# Plasma β-carotene and retinol concentrations of children increase after a 30-d supplementation with the fruit *Momordica cochinchinensis* (*gac*)[1–3]

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**ABSTRACT**

**Background:** In rural Vietnam, vitamin A deficiency is a concern. Among the indigenous fruit and vegetables, *Momordica cochinchinensis* (*gac*) fruit has been identified as having the highest β-carotene concentration. Locally, it is mixed with rice in a preparation called *xoi gac*.

**Objective:** The purpose of this study was to assess this β-carotene–rich rice preparation as a source of provitamin A for children in rural Vietnam.

**Design:** Preschoolers ($n$ = 185) participated in a 30-d controlled supplementation trial. Children with low hemoglobin concentrations were assigned to 1 of 3 groups: a fruit group, who received *xoi gac* that contained 3.5 mg β-carotene per serving; a powder group, who received rice mixed with 5.0 mg synthetic β-carotene powder; and a control group, who received rice without fortification.

**Results:** The mean increase in plasma β-carotene concentrations in the fruit and powder groups was significantly greater than that in the control group ($P < 0.0001$). After supplementation, the mean plasma retinol concentration in the fruit group was significantly higher than that in the control ($P = 0.006$) and powder ($P = 0.0053$) groups. Among the children with initial hemoglobin concentrations ≤110 g/L, the mean increase in hemoglobin concentrations in the fruit group was marginally higher than that in the control group ($P = 0.017$) but was not significantly different from that in the powder group.

**Conclusions:** β-Carotene from *xoi gac* is a good source of provitamin A carotenoids. Severely anemic children might particularly benefit from routine *xoi gac* consumption. *Am J Clin Nutr* 2002;75:872–9.

**KEY WORDS** β-Carotene, carotene-rich food, lycopene, retinol, preschoolers, vitamin A, vitamin A deficiency, food-based approach, β-carotene supplementation, *Momordica cochinchinensis*, *xoi gac*, Vietnam

## INTRODUCTION

Chronic vitamin A deficiency is a persistent nutritional problem among children in rural areas of Vietnam (1–3). Vitamin A capsule distribution programs may not represent a long-term solution, and food-based interventions are viewed as part of an effective strategy for reducing vitamin A deficiency (1). In this strategy, provitamin A carotenoids, particularly β-carotene, which provide vitamin A after enzymatic cleavage, play a key role. In Vietnam, the *gac* fruit (*Momordica cochinchinensis* Spreng 1826) is a promising candidate for food-based interventions (4). It is a large, bright-red fruit rich in β-carotene and lycopene (5, 6). The seed membrane and pulp of the *gac* fruit contain significant concentrations (≈7–10% by wt) of long-chain fatty acids, a key property for the efficient absorption and transport of β-carotene and other fat-soluble vitamins; this property is especially critical in the Vietnamese population, in whom fat intake is often low.

The *gac* vine is cultivated in home gardens and is commonly seen growing on lattices at the entrances to rural homes. Traditionally, *gac* seed and pulp are mixed with cooked rice to impart color and flavor (7). The local name of this dish is *xoi gac*. Preparation of *xoi gac*, which involves mixing the *gac* seed and pulp with rice and steaming the mixture in a covered container, causes a minimal loss of β-carotene (8). Because this dish is already well accepted, encouraging its consumption could produce a substantial increase in provitamin A intake.

The ability of β-carotene–rich foods to meet the vitamin A needs of at-risk subpopulations is currently under scrutiny. Although many studies showed a positive association between plasma β-carotene and intakes of natural food sources of provitamin A (9, 10), Bulux et al (11) reported little effect of increased consumption of certain β-carotene–rich foods on plasma β-carotene and retinol concentrations. Recently, de Pee et al (12) reported no improvement in serum retinol concentrations in lactating women in response to increased intake of dark-green leafy vegetables. The picture that has emerged from these controversies is that each potential food source needs to be individually evaluated in terms

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of its value as a provitamin A source. Accordingly, establishing the bioavailability of β-carotene in individual foods has become a high priority. The purpose of this study was to assess the bioavailability of β-carotene when given as *xoi gac* to a population of rural Vietnamese children with low hemoglobin concentrations. Changes in plasma concentrations of α-tocopherol, β-carotene, retinol and several other carotenoids, and hemoglobin were determined in response to a 30-d supplementation with *gac*.

## SUBJECTS AND METHODS

### Field location and demographics

The children's supplementation trial was conducted in the Hai-Hung province of northern Vietnam. Because Hai-Hung is one of the most populated provinces in the Red River delta, it is representative demographically and economically of communities in the lowlands of northern Vietnam. Two communes in the Thanh-Mien district were selected as the study site on the basis of their accessibility, their similarity to each other in socioeconomic status, and their ability to provide an adequate number of preschoolers. Each commune consists of ≈8000 residents.

### Selection of participants

Lists of all children aged 31–70 mo were generated from the registry of births kept at the health center of each commune. All the mothers of these children ($n$ = 752) were invited to come for a screening session. At the time of selection for the study, participating children had to be free of any clinical signs (including maternal report) of illness, fever, lesion, diarrhea, or upper respiratory infection. To select children likely to be at higher risk of vitamin A deficiency, only those with a hemoglobin concentration between 100 and 120 g/L were accepted into the trial (low hemoglobin is associated with vitamin A deficiency) (13–15). Of the children in the selected age group, hemoglobin concentrations were measured in 711 (95%) with a HemoCue (HemoCue, Angelholm, Sweden). Of the children screened, 185 met the selection criteria. Twenty-five children with hemoglobin concentrations < 100 g/L were referred to community health workers for possible treatment of anemia. After allowance for a 15% attrition rate, the minimum sample size of each of the 3 groups in the trial (fruit, powder, and control) required to detect a difference in plasma β-carotene concentrations of 0.6 μmol/L between any 2 groups (power = 0.80 and α = 0.05) was calculated to be 58. This calculation was based on the results of the β-carotene supplementation study carried out in preschoolers in Guatemala (11). Families who participated in the screening or the study received a compensation of 10 000 dong (≈$1).

### Group assignment procedure

In each commune, the treatment was assigned by using a list of all eligible children. The order of names in each list was produced arbitrarily, and names were then sequentially assigned a number from 1 to 3 by local assistants who were blinded to the meaning of the numbers. The diet treatment code corresponding to each number was assigned arbitrarily by the project leader.

### Human subjects protocol and informed consent

After being informed about the study, both parents of each selected child signed the consent form. The study protocol was approved by the Human Subjects Review Committee of the University of California at Davis and by the Ministry of Health and the National Institute of Nutrition (NIN) in Vietnam.

### Data collection

Before and after the supplementation period, the weight and height of each child was measured and a medical history and blood sample were collected. The same research assistants measured weight and height on both occasions. Written instructions in Vietnamese were followed to ensure consistency. Height was measured with the use of a measuring tape secured to a wall. The children were asked to remove their shoes and hats, stand with their feet together and their knees straight, and place their heels and shoulder blades in contact with the wall. Weight was measured with the use of a spring balance. The children were asked to remove their jackets and shoes before these measurements. $z$ Scores for height-for-age and weight-for-age were calculated for each child with the use of the Waterlow classification and 1983 World Health Organization guidelines (16).

To track possible dietary changes during the study, local research assistants obtained intake data for all the children by interviewing their mothers before and after the trial with the use of a semiquantitative 1-mo food-frequency questionnaire. Intake data during the month of the trial did not include the supplemental meals. The food-frequency questionnaire was generated from a descriptive study previously conducted among adults in the same province and was then revised with the assistance of local collaborators. The final version was pilot-tested with local women who had a child < 5 y of age. The food-frequency questionnaire contained 20 items and took ≈15–20 min to complete. Pictures of servings of foods and common measuring devices were used to assist respondents in answering questions about portion size. Actual weights of portion sizes of common local foods and volumes of household utensils in the studied areas had been documented by local researchers of the NIN and were used in determining the amounts of food consumed [eg, 1 *mui* (section) of an orange = 40 g, 1 *mieng* (slice) of papaya = 160 g, 1 *khuc* (round piece) of fish = 30 g, 1 *mieng* (piece) of chicken (with bone) = 18 g, 1 *mieng* (bite-sized piece) of liver = 5 g, 1 *lung bat* (one-half of a bowl) of rice = 60 g, 1 *thia con* of lard = 3 g]. Intakes of selected nutrients (energy, vitamin A, and β-carotene) were quantified with the use of published food-composition data (5, 6).

### Supplementation

The supplementation trial was conducted for 30 d starting in December 1997. Meals were served from 0630 to 0800. Each participant received ≈110–120 g cooked rice/d. No other foods or beverages were provided. The fruit and powder preparations were designed to contain 5.0 mg β-carotene per serving on the basis of the recommended dietary allowance for retinol of 500 retinol equivalents for children aged 3–6 y and with the use of a conversion ratio of 10 to 1 (17). For the children in the fruit group, the rice was mixed with 20 g *gac* fruit, which initial analyses of fruit samples (before the study started) had shown would contain 5.0 mg β-carotene; however, analysis of the fruit stock after the trial's completion indicated that the fruit meal actually contained only 3.5 mg β-carotene. The powder group received rice mixed with 5.0 mg synthetic β-carotene. For the control group, the rice contained no β-carotene but was colored red with food coloring. A small amount of sugar (7 g) was added to each portion to enhance the flavor.

To prepare the fruit supplement, fresh *gac* fruit was purchased at open markets in Hanoi just before the study began. The fruits were peeled and seeded, and all pulp was mixed thoroughly, weighed, and then frozen in individual packages containing enough to prepare meals for 1 d. β-Carotene powder (10% β-carotene beadlets from F Hoffmann-La Roche Ltd, Basel, Switzerland) was also weighed into 30 individual packages so that each child's portion of supplemented rice would contain ≈50 mg 10% β-carotene. Likewise, Schilling's red and yellow food colorants (McCormick & Co, Hunt Valley, MD) were measured into individual packages to be added to the rice given to the control group. Each portion of red rice contained 0.1 mL yellow color and 0.15 mL red color.

### Food preparation and distribution

In each commune, a household with adequate cooking facilities was selected to serve as the cooking center. One center prepared the rice for the fruit group and one-half the amount of rice for the control group. Another cooking center prepared the rice for the powder group and the remaining one-half for the control group. A standardized way of preparing *xoi gac* was used: rice was weighed and soaked in water overnight, excess water was drained from the rice, and the supplementing material (fruit, powder, or colorant) was mixed into the uncooked rice. The rice was placed in covered steamers and cooked for ≈45 min. After cooking, the rice was then divided into individual portions by weight, packed into a precoded bag, and distributed to the 3 feeding centers in each commune. Children were assigned to the feeding center closest to their house. The code for each child contained information about the child, supplementation group, and feeding center. During the feeding session, consumption of the rice was observed to avoid any replacement or discard. After the child finished eating, the coded bag and leftovers were collected and the leftovers were weighed. Food preparation and distribution were handled by local health workers and were monitored by the staff of the NIN and the project leader.

### Analyses of carotenoids and fatty acids in *gac* fruit

To determine the carotenoid and fatty acid content of *gac* fruit, 4 samples were taken from the mixture used in the supplementation trial. These samples were packed with butylated hydroxytoluene in sealed containers and shipped frozen to the University of California at Davis for analyses.

#### *Carotenoid analyses*

Carotenoids were extracted from *gac* fruit according to methods previously described (18). Individual carotenoids were identified and quantified with the use of reversed-phase HPLC. The system consisted of 2 Absorbosphere HS octadecylsilane columns (150-mm long × 4.6-mm internal diameter, 3-μm particle size; Alltech, Deerfield, IL) connected in series. The liquid chromatograph was a Hewlett-Packard (Palo Alto, CA) 1100 equipped with diode-array detection. The mobile phase conditions were as follows: 0–5 min, 100% acetonitrile:methanol:ammonium acetate (85:15:0.01, vol:vol:wt); 5–20 min, linear gradient to 30% 2-propanol at a flow rate of 0.9 mL/min. The absorbance at 450 nm was monitored. Compounds were identified by comparing their retention times and ultraviolet-visible absorbance spectra with those of authentic standards.

#### *Fatty acid analyses*

The total fatty acid composition of *gac* pulp was determined by analyzing alkali extracts of the pulp with gas chromatography after conversion of liberated fatty acids to methyl esters (19). The analyses were performed at the Clinical Nutrition Research Unit housed at the University of California at Davis. The total oil content of *gac* fruit was determined in freeze-dried pulp samples with the use of the Soxtec System HT 1043 Extraction Unit (Tecator, Inc, Herndon, VA).

### Biochemical analyses

Two 3-mL blood samples were collected from each child by venipuncture into EDTA-coated tubes. The plasma layers were removed and kept frozen at −20°C until they were transported on dry ice to the University of California at Davis for analysis ≈1 wk later.

#### *Standard solutions*

β-Carotene, retinol, retinal, γ-tocopherol, α-tocopherol nicotinate, *O*-ethylhydroxylamine, and *O*-butylhydroxylamine were purchased from Sigma (St Louis, MO). α-Carotene and α-tocopherol were obtained from Fluka (Ronkonkoma, NY). β-Cryptoxanthin, canthaxanthin, lutein, zeaxanthin, and β-apo-129-carotenal were gifts from F Hoffmann-La Roche Ltd. Lycopene was not commercially available and was therefore isolated from fresh tomatoes. All standards were dissolved in ethanol. The concentrations were determined either gravimetrically or from published molar extension coefficients (ε) with ethanol as the solvent (20). Oxime derivatives that served as internal standards for retinol (retinal-*O*-ethyl oxime) and the carotenoids (β-apo-129-carotenal-*O*-*t*-butyl oxime) were synthesized according to established methods (21). The 2 oximes were suspended together in hexane and diluted to an absorbance at 325 nm of ≈0.01 absorbance units and an absorbance at 450 nm of 0.0075 absorbance units by adjusting the dilution and relative proportions of the 2 compounds. α-Tocopherol nicotinate was resuspended in hexane to an absorbance at 290 nm of ≈0.08 absorbance units. All standard solutions were stored at −80°C with butylated hydroxytoluene added.

#### *Plasma sample analyses*

The analytic method was as previously described by Lin et al (22) with some modifications. A 100-μL plasma sample was placed in a tube and mixed with 500 μL ethanol and 10 μg butylated hydroxytoluene; 1.8 mL hexane was then added to the tube, after which oxime and α-tocopherol nicotinate internal standards (100 μL each) were also added with the use of a positive-displacement pipette. The tube was then mixed vigorously by vortex action for 1 min, 400 μL $H_2O$ was added, and the tube was mixed by vortex action for another 30 s and centrifuged at 500 × *g* for 30 s at room temperature. After centrifugation, a 1-mL portion of the organic phase was transferred to a glass vial and evaporated to dryness under argon gas. The residue was dissolved in 25 μL 2-propanol, the vial was mixed, and then the volume in the vial was brought up to 50 μL with acetonitrile. A 20-μL aliquot was separated by reversed-phase HPLC on the system described above for the analysis of carotenoids from *gac* fruit. The analytes were quantified by relating their chromatographically integrated peak areas to the area of the appropriate internal standard. This ratio was then compared with the ratios obtained from 3 plasma calibrators of known concentrations of

**TABLE 1**
Estimated nutrient content of the study meals[1]

| | Control group | Powder group | Fruit group |
|---|---|---|---|
| Energy (kJ) | 920 | 920 | 1025 |
| Protein | | | |
| (g) | 4.02 | 4.02 | 4.44 |
| (% of energy) | 7 | 7 | 7 |
| Carbohydrate | | | |
| (g) | 48.8 | 48.8 | 50.9 |
| (% of energy) | 91 | 91 | 85 |
| Fat | | | |
| (g) | 0.42 | 0.42 | 2 |
| (% of energy) | 2 | 2 | 8 |
| β-Carotene (mg) | 0 | 5 | 3.5 |
| Total carotenoids (mg) | 0 | 6 | 85 |
| Thiamine (mg) | 0.2 | 0.2 | 0.2 |
| Riboflavin (mg) | 0.02 | 0.02 | 0.02 |
| Niacin (mg) | 2.22 | 2.22 | 2.22 |
| Vitamin B-6 (mg) | 0.14 | 0.14 | 0.14 |
| Folate (μg) | 4.50 | 4.50 | 4.50 |
| Vitamin E (mg) | 0.34 | 0.34 | 0.34 |
| Calcium (mg) | 16.6 | 16.6 | 27.8 |
| Iron (mg) | 1.66 | 1.66 | 1.66 |
| Zinc (mg) | 0.69 | 0.69 | 0.69 |

[1] Calculated on the basis of food-composition tables of local foods (6). The study meals consisted of 120 g rice mixed with food coloring (control group), 5.0 mg β-carotene powder (powder group), or 3.5 mg β-carotene (fruit group). Apart from the expected differences in carotenoid content, the study meals did not differ significantly in estimated nutrient content.

retinol, carotenoid, and α- and γ-tocopherol that were prepared in parallel. (The in-house control material was calibrated against standard reference material 968 purchased from the National Institute of Standards and Technology, Gaithersburg, MD.) Carotenoid, tocopherol, and retinol concentrations were expressed as μmol/L plasma. Hemoglobin concentrations were measured with the use of a HemoCue (HemoCue).

### Statistical methods

Statistical analyses were performed with the use of the STATVIEW program (version 5.01; SAS Institute Inc, Cary, NC) and SAS for WINDOWS software (release 7.0; SAS Institute Inc). Differences between anthropometric and biochemical indicators before and after supplementation were calculated, and group means were compared with the use of analysis of variance and analysis of covariance. Covariates were the initial values of the indicators, such as initial body weight or initial retinol concentration. Bonferroni or Dunn post hoc adjustment for multiple comparisons between the groups was applied for all variables. Significance at the $\alpha = 0.05$ level was assigned at $P < 0.0167$ (0.05/3); marginal significance was assigned at $P < 0.0333$ (0.10/3). Log-transformed data were specified in the analyses of nonnormally distributed variables (plasma retinol and hemoglobin concentrations). Potential outliers were detected in a box plot for each variable and were deleted from the data set if they were not biologically possible. In the analyses of plasma micronutrients, a subset (without outliers) of the data set was used. Total sample size was reduced by 9; however, the group size was still sufficient to achieve the desired statistical power ($n \geq 58$). Multiple regression techniques were used to explore the correlation between variables.

## RESULTS

### Diet and *gac* analyses

The estimated nutrient content of the study meals is shown in **Table 1**; the estimates are based on published food-composition tables (6) plus analyzed values for β-carotene and total carotenoids. Apart from the expected differences in carotenoid content, the meals consumed by the fruit group were moderately higher in energy, fat, and calcium than were those consumed by the control and powder groups, but these differences were not significant. β-Carotene was the dominant provitamin A carotenoid present in the *gac* fruit (175 μg/g edible portion; **Table 2**). The lycopene concentration was 802 μg/g edible portion. Concentrations of other carotenoids were negligible. Concentrations reported by other authors and the carotenoid concentrations of other fruit and vegetables available in the study areas are also listed for comparison. The fatty acid composition and total oil content of the *gac* pulp is listed in **Table 3**. The pulp contained 102 mg oil/g edible portion. The water content of the pulp was 78% (by wt).

### Sample size and anthropometric measures

As shown in **Table 4**, the sample of 185 children was evenly distributed across the 3 intervention groups and the 3 age groups. All children completed the study, and subjects consumed the entire study meal 77% of the time. There was no significant difference between groups in compliance. Before the study, 60% of the children were stunted (height-for-age $z$ score $< -2$), 49% were underweight (weight-for-height $z$ score $< -2$), and there was no significant difference in weight and height between the 3 groups.

After initial height was controlled for, mean height after supplementation was significantly lower in the powder group than in the control and fruit groups ($P < 0.0001$), but there was no significant difference in mean height between the fruit and control groups. After initial weight was controlled for, mean weight after supplementation was significantly higher in the control group than in the fruit ($P < 0.0001$) and powder ($P < 0.0001$) groups, and mean weight in the powder group was significantly lower than that in the fruit group ($P = 0.0142$). At the end of the study, 55% of the children were still stunted and 42% were underweight.

### Intake of β-carotene and vitamin A

The dietary intake data collected from mothers are shown in Table 4 and do not include the contribution of supplemented meals. Before the supplementation, there were no significant differences between the 3 groups in daily intakes of either β-carotene or vitamin A. Mean intakes of vitamin A were well below both the FAO/WHO safe level of intake for children 1–6 y of age (400 μg retinol equivalents/d) and the basal requirement (200 μg/d) (23). After supplementation, intakes of β-carotene from foods other than the supplemented meals increased in both the control and powder groups but declined slightly in the fruit group. Vitamin A intakes increased remarkably in the control group, from 93 to 199 μg retinol equivalents/d.

### Plasma micronutrient concentrations

#### β-*carotene*

The mean initial β-carotene concentration for the children in all 3 groups (0.198 μmol/L) was within the normal range

**TABLE 2**
Carotenoid content of *gac* and commonly consumed fruit and vegetables in the study areas

| Local name (English name) | Scientific name | β-Carotene[1] | Total carotenoids[1] |
|---|---|---|---|
| | | *μg/g* | *μg/g* |
| Fruit | | | |
| *Gac* | *Momordica cochinchinensis* Spreng | 175 | 977 |
| | | 188.1 (5) | 891.5 (5) |
| | | 457.80 (6) | |
| *Du-du* (papaya) | *Carica papaya* | 12.1 (5) | 29.6 (5) |
| | | 7.50 (6) | |
| *Quat* (mandarin orange) | *Citrus japonica* | 4.65 (6) | |
| *Chuoi* (banana) | *Musa sapientum* | 2.90 (6) | |
| *Tao ta* (jujube, Chinese date) | *Ziziphus jujuba* | 0.40 (6) | |
| Vegetables | | | |
| *Rau san tuoi* (cassava leaves) | *Manihot esculenta* | 82.80 (6) | |
| *Rau day* (jute potherb) | *Corchorus olitonus* | 78.50 (6) | 60.8 (5) |
| *Bap cai* (cabbage) | *Brassica oleracea* | 51.00 (6) | |
| *Rau cai cuc* (crown daisy) | *Chrysanthemum cronarium* | 31.60 (6) | |
| *Rau muong* (water spinach) | *Ipomoea aquatica* | 28.65 (6) | |
| *Rau lang* (sweet potato leaves) | *Ipomoea batatas* | 27.00 (6) | 32.9 (5) |
| *Rau cai xanh* (mustard green) | *Brassica juncea* | 18.25 (6) | |
| *Ca chua* (tomato) | *Solanum lycopersicum* | 6.00 (6) | |
| *Khoai lang* (yam) | *Opomoea batatas* | 14.70 (6) | 13.6 (5) |
| *Xu hao* (kohlrabi) | *Brassica oleracea* var. *gongylodes* | 3.13 (6) | |
| *Khoai tay* (potato) | *Solanum tuberrosum* | 0.29 (6) | |
| *Bi* (sweet gourd, wax gourd) | *Benincasa cerifera* | 0.05 (6) | |

[1] Reference numbers in parentheses.

(0.093–0.465 μmol/L; 24) (**Table 5**). There was no significant difference in initial β-carotene concentration between the groups. After initial weight and initial concentration were controlled for, mean plasma β-carotene concentrations after supplementation were significantly higher in the fruit and powder groups than in the control group ($P < 0.0001$). The difference between the fruit and powder groups in the mean increase in plasma β-carotene concentration was also significant ($P = 0.0131$). The plasma β-carotene concentrations of all the children in the fruit group and of 93% of the children in the powder group were >0.465 μmol/L after supplementation.

### *Other carotenoids*

After initial concentration and initial weight were controlled for, mean plasma α-carotene concentrations after supplementation were significantly higher in the fruit group than in the control ($P < 0.0001$) and powder ($P = 0.0018$) groups. After supplementation, mean plasma lycopene and zeaxanthin concentrations were significantly higher in the fruit group than in the control ($P < 0.0001$) and powder ($P < 0.0001$) groups. There was no significant group effect for changes in other carotenoids or between the changes in the control and powder groups.

### *Retinol*

The mean plasma retinol concentration (0.76 ± 0.33 μmol/L) of the children before supplementation was marginally adequate (24). Twenty-three percent of the children ($n = 42$) had retinol concentrations below the lower limit of the normal range (≤0.70 μmol/L) (24). There were no significant differences between the 3 groups in initial retinol concentrations. After supplementation, plasma retinol concentrations increased in all 3 groups (Table 5). After initial concentration and initial weight were controlled for, mean plasma retinol concentrations after supplementation were significantly higher in the fruit group than in the control ($P = 0.006$) and powder ($P = 0.0053$) groups. Mean retinol concentrations in the control and powder groups did not differ significantly. After supplementation, 4%, 12%, and 14% of children in the fruit, control, and powder groups, respectively, still had low retinol concentrations (≤0.70 μmol/L).

### *Hemoglobin*

Children were selected who had a hemoglobin concentration between 100 and 120 g/L. After supplementation, mean hemoglobin concentrations increased in all 3 groups. There was no significant group effect in children with initial concentrations >110 g/L; however, there was a significant ($P = 0.0034$) interaction

**TABLE 3**
Fatty acid composition and total oil content of *gac* pulp

| Fatty acid | Concentration | Percentage of total fatty acids |
|---|---|---|
| | *mg/g edible portion* | *%* |
| 14:0 | 0.89 | 0.87 |
| 16:0 | 22.48 | 22.04 |
| 16:1 | 0.27 | 0.26 |
| 18:0 | 7.20 | 7.06 |
| 18:1n−9 | 34.76 | 34.08 |
| 18:1n−7 | 1.15 | 1.13 |
| 18:2 | 32.06 | 31.43 |
| 18:3n−3 | 2.18 | 2.14 |
| 20:0 | 0.40 | 0.39 |
| 20:1 | 0.15 | 0.15 |
| 20:4 | 0.10 | 0.10 |
| 22:0 | 0.19 | 0.19 |
| 24:0 | 0.14 | 0.14 |
| Total | 101.98 | |

**TABLE 4**
Initial descriptive data and changes after supplementation[1]

| | Control group ($n$ = 38 F, 25 M) | | Powder group ($n$ = 25 F, 35 M) | | Fruit group ($n$ = 31 F, 31 M) | |
|---|---|---|---|---|---|---|
| | Inital | Change | Inital | Change | Inital | Change |
| Age ($n$) | | | | | | |
| 31–50 mo | 28 | | 26 | | 20 | |
| 51–60 mo | 19 | | 18 | | 21 | |
| 61–70 mo | 16 | | 16 | | 21 | |
| Height (cm) | 95.8 ± 6.3[2] | 1.052 ± 1.746[b] | 93.8 ± 5.8 | 1.343 ± 1.375[a] | 96.3 ± 5.5 | 0.850 ± 1.198[b] |
| Weight (kg) | 13.7 ± 1.5 | 0.427 ± 0.398[c] | 13.1 ± 1.5 | 0.362 ± 0.488[a] | 13.2 ± 1.4 | 0.376 ± 0.380[b] |
| Stunted (%)[3] | 46 | −5 | 70 | −9 | 64 | −2 |
| Underweight (%)[4] | 35 | −7 | 58 | −8 | 55 | −9 |
| Wasted (%)[5] | 3 | −1.5 | 1.6 | 1.4 | 0 | 1 |
| Daily intake[6] | | | | | | |
| β-Carotene (μg) | 52 ± 62 | 61 ± 17 | 39 ± 41 | 10 ± 16 | 44 ± 56 | 7 ± 10 |
| Vitamin A (μg retinol equivalent) | 93 ± 105 | 106 ± 27 | 86 ± 98 | 67 ± 65 | 87 ± 92 | 48 ± 37 |

[1] Means within a row with different superscript letters are significantly different, $P < 0.0167$ (analysis of covariance with Bonferroni correction).
[2] $\bar{x}$ ± SD.
[3] $z$ Score of height-for-age < −2.
[4] $z$ Score of weight-for-age < −2.
[5] $z$ Score of weight-for-height < −2.
[6] Data do not include the contribution from supplemented meals.

between group and anemia (initial hemoglobin concentration ≤110 g/L). In the children with anemia, a significant group effect was detected ($P = 0.0006$). In this subgroup, mean hemoglobin concentrations after supplementation in the control group were significantly lower than those in the fruit group ($P = 0.017$) but were not significantly different from those in the powder group; mean hemoglobin concentrations in the powder and fruit groups were not significantly different.

### *Tocopherols*

After initial concentration and initial weight were controlled for, plasma α-tocopherol concentrations after supplementation were marginally higher in the powder group than in the fruit group ($P = 0.03$). There was no significant difference between the control and powder groups or between the control and fruit groups. γ-Tocopherol increased slightly in the fruit and powder groups after supplementation, but the changes did not differ significantly between the groups (data not shown).

## DISCUSSION

The selection of *xoi gac* was based on the high β-carotene content of the *gac* fruit and the established acceptance of this dish within our target population. The β-carotene content of *gac* fruit is at least an order of magnitude higher than that of other fruit and vegetables available in the region (Table 2). More importantly, the *gac* fruit possesses 2 characteristics that suggest it can be a superior source of provitamin A. First, β-carotene derived from fruit appears to be more easily absorbed than that derived from dark-green vegetables, presumably because of a more efficient release of bound molecule from the matrixes of fruit than from those of vegetables (3). Second, the *gac* fruit is richer in oils than other common Vietnamese fruit and vegetables; the content and quality of the lipids in a meal are known to play a key role in β-carotene absorption (25). Our analyses of the *gac* pulp sample used in the supplementation provided a value of 101.98 mg oil/g pulp. The greater increase in plasma β-carotene concentrations after supplementation in the fruit group

**TABLE 5**
Initial values and changes in plasma micronutrient concentrations after supplementation[1]

| | Control group ($n$ = 59) | | Powder group ($n$ = 58) | | Fruit group ($n$ = 59) | |
|---|---|---|---|---|---|---|
| | Initial | Change | Initial | Change | Initial | Change |
| β-Carotene (μmol/L) | 0.190 ± 0.140 | 0.108 ± 0.184[a] | 0.192 ± 0.200 | 1.672 ± 1.246[b] | 0.255 ± 0.236 | 2.110 ± 0.898[c] |
| α-Carotene (μmol/L) | 0.073 ± 0.072 | 0.018 ± 0.062[a] | 0.067 ± 0.076 | 0.008 ± 0.060[a] | 0.072 ± 0.061 | 0.023 ± 0.060[b] |
| Canthaxanthin (μmol/L) | 0.051 ± 0.90 | 0.088 ± 0.116 | 0.103 ± 0.129 | 0.034 ± 0.180 | 0.033 ± 0.063 | 0.118 ± 0.108 |
| Lutein (μmol/L) | 0.170 ± 0.124 | 0.003 ± 0.125 | 0.164 ± 0.096 | 0.009 ± 0.090 | 0.197 ± 0.139 | 0.021 ± 0.138 |
| Lycopene (μmol/L) | 0.057 ± 0.130 | 0.073 ± 0.174[a] | 0.051 ± 0.118 | 0.197 ± 1.057[a] | 0.078 ± 0.142 | 0.729 ± 0.590[b] |
| Zeaxanthin (μmol/L) | 0.013 ± 0.170 | 0.021 ± 0.023[a] | 0.012 ± 0.028 | 0.002 ± 0.002[a] | 0.021 ± 0.025 | 0.066 ± 0.046[b] |
| Retinol (μmol/L) | 0.710 ± 0.333 | 0.100 ± 0.215[a] | 0.769 ± 0.294 | 0.102 ± 0.179[a] | 0.805 ± 0.364 | 0.128 ± 0.250[b] |
| Hemoglobin (g/L)[2] | 115 ± 4.80 | 4.714 ± 6.113 | 116 ± 4.00 | 5.667 ± 6.701 | 110 ± 4.50 | 6.484 ± 7.415 |
| α-Tocopherol (μmol/L) | 11.3 ± 3.44 | 0.201 ± 0.228 | 9.80 ± 2.73 | 0.304 ± 0.287 | 10.5 ± 3.24 | 0.216 ± 0.288 |

[1] $\bar{x}$ ± SD. Means within a row with different superscript letters are significantly different, $P < 0.0167$ (analysis of covariance with Bonferroni correction).
[2] For the entire population of children in the study, there were no significant differences between groups in changes in hemoglobin concentration; however, in the subgroup of children with initial hemoglobin concentrations ≤110 g/L, there was a significant group interaction ($P < 0.0034$). In this subgroup, after initial weight and initial concentration were controlled for, mean hemoglobin concentrations after supplementation in the control group were significantly lower than those in the fruit group ($P = 0.017$) but were not significantly different from those in the powder group (analysis of covariance with Bonferroni correction).

(1.86 μmol/L) than in the powder group (1.48 μmol/L) indicates improved absorption of β-carotene in the fruit group, which we attribute to the higher lipid content. This indication is strengthened by the discrepancy in the amount of β-carotene given to the 2 groups. The supplement given to the fruit group contained only 3.5 mg β-carotene, whereas that given to the powder group contained 5.0 mg β-carotene.

Mean retinol concentrations in all 3 groups increased from initial values after supplementation, and mean retinol concentrations after supplementation were significantly higher in the fruit group than in the control ($P = 0.006$) and powder ($P = 0.0053$) groups. Because there was a significant difference between the 2 treatment groups and the control group in the increase in plasma β-carotene concentration ($P < 0.0001$), it is surprising that the increase in plasma retinol concentration in the control and powder groups was so uniform. One explanation is the finding that the children in the control group consumed more preformed vitamin A during the study; indeed, the food-frequency data indicated that the children in the control group increased their consumption of condensed milk and meat during the study (data not shown). It is also plausible that the small amount of protein in the rice that was fed (≈4 g/d) led to mobilization of stored retinol and an increase in plasma concentrations. Interactions between protein deficiency and vitamin A status in children were noted by Sommer et al (26). The synthesis and circulating concentrations of retinol binding protein are sensitive to intakes of energy and protein. Accordingly, protein status can affect vitamin A serum concentrations by influencing the production and release of retinol binding protein (27). Because enrollment was restricted to children who were likely to be vitamin A deficient (by using hemoglobin concentration as a proxy for vitamin A status), another possible reason for the uniform increase in plasma retinol in all 3 groups is a regression-to-the-mean phenomenon (28–30).

There are no simple means of estimating the amount of retinol derived from dietary β-carotene when given with normal food products. Plasma vitamin A concentrations are homeostatically regulated by hepatic vitamin A status, and in well-nourished populations, plasma retinol concentrations are maintained within a narrow range and do not closely reflect recent dietary intake (31). Plasma vitamin A response is more useful as an indicator of vitamin A status in vitamin A–depleted populations and is sensitive to vitamin A supplementation in malnourished children or breast-feeding mothers. However, in marginally malnourished individuals, the plasma retinol concentration is not consistently responsive to an increase in vitamin A consumption. Changes in circulating β-carotene concentrations serve as the least problematic index for absorption because plasma carotenoid concentrations are reflective of dietary intake.

Other β-carotene supplementation trials in which children were fed vegetables and fruit have had mixed results. In their study in Guatemala, Bulux et al (11) reported a 3-fold increase in plasma β-carotene concentrations in the group supplemented with purified β-carotene; however, concentrations remained unchanged with carrot supplementation, and plasma retinol concentrations did not increase with supplementation with either purified β-carotene or carrots. The change in plasma retinol concentrations in children after supplementation with red sweet potato in a study in Indonesia was comparable to that seen in our study (9). The increase was improved with additional fat in the diet and when the children were dewormed. A recent β-carotene supplementation trial in lactating women in Indonesia showed a significant increase in the concentrations of plasma β-carotene and retinol in the group given an enriched wafer but no change in the group supplemented with dark-green leafy vegetables (12). The inconsistent results from β-carotene feeding studies may be because of differences in study design and length, the amount of the β-carotene supplements, the source of the β-carotene, and the age and physiologic status of the target groups. Because the bioavailability of β-carotene is influenced by a multitude of factors, it is difficult to make direct comparisons between studies.

An interaction between iron and vitamin A status was noted in several nutritional intervention studies (14, 32). Researchers studying animals have also observed that vitamin A supplementation has a positive effect on iron status (13, 15). In the present study, mean hemoglobin concentrations in all groups increased after supplementation. Although, some of the improvement may have been due to regression to the mean (low values of a measure are more likely to increase than decrease when measured again) (28–30), improved vitamin A status may have a positive effect on erythropoiesis or iron mobilization (15). An intriguing observation is that the difference in hemoglobin concentrations between the fruit and control groups was significant only among children with initial hemoglobin concentrations ≤110 g/L. β-Carotene might affect iron metabolism independently of the benefit of retinol and protein by improving iron absorption (33) or by protecting erythrocytes from oxidative damage by free radicals (34). Our results suggest that anemic children might benefit from β-carotene supplementation, but because the sample of children with hemoglobin concentrations <110 g/L in the present study was small ($n = 19$), larger studies are needed to confirm this finding.

In conclusion, the *gac* fruit was selected for the present study because of its high concentrations of β-carotene and lipids and local availability. *Xoi gac* was as effective at increasing plasma β-carotene concentrations as was a proportionally larger daily dose of synthetic β-carotene. Increases in plasma retinol concentrations were higher in the fruit group than in the control and powder groups. Because the differences between groups were marginal, functional tests and isotope-dilution techniques are advisable to determine a conclusive change in vitamin A status. Although seldom used in vitamin A intervention studies, fruit has been shown to be a good source of β-carotene. The *gac* fruit may be underutilized in the diet because of its seasonality and regional unavailability and a lack of awareness concerning its potential health value. The significant increase in plasma β-carotene concentrations in children after supplementation with *xoi gac* for 30 d indicates that *gac* fruit should be considered as part of a sustainable, food-based strategy for reducing the incidence of vitamin A deficiency in Vietnam.

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