-C). In this study the yellow maize β-carotene had the highest enrichment abundance of [2H]β-carotene at 546 (M+H++9), and the deuterium enrichment was randomly distributed to all possible positions of the β-carotene molecule as determined by Nuclear Magnetic Resonance (24). This intrinsic labeling (partial replacement of protons with deuterium with a random distribution in carotenoids) enables an easy differentiation of isotopeomers from yellow maize plant food and endogenous, unlabeled carotenoids and their cleavage products. The labeled β-carotene isomers had a mass range of from 540 (M+H+++3) to 553 (M+H+++16). However, to ensure optimal detection of labeled β-carotene and labeled retinol in serum, we selectively monitored only the labeled β-carotene isomers 544 (M+H+++7), 545 (M+H+++8), 546 (M+H+++9), 547 (M+H+++10), and 548 (M+H+++11), along with labeled Mretinol + 4 and Mretinol + 5, corresponding to the predominant retinol isotopeomers cleaved from these labeled β-carotene isomers. Because the m/z 544–548 isomers of labeled β-carotene represent 60% of the entire yellow maize β-carotene [i.e., mass 540 (M+H+++3) to mass 553 (M+H+++16)], an adjustment of 0.6 was used for the total enrichment of retinol from the labeled maize β-carotene. The percentage enrichments measured by GC–MS and the concentration of retinol in serum were used to calculate the concentration of labeled retinol in the circulation. The m/z of Mretinol +4 and Mretinol +5 = m/z 272 and 273 could be clearly detected and integrated to represent the formation of retinol from the labeled maize β-carotene.

Enrichment of labeled retinol from yellow maize β-carotene (25):

\[
\frac{\sum \text{areas of } m/z \ 272 - 273/60\%}{\sum \text{areas of } m/z \ 268 - 280} = (L)
\]

GC–electron capture negative chemical ionization–MS was used to measure retinol enrichment from the reference dose (Mretinol +10 m/z 278) and yellow maize (Mretinol +4 and Mretinol +5 = m/z 272 and 273, respectively).

Because the in vivo conversion of β-carotene to vitamin A is a dynamic process that combines the absorption, conversion, and metabolism of β-carotene, an appropriate way to determine the amount of labeled retinol (vitamin A) from the labeled yellow maize β-carotene is to use a reference dose with a known amount of vitamin A that is differentially labeled. In this study, 1 mg of [13C10]retinyl acetate was used as a reference dose and it was given a week after the yellow maize dose. [Here, Mretinol − H2O equals m/z 268 and M13C10retinol equals m/z 268 + 10 = m/z 278 (22).] The total enrichment of labeled retinol was determined by evaluation of the negative ions at Mretinol [m/z 268–270 ([13C10]–13C2), Mretinol + 4 and Mretinol + 5 [m/z 272–273 ([13C17]H2)] and Mretinol +10 [m/z 278–280 ([13C10]–13C2)]. The retinol m/z values are reduced by the mass of H2O, because a water molecule equivalent is removed from retinol during ionization in the mass spectrometer (22). The percentage enrichment of labeled retinol derived from [13C10]retinyl acetate was calculated by integrating the peak area under the reconstructed mass chromatogram of the negative ions at m/z 278, 279, and 280, divided by the total area response of labeled and unlabeled retinol fragment ions, as shown by Equation 2:

\[
\text{Enrichment of } ^{13}\text{C} \text{retinol from } [^{13}\text{C}_{10}] \text{ retinyl acetate} (25):
\]

\[
= \left( \sum \text{areas of } m/z \ 278 - 280 / \sum \text{areas of } m/z \ 268 - 280 \right)
\]

Areas under the curve of labeled retinol [2H] and [13C10] retinol in the serum

Total serum responses (nmol) to the [2H]β-carotene dose and the [13C10],retinyl acetate dose were determined by multiplying the total serum volume (estimated at 0.0435 L per kilogram body weight) by the concentration of [2H]β-carotene and [2H] retinol and [13C10] retinol in the circulation (nmol/L, determined for each time point of serum sampling by adding of all of the enrichment masses). Areas under the curve (AUC) in nmol · d for the serum labeled retinol responses after the ingestion of [2H] β-carotene dose and the [13C10] retinyl acetate dose were calculated with the use of the curves of total serum responses (expressed as nmol on the y axis) compared with time (expressed as d on the x axis) via Integral-Curve of Kaleidagraph (Synergy Software, Reading, PA). The conversion of the AUC unit from “nmol · d” to “μg · d” was done with the use of Mretinol = 291 for [2H]retinol and Mretinol = 296 for [13C10]retinol. Because of the 7-d delay in the administration of the retinyl acetate dose, the AUCs were calculated for 21 d after each labeled tracer.

Retinol equivalence calculations

The retinol equivalence was calculated by comparison of the AUC of serum [2H]retinol response from labeled yellow maize β-carotene (nmol) with the AUC of 1 mg [13C10]serum response, as shown by Equation 3.

\[
[2H] \text{retinol from } [^{13}\text{C}_{10}] \text{yellow maize } \beta \text{-carotene (nmol)} (25):
\]
TABLE 2

Subjects’ fasting serum concentrations of carotenoids, vitamin A, and vitamin E at baseline.

<table>
<thead>
<tr>
<th>Serum analyte</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis Lutein</td>
<td>4.1 ± 2.6</td>
</tr>
<tr>
<td>Lutein</td>
<td>33.9 ± 21.3</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>2.7 ± 0.7</td>
</tr>
<tr>
<td>Cryptoxanthin</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>2.3 ± 2.1</td>
</tr>
<tr>
<td>13 cis β-Carotene</td>
<td>10.8 ± 11.8</td>
</tr>
<tr>
<td>all-trans-β-Carotene</td>
<td>32.9 ± 17.5</td>
</tr>
<tr>
<td>Lycopene</td>
<td>19.4 ± 10.1</td>
</tr>
<tr>
<td>Retinol</td>
<td>59.2 ± 17.1</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>93.0 ± 38.6</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>896.2 ± 341.9</td>
</tr>
</tbody>
</table>

All values are means ± SDs. n = 8 subjects aged 48.4 ± 10 y (range: 40–67 y) with a BMI (in kg/m²) of 22.4 ± 3.1, respectively. The major carotenoids detected in serum were lutein, cis-lutein isomers, zeaxanthin, cryptoxanthin, α-carotene, all-trans-β-carotene, and 13 cis-β-carotene isomers. The mean value for baseline all-trans-β-carotene was 32.9 μg/dL (range: 8.8–57.2 μg/dL) and for baseline vitamin A (retinol) was 59.2 μg/dL (range: 39.5–93.1 μg/dL) for the 8 subjects.

RESULTS

Serum analyte at baseline

TABLE 2. The mean age and BMI were 48.4 ± 10.0 y and 22.4 ± 3.1, respectively. The major carotenoids detected in serum were lutein, cis-lutein isomers, zeaxanthin, cryptoxanthin, α-carotene, all-trans-β-carotene, and 13 cis-β-carotene isomers. The mean value for baseline all-trans-β-carotene was 32.9 μg/dL (range: 8.8–57.2 μg/dL) and for baseline vitamin A (retinol) was 59.2 μg/dL (range: 39.5–93.1 μg/dL) for the 8 subjects.

The carotenoid concentration of labeled yellow maize flour was determined before and after cooking by HPLC with a C30 column (Figure 2, Table 1). The main carotenoids in this labeled yellow maize were shown to be lutein, zeaxanthin, β-cryptoxanthin, 13 cis-β-carotene, all-trans-β-carotene, and 9 cis-β-carotene. To confirm the concentration of total provitamin A carotenoids in the yellow maize, a C18 column that could separate α- and β-cryptoxanthin was used in the HPLC system. No α-cryptoxanthin (which lacks vitamin A activity) was detected in the labeled yellow maize used in this study. Therefore, the cryptoxanthin detected in the HPLC with the C30 column was β-cryptoxanthin. The yellow maize was also analyzed on receipt from Houston by LC-MS to determine the actual enrichment of labeled β-carotene at m/z = 536–558. The mass of unlabeled β-carotene was detected at m/z 537 (M+ H+) at a minimum amount (<1%). The highest abundance of enrichment was detected at m/z 546 (M+H⁺)+9 for labeled [2H]β-carotene (Figure 3). The enrichment profile and percentage enrichment were similar in the harvests of June and October 2006. Our results show that some intact [2H]β-carotene from the yellow maize dose was absorbed into the circulation (Figure 4). Our results also show that all 8 subjects converted labeled yellow maize [2H]β-carotene to [2H]vitamin A and absorbed and converted the [13C10]retinyl acetate that was given as a reference dose to [13C10]retinol. The serum retinol response to the labeled [2H]β-carotene and [13C10]retinyl acetate reference dose peaked after the administration of the yellow maize dose on day 1 and after the retinyl acetate dose on day 8 of the study (Figure 5).

The AUC for the serum labeled [2H]retinol and [13C10]retinol response curves (nmol · d), calculated over 21 d after the [2H]β-carotene dose and the [13C10]retinyl acetate dose, are presented in Table 3. The conversion factor and vitamin A equivalence were calculated by comparison of the AUC of [2H]retinol derived from the yellow maize dose with the AUC [13C10]retinol derived from the retinyl acetate reference dose over 21 d after the dose. The mean AUC for yellow maize dose [2H]retinol was 72.9 nmol · d and

Conversion factor calculations

The conversion factor of β-carotene to retinol by weight (25)

\[ (\text{β-carotene dose in yellow maize (nmol)} \times (539 + 9))/((\text{2H} \text{retinol from β-carotene dose (nmol)} \times (286 + 5)) \]

Statistical analysis

Descriptive statistics on age, body mass index (BMI; in kg/m²), serum carotenoids, retinoids, and tocopherols were performed with the use of SPSS, version 15.0.1 (SPSS Inc, Chicago, IL).

RESULTS

All enrolled subjects completed the study successfully. The subjects’ characteristics and baseline fasting serum concentrations of carotenoids, vitamin A, and vitamin E are presented in Table 2.
was 161.1 nmol · d for the reference dose [13C10]retinol. The calculated conversion factor of yellow maize [2H]β-carotene (1.2 mg) to vitamin A ([2H]retinol) was 3.2 ± 1.5 to 1 by weight, and the range among subjects was 1.5–5.3 to 1 by weight. A conversion factor of 3.2 to 1 by weight means that 0.38 mg of retinol can be derived from 1.2 mg of yellow maize all-trans-β-carotene equivalents.

**DISCUSSION**

In this study, the baseline characteristics of the subjects were within the expected normal values for their age and sex. The low initial serum carotenoid concentrations reflect a low intake of fruit and vegetables, which suggests that subjects complied with the study protocol, which required a 14-d abstinence from high-carotenoid and high–vitamin A foods. None of the 8 subjects

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**FIGURE 3.** Deuterium enrichment profiles of high–β-carotene yellow maize line DEexp × CI7 − F1 by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (positive ion mode) for June (A) and October 2006 (B) harvests. Hydroponic labeling produces a range of isotopeomers. The most abundant isotopeomer of labeled β-carotene with 9 deuterium atoms is represented by a mass-to-charge ratio (m/z) of 546 (M⁺H⁺²H₉). The first arrow on each profile points to the 537 peak, showing that the molecular mass of unlabeled β-carotene is 537 (M⁺H⁺). The second arrow on each profile points to peak 546 (M⁺H⁺²H₉), showing the highest abundance of enrichment.

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**FIGURE 4.** Chromatography and spectrum of labeled all-trans-β-carotene in serum of a representative subject 48 h after a yellow maize dose made from yellow maize line DEexp × CI7 − F1. A: The arrow points to the ultraviolet-visible spectrum of all-trans-β-carotene in the circulation, corresponding to the peak of all-trans-β-carotene in B. B: The HPLC chromatogram for the serum extract. C: The small arrow points to the 537 (M⁺H⁺) peak, showing the molecular mass of endogenous β-carotene, and the second, longer arrow shows the β-carotene enrichment peak at 546 (M⁺H⁺²H₉) from the dose of yellow maize β-carotene that was absorbed as intact β-carotene into the serum (collected 2 d after the yellow maize dose and analyzed by liquid chromatography–mass spectrometry with atmospheric pressure chemical ionization and the use of a positive ion mode). mAU, mini absorbance unit.
were vitamin A deficient as defined by the World Health Organization (<20 µg/dL or <0.07 nmol/L) (26).

The carotenoid profile of this yellow maize line is similar to what is expected from the carotenoid biosynthetic pathway in yellow maize (27–29). With the use of LC-atmospheric pressure chemical ionization–MS (positive mode), intact labeled [\(^{2}\text{H}\)] β-carotene was detected in 3 mL of serum pooled from a few 48-h time-point samples (several subjects had leftover serum samples), which were collected after the labeled yellow maize dose (Figure 4). It was difficult to detect [\(^{2}\text{H}\)]β-carotene at later time points (ie, on day 3 to day 36 after consumption of the labeled yellow maize dose). Thus, most of the yellow maize [\(^{2}\text{H}\)]β-carotene (1.2 mg) was converted to retinol in the intestine and very little was converted after absorption. In a recent human study, the absorption of intact β-carotene was also shown to be very minimal after consumption of biofortified maize (18). Other studies that used similar techniques have detected post-absorption intact β-carotene when vegetables or spirulina were fed that contained higher amounts of β-carotene in the dose (≥4.5 mg β-carotene), relative to this study (1.2 mg maize β-carotene) (30, 31).

In Zimbabwe, maize is consumed ≥5 times/wk, and the daily intake by adults is ≈330 g/d (13, 32). In this study, sadza was made from seeds of a yellow maize breeding line that contained 1.2 mg of all-trans-β-carotene equivalents in a 300-g (cooked) serving. The all-trans-β-carotene equivalents in this study were defined as the sum of all provitamin A carotenoids with β-cryptoxanthin, 9 cis-β-carotene, and 13 cis-β-carotene contributing half the value of all-trans-β-carotene (33). Conflicting results have been observed in animal studies and human studies that tried to determine the vitamin A value of β-carotene isomers. Studies that used gerbils showed that the retinol activity equivalent of 1 µg retinol was 12 µg, 19 µg, and 32 µg for all-trans-β-carotene, 9 cis-β-carotene, and 13 cis-β-carotene, respectively (34). Others that used a Caco-2 model showed that the all-trans-isomer of β-carotene is preferentially taken up over the cis-isomer (35, 36).

In this study, the average conversion factor for a 1.2-mg dose of labeled yellow maize [\(^{2}\text{H}\)]β-carotene to labeled [\(^{2}\text{H}\)]retinol was 3.2 ± 1.5 to 1 by weight. The conversion factor of yellow maize β-carotene to retinol was 1.5–5.3 to 1 by weight among the 8 subjects. The US Recommended Dietary Allowances of vitamin A for nearly all reference men and women are 900 µg and 700 µg retinol activity equivalents, respectively (33). Thus, 1.2 mg yellow maize all-trans-β-carotene equivalents (in a 300-g cooked serving of maize) can provide an equivalent of 0.38 mg (380 µg) of retinol, or 40–50% of the US Recommended Dietary Allowance for adults. For preschool children with a Recommended Dietary Allowance of 400 µg retinol activity equivalents, 150 g cooked yellow maize sadza would be able to provide 50% of their daily requirement for vitamin A. This shows that high-β-carotene yellow maize is a good source of vitamin A in humans. In genetically enriched Golden Rice consumed by American adults, the conversion factor of the rice β-carotene to retinol in the cooked rice was comparable to that of maize β-carotene (3.8 to 1 in rice compared with 3.2 to 1 in maize) by weight, with the use of similar stable isotope enrichment techniques (37).

A recent study that used a biofortified yellow maize line and a plasma triacylglycerol-rich lipoprotein fraction technique in American women showed a conversion factor of 6.48 to 1 by weight (18). The differences in conversion factors between this study and our study with a normal vitamin A status (mean serum retinol concentration of 59.2 µg/dL, with a range of 39.5 to 93.1 µg/dL) could be explained by differences in study techniques, the sex of the subjects (our subjects were all male), or our study population’s genetic make-up, among other factors. It was shown recently that genetic polymorphisms in the BCMO1 gene result in variations in β-carotene conversion to vitamin A (38).

### TABLE 3

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>AUC of [(^{2}\text{H})]retinol</th>
<th>Conversion factor By weight</th>
<th>Conversion factor By mol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>By weight</td>
<td>By mol</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>125.9</td>
<td>133.2</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
<td>107.7</td>
<td>393.7</td>
<td>5.3</td>
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<td>116.8</td>
<td>326.9</td>
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</tr>
<tr>
<td>7</td>
<td>36.6</td>
<td>42.5</td>
<td>1.7</td>
</tr>
<tr>
<td>8</td>
<td>34.1</td>
<td>89.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>72.9 ± 43.7</td>
<td>161.1 ± 128.3</td>
<td>3.2 ± 1.5</td>
</tr>
<tr>
<td>Range</td>
<td>23.2–125.9</td>
<td>42.5–393.7</td>
<td>1.5–5.3</td>
</tr>
</tbody>
</table>

FIGURE 5. Calculated labeled retinol in the circulation of a representative volunteer during the study period after consumption of labeled yellow maize β-carotene on day 1 and a reference dose of [\(^{1}\text{3}\text{C}_{10}\)] retinyl acetate on day 8. The continuous line and solid-circle data points show the serum [\(^{2}\text{H}\)]retinol response of a subject after consumption of [\(^{2}\text{H}\)] yellow maize β-carotene on day 1 of the study. The dashed line and solid-triangle data points show serum [\(^{1}\text{3}\text{C}_{10}\)] retinol after consumption of a labeled reference dose of [\(^{1}\text{3}\text{C}_{10}\)] retinyl acetate on day 8 of the study. The retinol in circulation measured in nanomoles is shown on the y axis, and time in days is shown on the x axis.
Animal studies have shown mixed results. Studies with Mongolian gerbils that were fed high-β-carotene biofortified maize showed conversion factors of 2.8 and 2.9 mg β-carotene to 1 mg retinol for groups fed high-β-carotene maize and β-carotene supplement, respectively (39). In pig studies, a conversion efficiency of yellow maize β-carotene to vitamin A of 40:1 on a weight basis was shown, whereas in other studies 1 mg corresponded to 37–52 μg (19:1–27:1 by weight) when 10 mg of yellow maize β-carotene per kilogram of diet was fed (40–42). The bioavailability of β-carotene and the conversion of β-carotene to vitamin A are affected by many factors, some of which are metabolic and some of which are study design related (30, 31, 42, 43). However, the stable isotope enrichment technique used in this study is currently the most direct and reliable method of determining the bioavailability of carotenoids and the bioconversion of provitamin A carotenoids into vitamin A.

Several factors can explain the good conversion factor observed in our study. The yellow maize dose given to the subjects was cooked by the addition of yellow maize flour into boiling water to produce sadza (17). The milling of the maize kernel into flour and subsequent cooking may increase the accessibility of maize food components to digestive enzymes and facilitate the release of carotenoids for absorption by the intestine (44–46). The amount of dietary fat consumed with the dose (20 g butter, 0.5 g corn oil) may also have contributed to the good conversion efficiency shown in this study. Several studies support the fact that dietary fat enhances the absorption and transport of β-carotene (47, 48). Also, maize naturally contains oil in the germ (49, 50).

It was shown from in vitro studies that in the presence of an antioxidant such as α-tocopherol, β-carotene is converted exclusively to retinol by central cleavage (ie, 2 molecules of retinol are formed from one molecule of β-carotene), but in the absence of an antioxidant, β-apo-carotenoids are produced (51). In this study the labeled yellow maize dose contained an average of 24.1 ± 4.5 μg/g γ-tocopherol and 18.5 ± 4.0 μg/g α-tocopherol (dry weight). The coexistence of tocopherols could also have played a role in the increase of the bioavailability and bioconversion of this maize β-carotene to vitamin A.

This study shows that biofortified yellow maize rich in β-carotene might be recommended as an efficient food source to combat VAD in countries where maize is already a staple food and where VAD is a public health problem. Our study was done with 8 Zimbabwean men. Of the 8 subjects, 6 were black, one was Indian, and one was white. There were no significant differences in the conversion factors among the races. Therefore, it would be useful to repeat the study with larger sample sizes with different groups of people who vary by age, sex, genetic background, vitamin A status, and disease status, including investigations on genetic variability in single-nucleotide polymorphisms of relevant genes.

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The authors’ responsibilities were as follows—GT: designed the study and supervised the data analysis; GT and TM: wrote the manuscript; TM: coordinated the study and collected and analyzed samples; AHS: mobilized subjects and supervised the sample collection; AHS, THG, MAG, and RMR: revised the manuscript; THG: assisted with the study design; MAG: designed the production methods for the labeled yellow maize, and grew and harvested the labeled yellow maize for the study; and RMR: served as the study physician. The authors did not declare any conflicts of interest.

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