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β-Carotene in Golden Rice is as good as β-carotene in oil at providing vitamin A to children[1–4]

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ABSTRACT

Background: Golden Rice (GR) has been genetically engineered to be rich in β-carotene for use as a source of vitamin A.

Objective: The objective was to compare the vitamin A value of β-carotene in GR and in spinach with that of pure β-carotene in oil when consumed by children.

Design: Children (n = 68; age 6–8 y) were randomly assigned to consume GR or spinach (both grown in a nutrient solution containing 23 atom% ^2H_2O) or [$^2H_8$]β-carotene in an oil capsule. The GR and spinach β-carotene were enriched with deuterium (^2H) with the highest abundance molecular mass (M) at $M_{\beta\text{-}C}+^2H_{10}$. [$^{13}C_{10}$]Retinyl acetate in an oil capsule was administered as a reference dose. Serum samples collected from subjects were analyzed by using gas chromatography electron-capture negative chemical ionization mass spectrometry for the enrichments of labeled retinol: $M_{retinol}$+4 (from [$^2H_8$]β-carotene in oil), $M_{retinol}$+5 (from GR or spinach [$^2H_{10}$]β-carotene), and $M_{retinol}$+10 (from [$^{13}C_{10}$]retinyl acetate).

Results: Using the response to the dose of [$^{13}C_{10}$]retinyl acetate (0.5 mg) as a reference, our results (with the use of AUC of molar enrichment at days 1, 3, 7, 14, and 21 after the labeled doses) showed that the conversions of pure β-carotene (0.5 mg), GR β-carotene (0.6 mg), and spinach β-carotene (1.4 mg) to retinol were 2.0, 2.3, and 7.5 to 1 by weight, respectively.

Conclusions: The β-carotene in GR is as effective as pure β-carotene in oil and better than that in spinach at providing vitamin A to children. A bowl of ~100 to 150 g cooked GR (50 g dry weight) can provide ~60% of the Chinese Recommended Nutrient Intake of vitamin A for 6–8-y-old children. This trial was registered at www.clinicaltrials.gov as NCT00680212. *Am J Clin Nutr* doi: 10.3945/ajcn.111.030775.

INTRODUCTION

Vitamin A is essential for the promotion of general growth, maintenance of visual function, regulation of differentiation of epithelial tissues, embryonic development, and immune system function (1, 2). Vitamin A also has a role in the prevention of morbidity and mortality from infectious diseases in children (3–6). Chronic consumption of diets that are low in vitamin A results in vitamin A deficiency (VAD).

To prevent clinical VAD, chemically synthesized vitamin A (~500 million vitamin A capsules per year) has been administered periodically to "at-risk" populations (7, 8). Fortification of foods such as cooking oil and sugar with vitamin A is also considered an efficient way to prevent and control VAD (9, 10). Also, food-based programs designed to increase the availability of foods rich in provitamin A carotenoids and to promote their consumption have been suggested as realistic and sustainable alternatives to overcome VAD globally (10). However, the efficacy of carotenoid-rich plant foods at preventing VAD has been shown to be lower than that previously hoped for or expected: the vitamin A equivalency of provitamin A carotenoid–rich foods varies from 2 μg β-carotene–to–1 μg retinol (for diets containing pure β-carotene in oil) to 27 μg β-carotene–to–1 μg retinol (for β-carotene in certain leafy vegetables) (11–19). Moreover, WHO reports published in 2009 and 2012 claim that ~190–250 million preschool children worldwide are still affected by VAD (3, 20) and that providing vitamin A supplements could reduce all-cause mortality in children younger than 5 y by 24–34% (4, 5, 8). A UNICEF report in 2010 (6) showed that about 8.1 million children younger than 5 y died in 2009. Thus, vitamin A availability for all children in undernourished settings could prevent ~1.9–2.7 million child deaths annually. In an effort to provide long-term and sustainable prevention of VAD, staple foods biofortified with provitamin A carotenoids might be a safe and effective additional approach (21, 22).

Scientists have engineered components of the provitamin A biosynthetic pathway into rice endosperm (23) so that Golden

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Rice (GR) contains up to 37 μg total provitamin A carotenoids ($\sim$30 μg β-carotene) in 1 g milled and uncooked rice (24). This biofortified crop was developed specifically to target populations for whom rice is a staple food and VAD is particularly severe (7, 23). Although a small-scale bioconversion study has been completed with GR fed to US adults (25), an evaluation of the bioconversion efficiency of GR β-carotene (to vitamin A) in children is also needed. Here we report a trial to determine the vitamin A value of intrinsically labeled GR compared with spinach (a representative, household-grown, dark-green leafy vegetable that is rich in β-carotene and is commonly eaten by Chinese children) or pure β-carotene in oil, by using schoolchildren of marginal or normal vitamin A status.

SUBJECTS AND METHODS

Subjects

The study was carried out in an elementary school in the Hunan province of China in healthy schoolchildren (with normal biochemical test results; *see* below) aged 6–8 y either initially free of parasitic infection or verified free of infection after treatment with 400 mg albendazole (GlaxoSmithKline). Most area residents were local, middle-income, rural, and working people. Forty-eight percent of the study subjects who were treated (no side effects) were recruited to participate in the study 1 mo before the start of the study meals. Thus, all of the study subjects at the time of study were free of parasitic infection by the laboratory test. Local annual health evaluations were performed in all children, and biomedical tests were performed (white blood cell count, hemoglobin count, platelet count, red blood cell count, and hematocrit).

To estimate the sample size for the study, we used our previous studies in the United States with spinach or β-carotene capsules (26, 27) and studies in China (28) for dark-green vegetables for the power calculations. We found that to determine the differences for 2 categories—marginal and normal vitamin A intakes—we would need a sample size of 30 per category, and to evaluate the effect of food matrix among sources (spinach, GR, and β-carotene capsules) we would need 10 per group. Considering the possibility of subject withdrawals from the study, we proposed 36 subjects for the marginal vitamin A group and 36 subjects for the normal vitamin A group, with 12 each per group for the spinach group, rice group, and β-carotene capsule group.

From a total of 112 subjects screened, 72 were enrolled in the study, and sufficient serum samples to facilitate both the HPLC and gas chromatography–mass spectrometry analyses were collected from 68 subjects (this varied from 1 to 5 time points per subject). The anthropometric measurements of the subjects were within the range of the data reported for all of China for this age group (29). The study recruitment processes and protocol were approved by the Institutional Review Board–Tufts Medical Center in the United States and by the Ethics Review Committee of Zhejiang Academy of Medical Sciences in China. Both parents and pupils consented to participate in the study.

Production of intrinsically labeled plant material and preparation of labeled doses

Intrinsically deuterium-labeled GR grains and spinach were harvested from a hydroponic plant system (housed in the USDA-Agriculture Research Service Children's Nutrition Research Center in Houston, TX) by growing the plants with heavy water (deuterium oxide, D_2O), introduced into the growth medium shortly after the start of seed fill (in the case of rice), or at the initiation of planting (spinach), as previously described (26). GR grains were harvested at maturity, air dried for 3 d, and then dehulled, milled, and stored at -70°C until being shipped to Boston for cooking, as previously described (25). Similarly, spinach plants were harvested and packed as fresh leaves (with ice) and were shipped the next day to Boston for cooking and processing as previously described (26).

Cooked GR was divided into 60-g portions (cooked weight) in individual food containers and kept at -70°C until used. On the day of feeding, the GR doses were brought to room temperature and then steam heated briefly before being served with lunch.

Cooked, labeled spinach was homogenized, mixed, and divided into 30-g portions in individual containers and stored at -70°C until used. On the day of feeding, the spinach doses were brought to room temperature and then steam heated briefly before being served with lunch.

The pure $^{2}H_8$-β-carotene dose was crystalline all-*trans*-[$^{2}H_8$]β-carotene (11,11′,19,19,19,19′,19′,19′-[$^{2}H_8$]β-carotene), which was synthesized by scientists of BASF. The chemical purity was 91.2% (this percentage was used to calculate the β-carotene in the dose). The [$^{2}H_8$]β-carotene in the oil capsule (gelatin, size 1) was made by weighing 0.50 mg β-carotene by using a Mettler-Toledo XP-26 microbalance with 0.001-mg measurement readability and dissolving it with 10 μL absolute ethanol before mixing it with 170 mg corn oil to form a clear oil solution. The enrichment profiles of β-carotene in these plant foods together with the pure β-carotene for the study were analyzed by liquid chromatography–atmospheric pressure chemical ionization mass spectrometry and are presented in **Figure 1**.

As a reference dose, 0.5 mg [$^{13}C_{10}$]retinyl acetate (8, 9, 10, 11, 12, 13, 14, 15, 19, 20-[$^{13}C_{10}$]retinyl acetate) with a chemical purity of 98% and an isotopic purity of 99% (synthesized by Cambridge Isotope Laboratory) in a 170-mg corn oil gelatin capsule (size 3) was taken by the volunteers during the breakfast meal. The study doses were packed in a cooler together with PolarPack ice bricks, prechilled to -70°C, and shipped to the study site in China, where the doses were kept at -20°C until shortly before use.

Study design

The subjects were randomly assigned (using a computer-generated random numbers table) to take spinach, GR, or β-carotene in oil capsule. The full study lasted 35 d and included a 14-d diet preparation period, during which time the children (without parasitic infection) tried study meals provided by the kitchen that was set up to provide these meals, and during which time the parents were informed on dietary restrictions for their children during the study. The full study also included a dosing day (day 0) and a 21-d period to draw up to 5 blood samples per subject, to study blood response curves. Details are presented in **Figure 2**.

At 0700–0800 on day 0 of the study, the children consumed a [$^{13}C_{10}$]retinyl acetate (0.5 mg) in oil capsule (gelatin, size 3) with 10 mL soymilk and their standardized breakfast containing

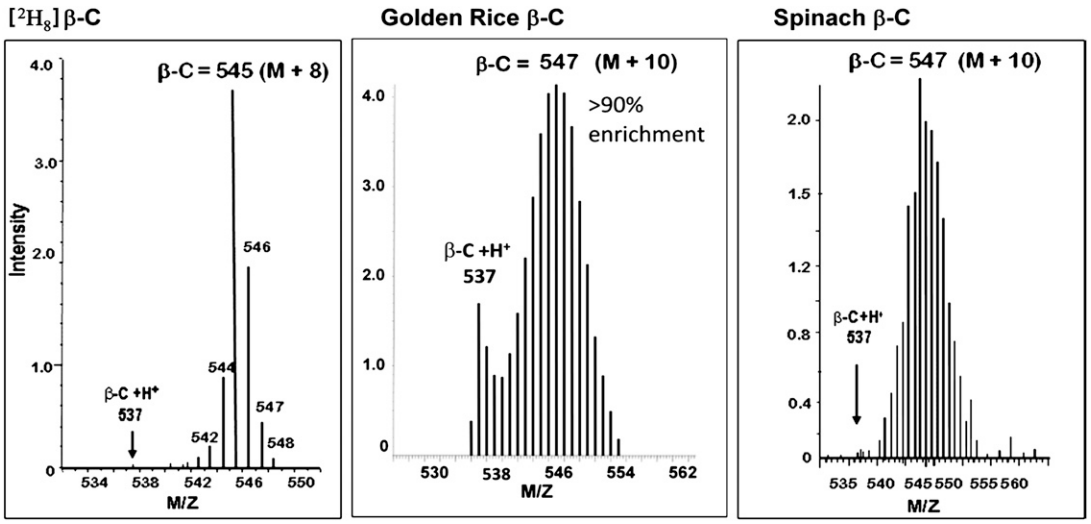

FIGURE 1. The enrichment profiles of β-carotene in chemically synthesized β-carotene and of the plant foods Golden Rice and spinach as analyzed by using liquid chromatography–atmospheric pressure mass spectrometry. β-C, β-carotene; M, molecular mass.

rice noodles (200 g, cooked), pork (110 g, cooked), one-half of an apple, and a 26-g cookie. The total energy of the meal was 630 kcal with ~20% energy from fat. Between 1145 and 1230, a standardized lunch [containing rice (250 g, cooked), pork meat (100 g, cooked), cabbage (100 g, cooked), and egg and tomato soup (100 g) with a total energy of ~560 kcal and ~20% of energy from fat] was taken by the subjects together with the study dose of either spinach (30 g), a capsule of β-carotene in oil, or GR (60 g). For the spinach or β-carotene capsule groups, an appropriate amount of white rice was added to the meal to ensure caloric equality with the 60-g GR group. The mean daily intake of vitamin A from subjects' routine meals was ~240 μg retinol per person based on the entire group's food records [34% of Chinese Recommended Nutrient Intake) for this age group = 700 μg retinol (30)]. During the 3-wk study period, the subjects ate all meals at school 5 d/wk. Instructions were given on vitamin A– or provitamin A–rich foods to be omitted over the weekend when meals were eaten at home (eg, liver products, excessive amounts of carrots). Foods eaten during the weekend were recorded in a take-home diary.

Fasting blood samples (<3 mL, less in some subjects) were attempted from each subject on the mornings of days 1, 3, 7, 14, and 21 over the experimental period (Figure 2) and analyzed as previously described (26).

Retinol equivalence calculations

The AUC (determined by using KaleidaGraph of Synergy Software, http://www.synergy.com) of the total serum [^{2}H]retinol response from each labeled intervention dose (μmol vs time) was compared with the AUC of the vitamin A reference dose (0.5 mg [$^{13}C_{10}$]retinyl acetate; molecular mass = 338).

The concentration of blood retinol was measured by using HPLC (26). The labeled retinol derived from each labeled β-carotene or [$^{13}C_{10}$]retinyl acetate dose was calculated as enrichment × retinol concentration × body weight × 0.0497, where body weight × 0.0497 L/kg (for children of this age group) was used to determine the total-body serum volume.

The amount of [^{2}H]retinol formed from the β-carotene dose was calculated as follows:

$$[^2\text{H}]\text{retinol formed from the } \beta\text{-carotene dose } (\mu\text{mol}) = \frac{\text{AUC of } [^2\text{H}]\text{retinol}}{\text{AUC of } [^{13}C_{10}]\text{retinol}} \times \frac{[^{13}C_{10}]\text{retinyl acetate (mg)}}{338} \times 1000 \quad (1)$$

Conversion factor calculations

The β-carotene to vitamin A conversion factor was defined as the amount of labeled β-carotene contained in a given oral dose of GR, spinach, or oil capsule, compared with the amount of vitamin A derived from each dose. Thus, the conversion factor of the labeled β-carotene oil capsule, GR, or spinach β-carotene (calculated as β-carotene from all-*trans* β-carotene plus one-half of all other provitamin A carotenoids) to vitamin A was determined as follows:

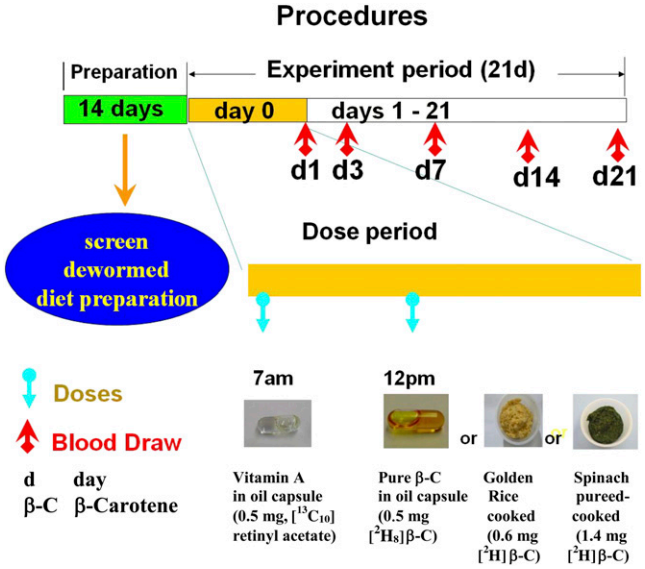

FIGURE 2. Diagram of the study timeline. β-C, β-carotene.

$$\text{Conversion factor of } \beta\text{-carotene to retinol by weight} = \frac{\beta\text{-carotene in plant or oil dose}(\mu\text{mol}) \times 536}{[^2\text{H}]\text{retinol formed from the labeled } \beta\text{-carotene dose}(\mu\text{mol}) \times 286} \quad (2)$$

Statistical analyses

Statistical analyses were performed to assess the significance of differences between subjects in the different groups (eg, based on anthropometric measures, sex, vitamin A status, subjects grouped into serum retinol concentration <24.9 or >25.0 μg/dL) by using SAS for Windows, version 9.2 (SAS Institute Inc).

Because of the limitation of obtaining serum samples for all 5 time points from each child (1–5 samples were obtained from each child), the following approach was used to assess the SE for conversion factors from each labeled dose: *1*) missing data points were considered to be missing completely at random because there was no reason to think that being missing was related to a subject's diet group or his or her response; *2*) there were enough missing data to compromise our ability to estimate individual AUCs; therefore, the MIXED procedure of SAS for Windows was used to estimate the mean AUC and its SE; *3*) the delta method (31) was used to estimate the SE of the ratio of the AUC formed from each β-carotene dose to the AUC of the reference dose under the assumption that the 2 AUCs are uncorrelated (this is a conservative approach that will overstate the SE if the AUCs are positively correlated); and *4*) ratios were compared by calculating *z* statistics as the difference in ratios divided by the square root of the sum of their squared SEs; the results were considered significantly different if a 2-sided observed significance level (*P* value) was <0.05.

RESULTS

The GR and spinach grown with heavy-water nutrient solutions generated material in which the enrichment profiles for β-carotene showed the most abundant enrichment peak at molecular mass+10 for GR β-carotene and spinach β-carotene (Figure 1); these labeled materials were suitable for study use.

Our volunteers were healthy, and the anthropometric measures for each group (a total of 68 subjects contributed sufficient blood samples for analysis) are listed in **Table 1**. No statistically significant differences in height and weight were observed between boys and girls, and their height and body weights were in the normal range for this age group in China (29). When evaluated by using the WHO growth reference data for ages 5–19 y (32), the *z* scores of weight-for-age ranged from a median to −2 for both girls and boys, whereas the *z* scores of height-for-age ranged from median to −1 for boys and from median to −2 for girls. As a population, our subjects' baseline serum vitamin A concentrations were marginal, with a mean concentration of 23.6 to 25.6 μg/dL (**Table 2**), and a range from 16.7 to 40.3 μg/dL. Boys in the β-carotene capsule treatment group had a significantly lower serum retinol concentration (22.2 μg/dL) than did girls in the β-carotene capsule ($P = 0.044$) and GR ($P = 0.025$) treatment groups but not significantly lower than that in any of the other subgroups. No significant difference in the serum retinol concentration was found between the girls in the β-carotene capsule treatment group and either sex of the GR or spinach group.

TABLE 1
Baseline anthropometric measures of the subjects contributing sufficient serum samples for vitamin A–enrichment analysis[1]

Dose groups	β-Carotene capsule	Golden Rice	Spinach
Age range (y)	6–8	6–8	6–8
Subjects (*n*)			
Boys	13	12	15
Girls	10	11	7
Height (cm)			
All	120.3 ± 3.3[2]	120.9 ± 4.1	119.8 ± 4.3
Boys	120.6 ± 3.5	122.0 ± 3.7	120.3 ± 5.2
Girls	120.0 ± 3.0	119.6 ± 4.4	118.7 ± 1.9
Weight (kg)			
All	20.8 ± 3.1	20.9 ± 2.7	20.5 ± 2.5
Boys	21.5 ± 3.8	21.4 ± 2.1	21.1 ± 2.8
Girls	19.8 ± 1.7	20.4 ± 3.4	19.0 ± 1.0

[1] No significant differences were found between groups or between sexes.
[2] Mean ± SD (all such values).

After ingestion of the labeled reference dose of [$^{13}C_{10}$]retinyl acetate in the morning and either the labeled β-carotene oil capsule, spinach, or GR at lunch (Figure 2), enrichment peaks of retinol were detected in all blood samples. The samples collected on days 1, 3, 7, 14, and 21 (after the labeled doses) were analyzed, and the mean values (in μmol) were determined for each time point. The AUCs of the enrichment responses were determined for each of the labeled diets and for the reference dose of labeled retinyl acetate (**Figure 3**).

The comparisons of the labeled vitamin A formed from the labeled β-carotene, GR, or spinach in the diet with that from the known amount of reference dose of [$^{13}C_{10}$]retinyl acetate enabled the calculation of equivalences of these labeled dietary provitamin A carotenoids to the labeled vitamin A dose; thus, the conversion efficiencies of the 3 dietary sources of labeled provitamin A were determined. The results show that the conversions of pure β-carotene (0.5 mg) in oil capsule, GR β-carotene (0.6 mg), and spinach β-carotene (1.4 mg) to retinol were 2.0 ± 0.9, 2.3 ± 0.8, and 7.5 ± 2.1 to 1 by weight, respectively, as presented in **Table 3**.

The conversion efficiencies of β-carotene in oil capsule, GR β-carotene, and spinach β-carotene to retinol in association with sex and the vitamin A nutrition status (<24.9 or >25 μg/dL retinol) are presented in Table 3. When further grouping of children in each of the 3 feeding groups into sex or vitamin A status, the number who provided blood samples to determine the AUC at the 5 time points for each sex ranged from 3 to 11 children and for each vitamin A status (ie, <24.9 or >25.0 μg/dL) ranged from 2 to 10 children. There was no significant difference in conversion efficiency between sexes nor between vitamin A status groupings.

DISCUSSION

The isotope reference method has been previously used to study adult volunteers ingesting β-carotene in oil or from spinach, carrots, spirulina, GR, and yellow maize (26, 27, 33–35). These previous studies usually involved 2 labeled doses (study dose and reference dose) given a few days or a week apart and frequent blood samples collected after each of the labeled

TABLE 2
Baseline vitamin A (retinol) concentrations in children for each labeled dose, sex, and vitamin A status (> or <25 μg/dL retinol) groups[1]

Dose groups	β-Carotene capsule (n = 23)	Golden Rice (n = 23)	Spinach (n = 22)
Serum retinol (μg/dL)	23.6 ± 4.3 (17.5–31.0)	25.6 ± 5.8 (16.7–40.3)	24.2 ± 3.5 (19.1–30.8)
Serum retinol by sex (μg/dL)			
Boys	22.2[a] ± 4.1	25.0[a,b] ± 5.4	23.8[a,b] ± 3.7
Girls	25.5[b] ± 4.1	26.1[b] ± 6.4	24.9[a,b] ± 3.6
Serum retinol by vitamin A category (μg/dL)			
>25.0 μg/dL	28.0 ± 1.8	29.3 ± 4.8	28.2 ± 2.6
<24.9 μg/dL	20.3 ± 2.1	21.2 ± 2.7	22.5 ± 2.7

[1] All values are means ± SDs; ranges in parentheses. Means with different superscript letters are significantly different, $P < 0.05$.

doses. However, frequent blood draws are not suitable for studies involving children, so only 5 blood draws per subject were attempted in this study. Furthermore, we gave the reference dose of [$^{13}C_{10}$]retinyl acetate (0.5 mg) in the morning and provided the labeled β-carotene in oil capsule or from GR or spinach at lunch to avoid possible absorption competition between the retinyl acetate and β-carotene. This resulted in an approximate 5-h delay of the responses of absorbed and converted labeled retinols formed from the retinyl acetate dose compared with the labeled plant (GR or spinach) β-carotene or β-carotene in oil dose. Because the experimental period of the study lasted 21 d (504 h), this 5-h difference would not affect the outcome of the AUC analyses.

Our study was designed to determine the β-carotene to vitamin A conversion values from 2 plant foods rich in provitamin A carotenoids using a traditional plant food, spinach, or a specially created biofortified food, GR, in children. Across all our subjects, no side effects or abnormalities were observed during this study in any individual who consumed the labeled spinach, GR, or the β-carotene in oil capsule with their meal. Furthermore, no abnormalities or complaints were reported after the completion of the study during a 1-y follow-up period.

Our results indicate that spinach, GR, and β-carotene in oil capsule can all provide children with vitamin A nutrition. Of these 3 provitamin A sources, and at the doses administered, GR was as effective as the pure β-carotene in oil capsule ($P = 0.8$), and both were much more effective than spinach at contributing to the vitamin A intakes of the children (GR β-carotene compared with spinach β-carotene: $P = 0.018$; pure β-carotene in oil compared with spinach β-carotene: $P = 0.014$). In conjunction with previous studies in adults (25), these current results provide further evidence that GR β-carotene could be an effective food source to provide vitamin A.

It is well known that VAD is generally caused by chronically low vitamin A diets. In rice-eating areas of the world, especially Southeast Asian countries, VAD is common (6). Even though the intake of dark-green and yellow vegetables and fruit can provide some vitamin A, these foods are relatively inefficient sources of

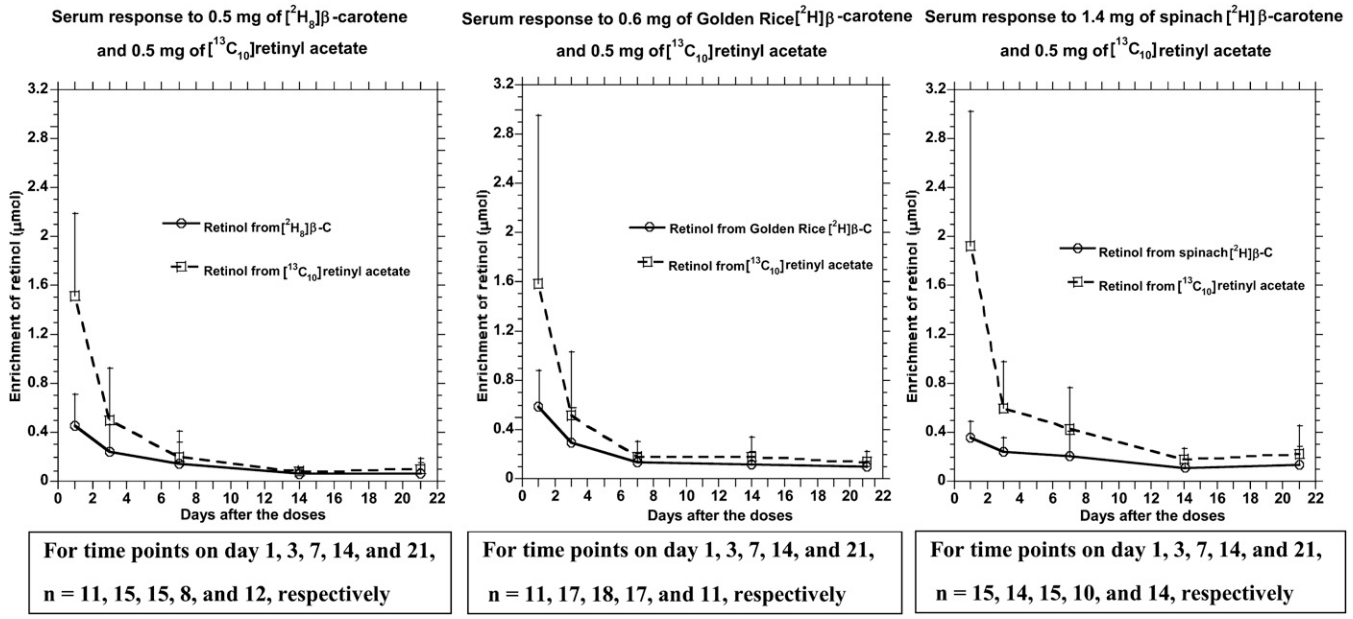

FIGURE 3. Mean enrichment (±SD) of serum retinols by treatment and time. Left panel: response to β-carotene in oil capsule (0.5 mg β-carotene) and [$^{13}C_{10}$]retinyl acetate (0.5 mg); middle panel: response to Golden Rice (0.6 mg β-carotene) and [$^{13}C_{10}$]retinyl acetate (0.5 mg); and right panel: response to spinach (1.4 mg β-carotene) and [$^{13}C_{10}$]retinyl acetate (0.5 mg). The number of children providing blood at the 5 time points is presented in the text box under the enrichment response graph of each group. β-C, β-carotene.

TABLE 3
Conversion factors for β-carotene in oil capsule, Golden Rice, and spinach to vitamin A in children by using AUC (μmol/d) calculated from Figure 3[1]

Dose groups	β-carotene capsule (0.5 mg) (n = 23)	Golden Rice (0.6 mg β-carotene) (n = 23)	Spinach (1.4 mg β-carotene) (n = 23)
AUC of retinol from dose (μmol/d)	3.3 ± 0.8	3.4 ± 0.5	3.4 ± 0.5
AUC of retinol from [$^{13}C_{10}$]retinyl acetate, 0.5 mg (μmol/d)	5.5 ± 2.1	5.9 ± 1.8	7.9 ± 1.9
Conversion factor by weight	2.0 ± 0.9[a]	2.3 ± 0.8[a]	7.5 ± 0.8[b]
Conversion factor by weight on sex			
Boys	2.4 ± 0.6	2.3 ± 1.3	7.3 ± 2.5
Girls	2.7 ± 1.8	2.4 ± 1.0	8.5 ± 4.7
Conversion factor by weight on vitamin A category			
>25.0 μg/dL	2.4 ± 1.2	1.7 ± 0.7	8.7 ± 2.7
<24.9 μg/dL	1.1 ± 1.2	3.1 ± 1.2	7.2 ± 3.1

[1] All values are means ± SEs. The SEs were estimated by using the delta method, and the ratios were compared by calculating z statistics. The conversion factors were considered significantly different (shown with different superscript letters) if the 2-sided observed significance level (P value) was <0.05. The conversion factor between the β-carotene capsule and spinach groups was P = 0.014, between the Golden Rice and spinach groups was P = 0.018, and between the β-carotene capsule and Golden Rice groups was P = 0.8.

vitamin A (16, 17). However, on the basis of our current findings, a single 50-g dry-weight serving of GR (a bowl of ~100 to 150 g cooked weight) can provide ~1 mg β-carotene, which would then be converted to ~435 μg retinol. This would represent ~60% of the Chinese vitamin A Recommended Nutrient Intake (700 μg) for a 7-y-old child.

The limitations of this study were that only a few individual subject kinetic curves including all 5 blood drawing time points were obtained. Thus, we chose to construct one AUC for each grouping, with SDs around the various points on the curve. This is similar to the "super-child" model that was developed by collecting samples at selected times from many individuals to make up a complete sample set. The super-child model approach has been used in a vitamin A study of preschool-age Peruvian children (36, 37). In our current study, these results generated one conversion factor for each dietary group, sex group, and vitamin A status group. These single group curves provide estimations for conversion efficiency for a group as a whole, but individually they are not suitable (because they do not provide suitable estimate of the SE) for the statistical analysis of differences in conversion efficiencies between the groups. However, by using the MIXED procedure of SAS to estimate the mean AUC and its SE for each intervention group, including the subgroups in sex and vitamin A status (> or <25 μg/dL) and for the reference dose, the ratios and their SEs could be estimated and z statistics calculated. The results showed a trend of the conversion factor for the boys group to be greater than that for the girls group but not statistically different. Both the β-carotene and spinach groups showed a trend (not statistically significant) of better conversion factor for the <24.9 μg/dL subgroup but not the same trend with the vitamin A status subgroups associated with the Golden Rice group, where the n value was smaller (Table 2).

Previous data have shown that the conversion efficiency of spinach β-carotene to vitamin A is low (26). In a study conducted in US adults, the conversion factor of spinach containing 11 mg β-carotene (300 g cooked spinach) was 21 to 1 by weight and that of β-carotene in oil capsule containing 6 mg β-carotene was 9 to 1 by weight. Other studies (16, 38) that evaluated the leafy vegetable β-carotene conversion efficiency also showed the conversion factors from 10:1 (Indian spinach) to 26:1 (mixed dark-green leafy vegetables). To ensure that the vitamin A formed from the spinach dose would be detectable, we gave a spinach dose containing 1.4 mg β-carotene (30 g cooked spinach), which is still a physiologic (dietary) dose. The average total serum response to the spinach dose (1.4 mg β-carotene) reached 1.9 μmol on day 1 and was higher (but not significantly so) than the serum response to the oil (0.5 mg β-carotene) or GR (0.6 mg β-carotene) doses (1.5 and 1.6 μmol, respectively), but these were not proportional with the contents of the β-carotene in each of the doses. Furthermore, compared with US adults (25), the Chinese children converted GR β-carotene to vitamin A (2.3 to 1 by wt) more efficiently than did US adults (3.8 to 1 by wt). This might have been related to one or both of the following possibilities: *1*) the age differences between individuals in the 2 populations or *2*) the differences in vitamin A status because these children have a relatively lower vitamin A status than do US adults.

In summary, the high bioconversion efficiency of GR β-carotene to vitamin A shows that this rice can be used as a source of vitamin A. GR may be as useful as a source of preformed vitamin A from vitamin A capsules, eggs, or milk to overcome VAD in rice-consuming populations. Awareness of the vitamin A equivalence of plant foods provides a scientific basis for designing food-based nutritional programs to improve vitamin A status in many regions of the world where VAD is still common.

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