Golden Rice is an effective source of vitamin A1–4

Guangwen Tang, Jian Qin, Gregory G Dolnikowski, Robert M Russell, and Michael A Grusak

ABSTRACT
Background: Genetically engineered “Golden Rice” contains up to 35 μg β-carotene per gram of rice. It is important to determine the vitamin A equivalency of Golden Rice β-carotene to project the potential effect of this biofortified grain in rice-consuming populations that commonly exhibit low vitamin A status.

Objective: The objective was to determine the vitamin A value of intrinsically labeled dietary Golden Rice in humans.

Design: Golden Rice plants were grown hydroponically with heavy water (deuterium oxide) to generate deuterium-labeled [2H]β-carotene in the rice grains. Golden Rice servings of 65–98 g (130–200 g cooked rice) containing 0.99–1.53 mg β-carotene were fed to 5 healthy adult volunteers (3 women and 2 men) with 10 g butter. A reference dose of [13C10]retinyl acetate (0.4–1.0 mg) in oil was given to each volunteer 1 wk before ingestion of the Golden Rice dose. Blood samples were collected over 36 d.

Results: Our results showed that the mean (±SD) area under the curve for the total serum response to [2H]retinol was 39.9 ± 20.7 μg·d after the Golden Rice dose. Compared with that of the [13C10]retinyl acetate reference dose (84.7 ± 34.6 μg·d), Golden Rice β-carotene provided 0.24–0.94 mg retinol. Thus, the conversion factor of Golden Rice β-carotene to retinol is 3.8 ± 1.7 to 1 with a range of 1.9–6.4 to 1 by weight, or 2.0 ± 0.9 to 1 with a range of 1.0–3.4 to 1 by moles.

Conclusion: β-Carotene derived from Golden Rice is effectively converted to vitamin A in humans. This trial was registered at clinicaltrials.gov as NCT00680355. Am J Clin Nutr 2009;89:1776–83.

INTRODUCTION
The intake of vitamin A provides humans with an important nutrient for vision, growth, reproduction, cellular differentiation and proliferation, and integrity of the immune system. Vitamin A deficiency can result in visual or ocular malfunctions such as night blindness and xerophthalmia (1) and can reduce immune responsiveness (2), which can result in an increased incidence or severity of respiratory infections, gastrointestinal infections (3), and measles (4). Vitamin A can be obtained from food, either as preformed vitamin A in animal products (eg, eggs and dairy products) or as provitamin A carotenoids, mainly β-carotene in plant products (eg, dark-green leafy vegetables and fruit).

Clinical and subclinical vitamin A deficiency is still a problem, affecting 250 million schoolchildren worldwide (5, 6). To prevent clinical vitamin A deficiency in developing countries, chemically synthesized vitamin A supplements have been distributed periodically to deficient populations (7–9). This has been shown to be an efficient and generally safe strategy. However, supplementation programs with a periodic mass distribution have been difficult to sustain because of high distribution costs. Recently, food-based interventions to increase the availability of provitamin A–rich foods and their consumption have been suggested as a realistic and sustainable alternative to overcome vitamin A deficiency globally (10). However, the efficacy of carotenoid-rich foods in the prevention of vitamin A deficiency has been questioned in several recent studies, which reported little or no nutritional benefit of vitamin A from the increased consumption of dark-green or yellow vegetables (11, 12). Recently, studies have shown that the equivalency of vegetable provitamin A carotenoids to vitamin A is in the range of 10–27 μg all-trans β-carotene to 1 μg retinol activity (13–16). These studies showed that food matrices greatly affect the bioavailability of vitamin A and carotenoids.

In recent years, scientists have introduced the biosynthetic pathway for provitamin A carotenoids into staple foods, including genetically engineered Golden Rice, which contains 1.6–35 μg β-carotene per gram of dry rice. Golden Rice–1, which was transformed with a construct containing a phytoene synthase gene from daffodil, contains 1.6 μg carotenoids (0.8 μg β-carotene) per gram of dry rice (17). Golden Rice–2 was transformed with a construct containing a phytoene synthase gene from maize and contains up to 35 μg β-carotene per gram of dry rice (18). Because the vitamin A equivalency of various foods and supplements varies from 2 μg β-carotene to 1 μg retinol (when provided as a β-carotene supplement in oil) to 27 μg β-carotene to 1 μg retinol (when provided as vegetable β-carotene) (11, 13), and this equivalency is matrix dependent, it is important to de-
termine the vitamin A equivalency of β-carotene from Golden Rice. This information is critical for the purpose of designing informed, food-based nutritional strategies for rice-eating regions throughout the world where vitamin A deficiency is common. Because vitamin A is homeostatically regulated in the circulation of healthy subjects and it is impossible to distinguish the newly formed vitamin A from endogenous vitamin A (19), we chose intrinsic labeling of the provitamin A carotene as the optimal approach to determine its vitamin A equivalence. We produced intrinsically labeled Golden Rice, fed the rice to healthy volunteers, and used an isotope reference method to determine the conversion factor of Golden Rice β-carotene to vitamin A.

MATERIALS AND METHODS

Production of intrinsically labeled Golden Rice

Rice seeds were imbibed and germinated on cheesecloth suspended over distilled water. After 4 d, seedlings were planted in trays suspended over 20-L tubs of nutrient solution containing the following macronutrients: KNO₃, 1 mmol/L; KH₂PO₄, 1 mmol/L; Ca(NO₃)₂, 1 mmol/L; MgSO₄, 1 mmol/L; K₂SiO₄, 0.1 mmol/L; CaCl₂, 25 μmol/L; H₂BO₃, 25 μmol/L; MnSO₄, 2 μmol/L; ZnSO₄, 2 μmol/L; CuSO₄, 0.5 μmol/L; H₂MoO₄, 0.5 μmol/L; and NiSO₄, 0.1 μmol/L. No deuterium oxide was added. Iron was added in chelated form as Fe(III)HEDTA (N-hydroxyethyl-ethylenediamine-triacetic acid) at 20 μmol/L. MES buffer (adjusted with potassium hydroxide) was added at 2 mmol/L to maintain the nutrient solution pH between 5.4 and 5.8. Plants were maintained on this solution until flowering (~5.5 mo after planting) in a greenhouse in Houston, TX; the nutrient solution was topped off, as needed, with a refill solution containing the same macronutrients as above, but without additional MES buffer or K₂SiO₄. A complete change-out of the solution (using starting solution) was performed at 6-wk intervals. Approximately 7 d after flowering, the plants were transferred to a new nutrient solution containing 23 atom%²H₂O, 20 μmol/L Fe(III)HEDTA, 2 mmol/L MES buffer, and the following macronutrients: KNO₃, 5 mmol/L; KH₂PO₄, 2 mmol/L; Ca(NO₃)₂, 2 mmol/L; MgSO₄, 1 mmol/L; K₂SiO₄, 0.1 mmol/L; CaCl₂, 25 μmol/L; H₂BO₃, 25 μmol/L; MnSO₄, 2 μmol/L; ZnSO₄, 2 μmol/L; CuSO₄, 0.5 μmol/L; H₂MoO₄, 0.5 μmol/L; and NiSO₄, 0.1 μmol/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 Golden Rice β-carotene (determined empirically in pilot studies). At this time, plants were also placed in a clear plastic-walled labeling system (Figure 1), which maintained an elevated ²H₂O concentration in the gas atmosphere surrounding the plants and panicles. Plants were maintained on this media, with the solution topped off as needed (using the same ²H₂O solution) until the panicles had matured (~3 wk later). During this labeling period, the temperature within the labeling system was maintained between 26 and 31°C, and the relative humidity was maintained between 45% and 55%. At maturity, whole panicles were collected and stored at −20°C until further processing. For processing, seeds were de-hulled with an Impeller Husker (model FC2K; Yamamoto Co, Ltd, Yamagata-ken, Japan); seeds were subsequently polished in small batches for 30 s with an electric grain polisher (model “Pearles;” Kett Electric Laboratory, Tokyo, Japan). Polished seeds were stored at −80°C until shipped to Boston, where they were cooked and analyzed before the clinical studies.

**Rice preparation**

The rice was cooked by using a rice cooker (SR-G18FG; Panasonic, Chachoengsao, Thailand) by adding water in a quantity of 150% weight of the rice. The rice was cooked for 30 min. Our analysis showed that the total amount of Golden Rice β-carotene in the dose was the same before and after it was cooked (0.99 or 1.53 mg β-carotene in a dose). The cooked rice was divided into portions (130 g cooked rice containing 0.99 mg β-carotene or 200 g cooked rice containing 1.53 mg β-carotene) and kept at −15°C until served within 1–3 mo. On the day of feeding, the rice was brought to room temperature and then heated with a microwave oven [60 s by using a Panasonic NN-Sensor 953 (Genius) 1350-W microwave oven, 2.2 cubic feet capacity].

**Volunteers and study design**

The study protocol was approved by the Tufts Medical Center Institutional Review Board. Persons who had not taken vitamin A or β-carotene supplements within the past month and who were not disqualified based on several exclusion criteria were eligible to become study volunteers. Potential subjects were accepted...
tested the use of 0.43 mg [13C10]retinyl acetate as a reference dose for the other 4 volunteers to ensure successful detection of labeled retinol in each volunteer, even those with a higher body mass. The [13C10]retinyl retinyl acetate in an oil capsule was given together with 200 g cooked white rice, 10 g butter, 50 g peeled cucumbers, 0.2 g salt, 5 g vinegar, and a 500-mL bottle of water at breakfast (time 0). The total calorie content of the meal was ~450 kcal (25% from fat). A second standardized meal (lunch) was eaten by all volunteers 4 h after the breakfast meal; this second meal contained 60 g turkey meat, 50 g white bread, 20 g roasted cashew, and 100 g cucumber (peeled) salad with 15 g corn oil and 5 g vinegar (total energy: 600 kcal, 40% from fat). On day 8 of the study, the volunteers consumed the same breakfast meal as on day 1, but 200 g white rice was replaced with labeled Golden Rice (either 130 g cooked Golden Rice together with 70 g cooked white rice containing 0.99 mg β-carotene or 200 g cooked Golden Rice containing 1.53 mg β-carotene). Also on day 8, the standardized lunch (as above) was eaten by all of the volunteers 4 h after the breakfast meal. The amount of Golden Rice in the breakfast meal varied because we were trying to study as many subjects as possible with a limited amount of intrinsically labeled rice. Our results showed that our method can effectively assess the vitamin A equivalency of β-carotene doses as low as 1 mg in rice.

A total of 30 serum samples (10 mL each) were obtained from each subject at the following time points: day 1 at 0 (just before the breakfast dose), 5, 8, 11, and 13 h (after the dose); day 2 at 0 (24 h after the dose taken before the day 2 breakfast), 5, and 11 h (after the day 2 breakfast); day 3 at 0 (48 h after the dose taken before the day 3 breakfast) and 11 h (after the day 3 breakfast); days 4, 6, and 7 at 0 h (before each day’s breakfast); day 8 at 0 (before the Golden Rice breakfast dose), 5, 8, 11, and 13 h (after the Golden Rice dose taken); day 9 at 0 (24 h after the dose taken and before the day 9 breakfast), 5, and 11 h (after the breakfast); day 10 at 0 (48 h after the dose taken and before the day 10 breakfast) and 11 h (after the day’s breakfast); days 11, 13, 15, 19, 22, 29, and 36 at 0 h (before each day’s breakfast) (Figure 2). Fasting serum samples were collected at the 0-h time points. The serum samples were kept at −70°C until analyzed. The retinol (Mretinol + 10) derived from the [13C10]retinyl acetate dose, the retinol (Mretinol + 5) formed from the labeled Golden Rice β-carotene, and the intact Golden Rice β-carotene (M + 9) were followed in all samples up until the end of the study.

[13C10] Vitamin A as an isotope reference

To quantify the amount of vitamin A formed from the Golden Rice β-carotene, a known amount of vitamin A that is differently labeled can be used as a reference dose. We used 1 mg [13C10]vitamin A [in the present study: Mretinol = retinol − H2O = m/z (mass/ charge, a unit for mass spectrometry) 268, M [13C10]retinol = m/z 268 + 10 = m/z 278] in an oil capsule as a reference dose given 1 wk before the Golden Rice meal. Our initial test showed that our method can trace the vitamin A body response after ingestion of 0.43 mg [13C10]vitamin A—a physiologic dose.

**FIGURE 2.** Experimental design for the Golden Rice human study. Three sets of dashed lines are used to amplify the overall time period to progressively larger scales. The first order (upper scale) includes the entire 36-d study and sampling period. The second order (middle scale) represents days 1–15 of more frequent blood sampling. Each cell on the horizontal time axis represents a 24-h period, with the division line representing the fasting blood collection time (0 h). The cells in black represent days 1 and 8, the days on which the tracers were ingested. The third order (lower scale) is common for these 2 dosing and high-multiple blood-sampling days (days 1 and 8), with each cell on the time axis representing 1 h. The 0 time represents the first blood sampling of the day, and all other numbers represent the times of subsequent sampling (in h) relative to the time of first sampling. The anchor symbols represent the oral dosing of the tracer. The arrows indicate the times at which blood samples were collected. d, day in the study; h, hour after study dose or after fasting blood sample, RAc, retinyl acetate.
Blood sample analysis

An HPLC instrument equipped with a C18 column was used to separate the serum retinol (20). The fractions containing retinol in the HPLC eluent were collected individually and derivatized for gas chromatography/electron capture negative chemical ionization–mass spectrometry (GC/ECNCI-MS) (21) to measure retinol enrichment from the reference vitamin A dose ($M_{\text{retinol}} + 10 = m/z \ 278$) or Golden Rice β-carotene ($M_{\text{retinol}} + 5 = m/z \ 273$) dose. The total enrichment of labeled retinol was determined by the evaluation of negative ions at $M_{\text{retinol}} \ [m/z \ 268-270 \ (^{13}C_0 - ^{13}C_2)]$, $M_{\text{retinol}} + 5 \ [m/z \ 273-277 \ (^{2}H_4 - ^{2}H_8)]$, and $M_{\text{retinol}} + 10 \ [m/z \ 278-280 \ (^{13}C_0 - ^{13}C_2)]$. The whole enrichment of the retinol from the Golden Rice β-carotene was calculated as 2 times the sum of the enrichment of $M_{\text{retinol}} + 5$, $M_{\text{retinol}} + 6$, $M_{\text{retinol}} + 7$, $M_{\text{retinol}} + 8$, and $M_{\text{retinol}} + 9$ based on the assumption of the symmetric distribution of the labeled Golden Rice β-carotene.

Concentrations of serum carotenoids and retinoids were determined using HPLC equipped with a C30 column (22). For enrichment of intact β-carotene, liquid chromatography/atmospheric pressure chemical ionization–mass spectrometry (23) was used to determine the absorption of intact β-carotene after consumption of the cooked Golden Rice.

Areas under the curve of labeled retinol or β-carotene in the serum

Total serum responses (nmol) to the $[^2H]β$-carotene dose and the $[^{13}C_{10}]$retinyl acetate dose were determined by multiplying the total serum volume ($0.0435 \ \text{L/kg body wt}$) by the concentration of $[^2H]β$-carotene and $[^2H]$retinol and $[^{13}C_{10}]$retinol in the circulation (nmol/L, determined for each time point of serum sampling by adding all of the enrichment masses). Areas under the curve (AUCs) for serum labeled retinol or β-carotene responses (in nmol·d) after the $[^2H]$β-carotene dose and the $[^{13}C_{10}]$retinyl acetate dose were calculated by using the curves of total serum responses (in nmol; y axis) compared with time (in d; 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 by using $M_{\text{retinol}} = 291$ for $[^3H]$retinol and $M = 296$ for $[^{13}H]$retinol. Because of the 7-d delay in the administration of the Golden Rice dose, the AUCs were calculated for 21 d after each labeled tracer.

Retinol equivalence calculations

The AUC of serum $[^2H]$retinol response (from the labeled Golden Rice) was compared with the AUC of the vitamin A reference dose (0.4–1.0 mg $[^{13}C_{10}]$retinyl acetate; molecular mass = 336). The amount of $^2H$ retinol was calculated as follows:

$$[^2H]\text{Retinol formed from the β-carotene dose (nmol)} = \frac{\text{AUC of }[^2H]\text{retinol}/\text{AUC of }[^{13}C_{10}]\text{retinol}}{\left(\frac{[^{13}C_{10}]\text{retinyl acetate}}{336}\right)}$$

(1)

Conversion factor calculations

The amount of a given oral dose of Golden Rice β-carotene (0.99–1.53 mg) compared with the amount of vitamin A derived from the β-carotene dose was defined as the β-carotene to vitamin A conversion factor. Thus, the conversion factor of Golden Rice β-carotene (calculated β-carotene from all-trans β-carotene plus one-half of all other provitamin A carotenoids) to vitamin A was determined as follows:

Conversion factor of β-carotene to retinol (by weight)

$$= \frac{[^{13}C_{0}-^{13}C_{2}]\text{β-carotene in vegetable dose (nmol)}}{536} \times \frac{[^{13}C_{0}-^{13}C_{2}]\text{β-carotene dose (nmol) × 286}}{[^{13}C_{0}-^{13}C_{2}]\text{β-carotene dose (nmol)}}$$

(2)

where 536 is the molecular mass of β-carotene, and 286 is the molecular mass of retinol.

Statistical analyses

Statistical analyses was performed to assess the significance of differences between vitamin A conversion factors by sex and to determine correlations between conversion factors and the BMI of each subject. Systat version 10.2 (Systat Software Inc) was used for data analysis.
RESULTS

Confirmation of intrinsically labeled Golden Rice with enriched \( \beta \)-carotene

We grew Golden Rice hydroponically with mineral nutrients and introduced 23 atom% heavy water (\( ^2\)H\(_2\)O) to the hydroponic medium after flowering (Figure 1) to intrinsically label the Golden Rice \( \beta \)-carotene with deuterium (\( ^2\)H). Our HPLC analysis showed that, in the grains, all-trans \( \beta \)-carotene was the dominant carotenoid (\( \approx 20 \mu g \) \( \beta \)-carotene in a gram of dry rice) together with small amounts of lutein, anhydrolutein, zeaxanthin, cryptoxanthin, 13-cis \( \beta \)-carotene, and 9-cis \( \beta \)-carotene (Figure 3). With this labeling method, the intrinsically labeled Golden Rice \( \beta \)-carotene showed a protonated molecule of \( m/z \) of \( M_{\text{RC}} + H^+ = 536 + 1 \) (representing unlabeled \( \beta \)-carotene) and a range of isotopomers with the most abundant showing an enrichment of 9 deuterium at \( M_{\text{enrich-RC}} = M_{\text{RC}} + H^+ + 9 \) mass units (Figure 4) as analyzed by using the liquid chromatography/mass spectrometry with a positive atmospheric pressure chemical ionization interface (the total \( m/z \) was \( M_{\text{RC}} + 9 + H^+ = 536 + 9 + 1 = m/z \) 546) (23). This labeled Golden Rice \( \beta \)-carotene produces retinol with a most abundant peak mass at the ionized retinol plus 5 mass units derived from deuterium minus water (the result of ionization in the mass ionization chamber).

Characteristics of volunteers

Five healthy nonsmokers (2 men and 3 women; age: 41–70 y; BMI: 22–29) were recruited as study volunteers. The characteristics of volunteers are presented in Table 1. Concentrations of carotenoids and retinoids in serum samples collected at baseline and analyzed by using HPLC (22) are presented in Table 2. These data showed that the vitamin A and carotenoid concentrations of the volunteers were in the normal range.

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<td>73.6 ± 13.2</td>
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Blood response to a Golden Rice meal

Retinoids labeled at \( M_{\text{retinol}} + 5 \) or \( M_{\text{retinol}} + 10 \) (\( M_{\text{retinol}} = m/z \) 268, representing unlabeled endogenous retinol) were detected in serum extracts, as shown in the middle and bottom panels of Figure 5. The mass distribution of circulating retinol at baseline (day 1 at 0 h before the reference dose; top panel), the enrichment of \( M_{\text{retinol}} + 10 = m/z \) 278 from the reference dose \( ^{13}\)Cretinyl acetate (day 1 at 13 h after the dose; middle panel), and the enrichment of \( M_{\text{retinol}} + 5 = m/z \) 273 and \( M_{\text{retinol}} + 10 = m/z \) 278 retinol (day 9 at 0 or 24 h after the Golden Rice meal; bottom panel) are shown in the figure. The kinetic responses of labeled \( M_{\text{retinol}} + 5 = m/z \) 273 and \( M_{\text{retinol}} + 10 = m/z \) 278 retinol up to 32 d from one subject are presented in Figure 6.

The responses of the volunteers who consumed a reference dose on day 1 and a labeled Golden Rice dose (130 or 200 g cooked weight containing 0.99 or 1.53 mg labeled \( \beta \)-carotene, respectively) on day 8, together with 10 g butter, are presented in Table 3.

For Golden Rice \( \beta \)-carotene, analysis of serum samples by liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (23) showed that the labeled \( \beta \)-carotene was absorbed intact after consumption of the cooked Golden Rice. Serum response kinetics of intact \( \beta \)-carotene are presented in Figure 7.

Conversion factor of Golden Rice \( \beta \)-carotene to vitamin A

The AUC response of vitamin A (\( M_{\text{retinol}} + 5 \)) formed from the dose of labeled Golden Rice \( \beta \)-carotene was compared with the AUC of the reference \( ^{13}\)C\(_{10}\)vitamin A, up to 21 d (Table 3). The Golden Rice containing 0.99 mg \( \beta \)-carotene provided 0.24–0.51 mg retinol, and the Golden Rice containing 1.53 mg \( \beta \)-carotene provided 0.24–0.94 mg retinol. It should be noted that at these physiologic doses (0.99–1.53 mg \( \beta \)-carotene), it is unlikely that the differences in dose would influence the outcome of retinol equivalence per milligram of \( \beta \)-carotene. Acknowledging that we had a limited number of subjects (\( n = 5 \)), statistical analysis showed that there was no difference in the calculated conversion factors between subjects taking 0.99 or 1.53 mg Golden Rice \( \beta \)-carotene. Altogether, our results show that the conversion factor of Golden Rice \( \beta \)-carotene to retinol is 3.8 ± 1.7 (mean ± SD) to 1 with a range of 1.9–6.4 to 1 by weight, or 2.0 ± 0.9 to 1 with a range of 1.0–3.4 to 1 by mol, as presented in Table 3. The conversion factors between men (\( n = 2 \)) and women (\( n = 3 \)) were not different. In addition, in these 5 subjects, there was no correlation between the conversion factors and BMIs.

### Table 1

Characteristics of the 5 study subjects

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### Table 2

Carotenoid and retinol concentrations in the serum of each subject at the beginning of the study

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<th>( t )-( \beta )-C</th>
<th>9c-( \beta )-C</th>
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<td>0.06</td>
<td>0.13</td>
<td>0.08</td>
<td>0.17</td>
<td>0.17</td>
<td>2.24</td>
<td>0.03</td>
</tr>
<tr>
<td>SD</td>
<td>0.45</td>
<td>0.02</td>
<td>0.17</td>
<td>0.01</td>
<td>0.03</td>
<td>0.31</td>
<td>0.01</td>
<td>0.02</td>
<td>0.09</td>
<td>0.03</td>
<td>0.10</td>
<td>0.07</td>
<td>0.53</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\( ^1 \)Zeax, zeaxanthin; Crypt, cryptoxanthin; \( \beta \)-C, \( \beta \)-carotene; \( \pi \)-C, \( \pi \)-carotene; lyco, lycopene; \( e \), \( c \), \( t \), \( trans \); RE, retinyl esters.
Golden Rice is a bioengineered crop with yellow-colored endosperm that contains β-carotene (provitamin A). To produce Golden Rice, 2 enzymes are introduced into the endosperm [phytoene synthase (psy) and phytoene desaturase (crtl)] via an endosperm-specific glutelin (Gtl) promoter (15), to establish a β-carotene biosynthetic pathway in the rice grains. This is the first study on the vitamin A value of Golden Rice in humans, and our analysis showed a very efficient bioconversion of β-carotene to vitamin A (Table 3). Using a conversion factor in which 3.8 µg Golden Rice β-carotene provides 1 µg retinol, along with the level of Golden Rice β-carotene being 20–30 µg/g uncooked rice, we project that 100 g uncooked rice provides 500–800 µg retinol. This represents 80–100% of the estimated average requirement (EAR) for men and women and 55–70% of the Recommended Dietary Allowance (RDA, derived from the EAR) for men and women, as set by the US National Academy of Science (24). In the United States, the EAR and RDA for vitamin A were set based on the amount needed to provide 4 mo of vitamin A storage in the body. For children, additional study of Golden Rice β-carotene conversion to retinol is needed. However, we speculate that 50 g uncooked Golden Rice, which is a reasonable serving size for children aged 4–8 y in rice-eating regions, who eat ∼130–200 g rice/d (25), would be able to provide >90% of vitamin A EAR (275 µg retinol/d) or >60% of the RDA (400 µg retinol/d) (24).

In this study, we used a combination of state-of-the-art approaches to determine the vitamin A equivalence of Golden Rice in humans. Plants were grown hydroponically in heavy water to intrinsically label Golden Rice β-carotene with deuterium. The subjects were fed both the labeled rice and a reference dose of differently labeled retinol. Sensitive mass spectrometry approaches were used to analyze both β-carotene and retinol enrichment in serum with stable isotope labeling, which allowed us to easily discern Golden Rice and reference dose molecules from preexisting, endogenous β-carotene and retinol. Our subsequent results, based on a small number of US volunteers, showed the effective conversion of Golden Rice β-carotene, even though all individuals were of normal vitamin A status. Whether this conversion efficiency is a good approximation for rice-eating populations with marginal-to-severe vitamin A deficiency, or perhaps is a conservative underestimate, has yet to be determined. To provide information for public health or public policy purposes, a larger long-term trial targeting individuals with marginal vitamin A status is needed. Whether this conversion efficiency is a good approximation for rice-eating populations with marginal-to-severe vitamin A deficiency, or perhaps is a conservative underestimate, has yet to be determined. To provide information for public health or public policy purposes, a larger long-term trial targeting individuals with marginal vitamin A status is needed. Whether this conversion efficiency is a good approximation for rice-eating populations with marginal-to-severe vitamin A deficiency, or perhaps is a conservative underestimate, has yet to be determined. To provide information for public health or public policy purposes, a larger long-term trial targeting individuals with marginal vitamin A status is needed.
TABLE 3
Subject responses to a reference dose of [\textsuperscript{13}C\textsubscript{10}]retinyl acetate and a Golden Rice meal with [\textsuperscript{2}H\textsubscript{9}]\beta-carotene

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>GR (\beta)-C</th>
<th>[\textsuperscript{13}C\textsubscript{10}]RAc</th>
<th>AUC [\textsuperscript{2}H\textsubscript{9}]retinol</th>
<th>AUC [\textsuperscript{13}C\textsubscript{10}]retinol</th>
<th>Retinol equivalent</th>
<th>Conversion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>mg</td>
<td>(\mu g) (\cdot) d</td>
<td>(\mu g) (\cdot) d</td>
<td>(\mu g)</td>
<td>By weight</td>
</tr>
<tr>
<td>1</td>
<td>0.99</td>
<td>1.01</td>
<td>19.4</td>
<td>67.5</td>
<td>0.25</td>
<td>4.0</td>
</tr>
<tr>
<td>2</td>
<td>1.53</td>
<td>1.01</td>
<td>38.8</td>
<td>57.9</td>
<td>0.94</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>0.99</td>
<td>0.43</td>
<td>74.3</td>
<td>53.9</td>
<td>0.51</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>0.99</td>
<td>1.01</td>
<td>34.4</td>
<td>124.2</td>
<td>0.24</td>
<td>4.1</td>
</tr>
<tr>
<td>5</td>
<td>1.53</td>
<td>1.01</td>
<td>32.8</td>
<td>119.9</td>
<td>0.24</td>
<td>6.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.21 ± 0.30</td>
<td>0.89 ± 0.26</td>
<td>39.9 ± 20.5</td>
<td>84.7 ± 34.5</td>
<td>0.36 ± 0.17</td>
<td>3.8</td>
</tr>
</tbody>
</table>

\(1\) GR \(\beta\)-C, Golden Rice \(\beta\)-carotene; RAc, retinyl acetate; AUC, area under the curve.

The food matrix plays an important role in determining the bioavailability of vitamin A from provitamin A carotenoids in a particular food. Rice has a simple and easily digestible food matrix, which allows for a high bioavailability and bioconversion of \(\beta\)-carotene to vitamin A. Similarly, spirulina, with its simple food matrix, has also shown a highly efficient conversion factor for \(\beta\)-carotene to vitamin A of 4.5 to 1 by weight in humans (27). To combat vitamin A deficiency, consumption of locally available vegetables, fruit, and other plant foods, such as algae products, should be encouraged. Each of these plant foods can contribute to vitamin A nutrition, although the conversion of the provitamin A carotenoids within them may not be equivalent. Conversion factors for provitamin A carotenoids from various plants have been reported as 12 to 1 for fruit (14, 28), 13 to 1 for sweet potato (15), 15 to 1 for carrots (16), and 10 to 1 (15), 21 to 1 (16), 26 to 1 (14), 27 to 1 (13), and 28 to 1 (28) for green leafy vegetables. Thus, comparatively speaking, Golden Rice has a very favorable conversion ratio.

It should be noted that we closely monitored our subjects for any possible adverse effects after the consumption of Golden Rice and found no evidence of any problems, including allergic reactions or gastrointestinal disturbance. Although this attests to the probable safety of Golden Rice, we acknowledge that only a single serving was fed to each study subject. A much longer exposure with a larger cumulative consumption of Golden Rice would be needed to make definitive assertions regarding the inherent safety of this food for human use.

Staple foods should not only provide energy but also nutrients in a bioavailable form. Thus, Golden Rice may be a cost-effective staple food for combating vitamin A deficiency in rice-eating populations (29). Other provitamin A–containing staple foods, such as corn, cassava, sweet potato, and sorghum should be developed to serve other vitamin A–deficient populations with different food cultures.

We thank the Metabolic Research Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging for recruiting our volunteers and for performing the human study procedures and David Dworak and Chee-Ming Li of the USDA/ARS Children’s Nutrition Research Center for helping to label and produce the Golden Rice.

The authors’ responsibilities were as follows—GT: designed the study, supervised the data collection, analyzed the data, and wrote the manuscript; JQ: collected and analyzed the samples; GGD: supervised the mass spectrometric analysis and revised the manuscript; RMR: supervised the human study as the study physician and revised the manuscript; and MAG: designed the production methods for the labeled Golden Rice, harvested the labeled Golden Rice for the study, and revised the manuscript. No financial benefit was obtained from this research study.

REFERENCES