Carotene-rich plant foods ingested with minimal dietary fat enhance the total-body vitamin A pool size in Filipino schoolchildren as assessed by stable-isotope-dilution methodology\textsuperscript{1–3}

Judy D Ribaya-Mercado, Cherry C Maramag, Lorena W Tengco, Gregory G Dolnikowski, Jeffrey B Blumberg, and Florentino S Solon

\textbf{ABSTRACT}

\textbf{Background:} Strategies for improving the vitamin A status of vulnerable populations are needed.

\textbf{Objective:} We studied the influence of the amounts of dietary fat on the effectiveness of carotene-rich plant foods in improving vitamin A status.

\textbf{Design:} Schoolchildren aged 9–12 y were fed standardized meals 3 times/d, 5 d/wk, for 9 wk. The meals provided 4.2 mg provitamin A carotenoids/d (mainly β-carotene) from yellow and green leafy vegetables [carrots, pechay (bok choy), squash, and kangkong (swamp cabbage)] and 7, 15, or 29 g fat/d (2.4, 5, or 10 g fat/meal) in groups A, B, and C (n = 39, 39, and 38, respectively). Other self-selected foods eaten were recorded daily. Before and after the intervention, total-body vitamin A pool sizes and liver vitamin A concentrations were measured with the deuterated-retinol-dilution method; serum retinol and carotenoid concentrations were measured by HPLC.

\textbf{Results:} Similar increases in mean serum β-carotene (5-fold), α-carotene (19-fold), and β-cryptoxanthin (2-fold) concentrations; total-body vitamin A pool size (2-fold); and liver vitamin A (2-fold) concentrations were observed after 9 wk in the 3 study groups; mean serum retinol concentrations did not change significantly. The total daily β-carotene intake from study meals plus self-selected foods was similar between the 3 groups and was 14 times the usual intake; total fat intake was 0.9, 1.4, or 2.0 times the usual intake in groups A, B, and C, respectively. The overall prevalence of low liver vitamin A (<0.07 μmol/g) decreased from 35% to 7%.

\textbf{Conclusions:} Carotene-rich yellow and green leafy vegetables, when ingested with minimal fat, enhance serum carotenoids and total-body vitamin A pool size and can restore low liver vitamin A concentrations to normal concentrations. \emph{Am J Clin Nutr} 2007; 85:1041–9.

\textbf{KEY WORDS} Vitamin A, deuterated-retinol dilution, stable-isotope dilution, retinol, plant carotenoids, β-carotene, bioavailability, dietary fat, school-age children, Philippines

\textbf{INTRODUCTION}

Vitamin A deficiency results in growth retardation, anemia, reduced resistance to infection, impaired cellular differentiation, xerophthalmia, and, ultimately, blindness and death (1). Plant foods containing provitamin A carotenoids often are the primary sources of vitamin A in some populations in whom vitamin A deficiency is prevalent (2–4); thus, strategies for improving the absorption and bioconversion of plant carotenoids are essential. It is well known that dietary fat enhances carotene uptake from foods (2). In certain groups, the concomitant consumption of plant carotenoids and dietary fat enhances serum (or plasma) retinol concentrations (2–8). However, because serum retinol is subject to homeostatic control over the physiologic range of liver vitamin A concentrations (9), serum retinol could remain unchanged with dietary intervention (10–14). During the past decade, stable-isotope-dilution methods have emerged as the state-of-the-science methods for the biochemical assessment of vitamin A status (15, 16). Deuterated-retinol-dilution (DRD) techniques that use deuterium-labeled vitamin A (17–19) have been used to assess the total-body vitamin A pool size (also referred to as total-body vitamin A stores) in adults and children in the United States (17, 20), Bangladesh (14, 20–22), Guatemala (23, 24), Philippines (8, 24, 25), China (13), Peru (26), and Nicaragua (27).

The aims of this study were to assess the influence of amounts of dietary fat on the bioavailability (ie, change in serum concentrations) of provitamin A carotenoids in yellow and green-leafy vegetable meals, and the effectiveness of plant carotenoids in improving the vitamin A status of marginally nourished school-age children as assessed by the DRD method.

\textbf{SUBJECTS AND METHODS}

\textbf{Subjects}

The study participants were 116 children aged 9–12 y (x ± SD: 10.6 ± 0.8 y) enrolled in the elementary schools of Banawang and Overland, located in the adjacent rural communities of bustamante, 

1 From the Jean Mayer US Department of Agriculture Human Nutrition Research Center at Tufts University, Boston, MA (JDR-M, GGD, and JBB), and the Nutrition Center of the Philippines, Taguig City, Philippines (CCM, LWT, and FSS).

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of Banawang and Atillano Ricardo, in Bagac, Bataan province, Philippines. The study sites were chosen because of their low socioeconomic status and high prevalence of subclinical vitamin A deficiency based on the 1998 Philippine national nutrition survey (28). The study participants were generally in good health, had no major chronic illnesses, had no clinical signs of vitamin A deficiency, and did not take any nutritional supplements. During the time that vitamin A status was assessed, the participants had no acute illnesses, febrile conditions, or gastrointestinal problems. Selection of the study sites and screening of participants had no acute illnesses, febrile conditions, or gastrointestinal problems. Selection of the study sites and screening of the study participants were conducted between December 2003 and June 2004. Baseline tests, dietary intervention, and postintervention tests were conducted from July 2004 to November 2004. Written informed consent was obtained from the children and their caregivers. Approval to conduct the study was obtained from the Philippine Council for Health Research and Development National Ethics Committee and from the Tufts University–New England Medical Center Human Investigation Review Committee.

Study design: dietary intervention

The study protocol is given in Figure 1. The participants were randomly assigned to study groups A, B, or C (n = 39, 39, and 38, respectively) and were fed standardized meals at school 3 times daily on school days (5 d/wk) for 9 wk. The meals consisted of 3-d rotating menus consisting of traditionally accepted recipes with different fat contents: 2.4 (group A), 5 (group B), or 10 (group C) g/meal. In the groups fed more fat, carbohydrate was reduced to provide similar amounts of energy. There were no dietary restrictions imposed on the study participants; they were free to eat their usual self-selected snacks and other foods during the 9-wk period. However, participants and their caregivers were asked to record all self-selected foods not provided in the study meals. These food records were submitted daily to dietitians from the Nutrition Center of the Philippines (NCP), who verified the kinds and amounts of foods eaten by interviewing the children. Philippine food-composition tables (29) were used to assess intakes of retinol, β-carotene, energy, fat, protein, and carbohydrate. Intakes of α-carotene and β-cryptoxanthin were assessed by using the US Department of Agriculture (USDA) nutrient database (30). Because many Philippine foods are not listed in the USDA database, the intakes of α-carotene and β-cryptoxanthin may have been underestimated.

The study meals contained the following vegetables: carrots, pechay, squash, and kangkong (swamp cabbage; Ipomea batatas aquatica). The meals provided the following amounts of provitamin A carotenoids per meal: β-carotene, 1123 μg; α-carotene, 271 μg; and β-cryptoxanthin, 14 μg. Small amounts of chicken and pork provided 4.2 μg preformed retinol per meal. Refined coconut oil, the most common source of dietary fat in rural Philippine communities, was used as the primary source of dietary fat for this study, and samples were analyzed for total fat content by the Adamson University Technology Research and Development Center (Manila, Philippines). Vegetables and other ingredients in the menu were boiled separately, and predetermined amounts were weighed and placed in individual food containers for each child; coconut oil was added by using custom-made ladles of different sizes that delivered the desired amounts. The food containers were color-coded depending on the child’s study group. Participants belonging to the same group ate together, separately from the other groups. NCP dietitians were responsible for all of the dietary aspects of the study, including the purchase, preparation, and cooking of foods and the weighing of meal components into the food containers. They supervised the study participants during meals at school and recorded their food intakes and any plate waste. They also verified and kept daily records of the self-selected foods eaten during school days and of foods eaten at home on weekends, as reported by the children or their caregivers. The daily intakes of retinol, provitamin A carotenoids, energy, fat, protein, and carbohydrates from self-selected foods eaten during the 45 school days plus the daily amounts provided by the study meals were added together to obtain the total dietary intakes per day. Foods eaten during the 16 weekend days of the study period were considered to represent the subjects’ usual dietary intakes.

Deuterated-retinol-dilution method: estimation of the total-body vitamin A pool size

The DRD technique was used to estimate total-body vitamin A pool size (17–19). Tetradeterated retinyl acetate [D4-retinyl acetate; all-trans-retinyl-10,19,19,19-[2H4]acetate] and octadeterated retinyl acetate [D8-retinyl acetate; all-trans-retinyl-10,14,19,19,20,20,20-[3H6]acetate] were synthesized by the

Cambridge Isotope Laboratories (Andover, MA). Capsules containing 5-mg amounts of these isotopes were prepared by first dissolving a known amount in absolute ethanol, adding a predetermined amount of corn oil, and evaporating off the ethanol under nitrogen as previously described (23). D4-retinyl acetate was administered at baseline, and D8-retinyl acetate was administered after the intervention to distinguish serum [\( ^{2}\text{H}_{4}\) ]-retinol (D8-retinol) from any residual [\( ^{2}\text{H}_{4}\) ]-retinol (D4-retinol). A 10-d stabilization period followed the food-intervention phase (during which time the subjects ate their usual diets) to allow the vitamin A consumed during the intervention period to equilibrate with endogenous vitamin A before the postintervention DRD procedure was initiated. The capsules of D4- or D8-retinyl acetate were administered to the children by NCP dietitians at school, and they were ingested with a high-fat, low-vitamin A breakfast consisting of glutinous rice and palm starch cooked in coconut milk and to which coconut oil (10 g) was added. The children were observed when they swallowed the capsule.

DRD involves the administration of an oral dose of deuterated retinyl acetate, determination of the ratio of deuterated to non-deuterated retinol (D/H) in serum after \( \approx 20 \) d when the administered isotope has mixed with the body’s vitamin A pool, and using the serum (D/H) value in a mathematical formula developed by Olson and coworkers to calculate the total-body vitamin A pool size (17). This formula, which is a modification of that developed by Bausch and Rietz (31) in rats, is as follows:

Total-body vitamin A pool size (in mmol retinol) =

\[
F \times \text{dose} \times \{S \times a \times [(1/D:H) - 1]\}
\]

where \( F \) is a factor that expresses the storage efficiency of an oral vitamin A dose and is considered to be 0.5 on the basis of studies in rats (31), dose is the amount of labeled vitamin A (in mmol retinol equivalents) administered orally, and \( S \) is 0.65, a correction for inequalities in specific activities in serum and liver (32). The factor \( a \) is the fraction of the absorbed deuterated retinol remaining in the body at the time of blood sampling and is based on the half-life of vitamin A turnover, which was estimated to be \( \approx 140 \) d in adults (33). In the formula, \( a \) was assumed to be independent of the size of the vitamin A stores and to be time invariant: \( a = e^{-kt} \), where \( k = 0.693/140 \) or 0.5%/d, and \( t \) is the time (in d) since the isotope dose was administered. The factor “−1” corrects for the contribution of the administered dose to the total-body vitamin A pool.

**Estimation of liver vitamin A concentrations**

Liver vitamin A concentration was estimated by assuming that, in this age group, liver weight is 3% of body weight (33) and that 90% of the total-body vitamin A is in the liver (9). It is recognized, however, that because the study participants had poorer vitamin A status at baseline than after the intervention, baseline hepatic vitamin A stores may have been <90% of the total-body vitamin A. In poorly or marginally nourished persons, nonhepatic tissues contain an appreciable amount (10–50%) of the total-body vitamin A (33, 34). In the present study, by choosing to make the same assumption regarding the percentage of total-body vitamin A present in liver (ie, 90%) for estimating liver vitamin A concentrations at baseline and after the intervention, we are reporting improvements in liver vitamin A concentrations that are conservative.

**Blood handling and tests**

Venous blood was drawn 4 times during the study period, ie, twice at preintervention (3 and 20 d after the oral dose of D4-retinyl acetate) and twice at postintervention (at the end of the 9-wk dietary intervention period and 20 d after administration of the oral dose of D8-retinyl acetate). Blood drawn 3 d after the isotope was administered at preintervention was used to study vitamin A kinetics 3 d after ingestion of deuterated retinyl acetate, and the data will be reported in a separate paper. Blood drawn 20 d after the isotope was administered at preintervention was used to measure serum (D4:H)-retinol, carotenoids, retinol, and C-reactive protein. Blood drawn at the end of the dietary intervention period was used to measure serum carotenoids, retinol, and C-reactive protein; that 20 d after the isotope was administered at postintervention was used to measure serum (D8:H)-retinol.

To prevent the photodegradation of retinoids and carotenoids, venous blood was extracted into aluminum-wrapped evacuated tubes, and subsequent procedures were carried out in a darkened room. Blood was allowed to clot and was then centrifuged at 2800 \times g for 30 min at room temperature. Aliquots (0.5 mL) of serum were transferred into aluminum-wrapped cryovials, frozen at \( -20 \) °C, and then transported within 24 h on dry ice to a freezer (\( -70 \) °C) in Manila, where they were kept until handled and carried on dry ice to Tufts University (Boston, MA), where they were stored at \( -70 \) °C until analyzed.

Carotenoids and retinol were extracted from serum with the use of chloroform:methanol (2:1, by vol) and then hexane (35), after the addition of internal standards (echinonene and retinyl acetate), and were analyzed simultaneously by a gradient reversed-phase HPLC procedure (36) with a YMC30 carotenoid column (3-\( \mu \)m particle size; internal diameter \( \times \) length: 4.6 \( \times \) 150 mm), 2 Waters 515 HPLC pumps (Waters Corp, Milford, MA), a Waters 717plus autosampler, and a Waters 2996 photodiode array detector, which was set to monitor the absorbance of these compounds at 450 and 340 nm, respectively. The peak areas were calibrated against known amounts of standards, and concentrations were corrected for extraction and handling losses by determining percentage recoveries of internal standards. Retinol isotopes were analyzed by gas chromatography electron capture negative chemical ionization mass spectrometry (GC-MS) after separation of retinol from other serum components by HPLC and derivatization of retinol into trimethylsilyl derivatives (37). All HPLC and GC-MS procedures were conducted at Tufts University.

Serum C-reactive protein was analyzed at the Bureau of Research and Laboratories, Department of Health, Manila, by solid-phase sandwich immunometric assay with a NycoCard READER II System (Axis-Shield Group, Oslo, Norway).

**Helmithic infections**

Fecal samples were analyzed at baseline for helminths (Ascaris lumbricoides, Trichuris trichiura, and hookworm) by using the Kato-Katz procedure (38). Those found positive for any of these intestinal parasites were treated with 400 mg chewable albendazole (Kopran Ltd, Mumbai, India) 1 wk before the DRD test was initiated at baseline. The Kato-Katz procedure was repeated midway and at the end of the intervention period to determine any new or recurrent helminthic infections. The thresholds proposed by a World Health Organization Expert...
Committee (39) were used to classify the intensity of infection (light, moderate, or heavy) for each helminth.

**Anthropometric measurements**
Prevaleces of underweight, stunting, and wasting were determined on the basis of weight-for-age, height-for-age, and body mass index—-for-age $z$ scores of less than $−2$, with the use of the US Centers for Disease Control and Prevention (CDC) growth charts (40). The $z$ scores were computed by using the STATA 9 statistical package (STATA Corp, College Station, TX).

**Statistical analyses**
Dietary intakes of the 3 treatment groups were analyzed by using one-factor analysis of variance (ANOVA) and Scheffe’s test. For variables measured at baseline and at postintervention, a 2-factor ANOVA was used to study the main effects of treatment group and repeated measures of variables and the interaction of these 2 main effects. Variables that were not normally distributed were logarithmically transformed. The above analyses were performed by using STATVIEW SE + GRAPHICS software (Abacus Concepts Inc, Berkeley, CA). When the interaction of treatment group $\times$ repeated measures was not significant ($P > 0.05$), no further subgroup analysis was performed, and only the main effects are described and discussed. The overall prevalences of underweight, stunting, wasting, and vitamin A deficiency at baseline were compared with those postintervention by using McNemar’s test with the SAS program (SAS Institute Inc, Cary, NC).

**RESULTS**
A total of 116 children participated in the dietary intervention phase of the study; however, only 106 children completed the procedures for the DRD test at postintervention. Furthermore, in 2 subjects who completed the postintervention DRD procedures, serum D8-retinol was undetectable, possibly because of the incomplete ingestion or malabsorption of the D8-retinyl acetate dose. Thus, baseline and postintervention DRD test results are available for only 104 of the children. At 112 d after administration of the D4-retinyl acetate dose, the mean $(± SD)$ enrichment of serum retinol with residual D4-retinol was $2.23 ± 0.82\%$.

**Dietary intakes**
The mean $(± SD)$ selected dietary intakes of the study participants are provided in Table 1 from the following sources: 1) standardized meals provided at school, after subtracting plate waste (a total of 135 meals were provided during 45 school days); 2) standardized meals provided at school, after subtracting plate waste, plus the contribution from self-selected foods eaten during the 45 school days; and 3) self-selected foods eaten during 16 non-school days (Saturdays and Sundays). The standardized meals provided to groups A, B, and C were similar in energy, provitamin A carotenoid, and retinol contents, but differed in fat and carbohydrate contents. The carbohydrate sources (rice, pasta) contained some protein; thus, as their amounts were decreased, dietary protein also decreased. However, the daily total protein intakes ingested by the 3 groups were not significantly different when the self-selected snacks and foods were included.

### Table 1

<table>
<thead>
<tr>
<th>Energy (kcal/d)</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>994 ± 33</td>
<td>967 ± 57</td>
<td>968 ± 48</td>
</tr>
<tr>
<td></td>
<td>1588 ± 178</td>
<td>1585 ± 234</td>
<td>1655 ± 349</td>
</tr>
<tr>
<td></td>
<td>1050 ± 341</td>
<td>1072 ± 386</td>
<td>1089 ± 453</td>
</tr>
</tbody>
</table>

**Fat (g/d)²**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1 ± 0.2</td>
<td>15.3 ± 0.8</td>
<td>29.2 ± 1.4</td>
</tr>
<tr>
<td>21.1 ± 5.4</td>
<td>29.3 ± 6.0</td>
<td>44.6 ± 11.1</td>
</tr>
</tbody>
</table>

**Carbohydrate (g/d)³**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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</thead>
<tbody>
<tr>
<td>36.6 ± 1.4</td>
<td>34.9 ± 2.5</td>
<td>32.9 ± 1.9</td>
</tr>
<tr>
<td>53.2 ± 8.4</td>
<td>51.3 ± 8.6</td>
<td>52.8 ± 13.1</td>
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</tbody>
</table>

**Retinol (μg/d)⁴**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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</thead>
<tbody>
<tr>
<td>12.7 ± 0.5</td>
<td>12.6 ± 1.0</td>
<td>12.7 ± 0.8</td>
</tr>
<tr>
<td>81.5 ± 47.0</td>
<td>75.6 ± 43.4</td>
<td>89.0 ± 76.2</td>
</tr>
</tbody>
</table>

**β-Carotene (μg/d)⁴**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>3372 ± 120</td>
<td>3360 ± 216</td>
<td>3372 ± 168</td>
</tr>
<tr>
<td>3552 ± 168</td>
<td>3528 ± 288</td>
<td>3540 ± 216</td>
</tr>
</tbody>
</table>

**α-Carotene (μg/d)⁴**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>811 ± 36</td>
<td>811 ± 60</td>
<td>816 ± 48</td>
</tr>
<tr>
<td>838 ± 55</td>
<td>838 ± 96</td>
<td>838 ± 70</td>
</tr>
</tbody>
</table>

**β-Cryptoxanthin (μg/d)⁴**

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>43 ± 2.4</td>
<td>43 ± 2.4</td>
<td>43 ± 2.4</td>
</tr>
<tr>
<td>67 ± 24</td>
<td>60 ± 22</td>
<td>70 ± 29</td>
</tr>
</tbody>
</table>

¹ All values are $\bar{x} ± SD$ for 39 children in group A (20 M, 19 F), 39 in group B (21 M, 18 F), and 38 in group C (21 M and 17 F). Groups A, B, and C were fed standardized caroten-rich meals containing 7, 15, and 29 g fat/d, respectively, 5 d/wk, for 9 wk at school. Mean values in a column with different superscript letters are significantly different, $P < 0.05$ (ANOVA and post hoc comparisons with Scheffe’s test).

² From standardized meals provided at school (3 times/d, 5 d/wk, for 9 wk) after subtraction of plate waste. A total of 135 meals were provided during 45 school days.

³ From standardized meals provided at school after subtraction of plate waste plus the contribution from self-selected foods eaten during 45 school days, as recorded daily and reported by study participants and caregivers.

⁴ From self-selected foods eaten during 16 non-school days (Saturdays and Sundays), as recorded daily and reported by study participants and caregivers. These dietary intakes are representative of the participants’ usual intakes.

⁵ Based on Philippine food-composition tables (29).

⁶ Based on the US Department of Agriculture National Nutrient Database for standard reference (30).

Examples of self-selected snacks and foods that provided retinol, carotenoids, macronutrients, and energy are as follows: fried eggs, hot dogs, fish, sautéed fermented shrimp, rice cakes with margarine, cheese curls, corn chips, chocolate, fried sweet potato (kamote), and sweet potato leaves. The daily mean total intakes of provitamin A carotenoids and retinol by groups A, B, and C.
Significant decreases in body weight, height, and BMI were observed in the study participants at postintervention; there were no significant differences in any of these measures between the 3 groups (Table 2).

**Anthropometric data**

Significant increases in body weight, height, and body mass index were observed in the study participants at postintervention; there were no significant differences in any of these measures between the 3 groups (Table 2). Based on McNemar's test, the overall prevalence of underweight decreased significantly from 47.8% to 41.6% at postintervention (P = 0.02); there were no significant differences from baseline (P > 0.05) in the prevalence of stunting (38.1% versus 39.8%) or of wasting (13.3% versus 9.7%) at postintervention.

**Serum carotenoids**

Serum carotenoid concentrations in the study participants at baseline and at postintervention are provided in Table 3. The values were logarithmically transformed for data analyses. There were significant increases in serum \( eta \)-carotene (5-fold), \( \alpha \)-carotene (19-fold), and \( \beta \)-cryptoxanthin (2-fold) in the study participants at postintervention; there were no significant differences in any of these measures between the 3 groups.

**Vitamin A status**

There were significant increases in the total-body vitamin A pool size (2-fold) and liver vitamin A concentration (2-fold) in study participants at postintervention; there were no significant differences in these measures between the 3 groups. There was no significant change in serum retinol (P = 0.07) at postintervention, and there were no significant differences in serum retinol between the 3 groups (Table 4).

At baseline, 36 children (34.6%) had liver vitamin A concentrations that are considered low, i.e., <0.07 \( \mu \)mol/L (9, 33) (Table 5); after the intervention, the number was reduced to 7 (6.7%; P = 0.0001): 1 in group A, 4 in group B, and 2 in group C. Furthermore, although the liver vitamin A concentration remained below the cutoff concentration in 7 children at postintervention, improvements from baseline values were observed in all of them. Serum retinol values <0.35 \( \mu \)mol/L (<10 \( \mu \)g/dL) are considered deficient, and values between 0.35 and <0.70 \( \mu \)mol/L (10 and <20 \( \mu \)g/dL) are considered low (41). None of the children had deficient serum retinol values. At baseline, 20 children (19.2%) had low serum retinol values; at postintervention, 11 children had deficient serum retinol values (10.6%), of whom 6 had adequate serum retinol concentrations at baseline. Based on McNemar's test, the prevalence of low serum retinol values at baseline, relative to those at postintervention, was not significantly different (P = 0.08).

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 39)</th>
<th>Group B (n = 39)</th>
<th>Group C (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>26.4 ± 6.2</td>
<td>26.5 ± 6.3</td>
<td>26.0 ± 4.5</td>
</tr>
<tr>
<td>After intervention</td>
<td>27.8 ± 6.2</td>
<td>27.8 ± 6.5</td>
<td>27.4 ± 4.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>130.8 ± 7.7</td>
<td>131.2 ± 8.0</td>
<td>130.1 ± 7.9</td>
</tr>
<tr>
<td>After intervention</td>
<td>132.7 ± 8.3</td>
<td>133.4 ± 8.5</td>
<td>132.2 ± 8.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.3 ± 2.0</td>
<td>15.2 ± 1.9</td>
<td>15.3 ± 1.5</td>
</tr>
<tr>
<td>After intervention</td>
<td>15.8 ± 2.2</td>
<td>15.7 ± 1.9</td>
<td>15.8 ± 1.5</td>
</tr>
</tbody>
</table>

1 All values are \( \bar{x} \pm SD \); groups A, B, and C were fed standardized carotene-rich meals containing 7, 15, and 29 g fat/d, respectively, 5 d/wk, for 9 wk at school. Including self-selected foods, the total daily fat intakes were 21, 29, and 45 g/d, respectively.

2 Significant difference from baseline (2-factor ANOVA): P = 0.0001 for repeated measures, \( P > 0.05 \) for treatment group, and \( P > 0.05 \) for interaction of treatment group \( \times \) repeated measures.

**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 39)</th>
<th>Group B (n = 39)</th>
<th>Group C (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>all-trans-( \beta )-Carotene (( \mu )mol/L)</td>
<td>0.24 ± 0.12 (0.06–0.54)</td>
<td>0.19 ± 0.12 (0.07–0.67)</td>
<td>0.20 ± 0.07 (0.07–0.37)</td>
</tr>
<tr>
<td>After intervention</td>
<td>1.11 ± 0.48 (0.21–2.48)</td>
<td>1.12 ± 0.61 (0.23–3.07)</td>
<td>1.06 ± 0.41 (0.30–2.36)</td>
</tr>
<tr>
<td>( \alpha )-Carotene (( \mu )mol/L)</td>
<td>0.03 ± 0.02 (0.01–0.08)</td>
<td>0.03 ± 0.02 (0.01–0.11)</td>
<td>0.03 ± 0.02 (0.01–0.07)</td>
</tr>
<tr>
<td>After intervention</td>
<td>0.60 ± 0.24 (0.12–1.29)</td>
<td>0.56 ± 0.23 (0.15–1.16)</td>
<td>0.56 ± 0.20 (0.18–1.06)</td>
</tr>
<tr>
<td>( \beta )-Cryptoxanthin (( \mu )mol/L)</td>
<td>0.07 ± 0.05 (0.01–0.31)</td>
<td>0.07 ± 0.08 (0.01–0.41)</td>
<td>0.06 ± 0.05 (0.01–0.22)</td>
</tr>
<tr>
<td>After intervention</td>
<td>0.12 ± 0.07 (0.03–0.31)</td>
<td>0.12 ± 0.11 (0.03–0.66)</td>
<td>0.12 ± 0.09 (0.04–0.40)</td>
</tr>
<tr>
<td>13-cis-( \beta )-Carotene (( \mu )mol/L)</td>
<td>0.02 ± 0.01 (0.01–0.04)</td>
<td>0.02 ± 0.01 (0.01–0.06)</td>
<td>0.02 ± 0.01 (0.01–0.04)</td>
</tr>
<tr>
<td>After intervention</td>
<td>0.07 ± 0.03 (0.02–0.17)</td>
<td>0.07 ± 0.04 (0.02–0.20)</td>
<td>0.07 ± 0.03 (0.01–0.14)</td>
</tr>
</tbody>
</table>

1 All values are \( \bar{x} \pm SD \); range in parentheses. Groups A, B, and C were fed standardized carotene-rich meals containing 7, 15, and 29 g fat/d, respectively, 5 d/wk, for 9 wk at school. Including self-selected foods, the total daily fat intakes were 21, 29, and 45 g/d, respectively.

2 Significant difference from baseline (2-factor ANOVA): P = 0.0001 for repeated measures, \( P > 0.05 \) for treatment group, and \( P > 0.05 \) for interaction of treatment group \( \times \) repeated measures.
TABLE 4

Vitamin A status at baseline and after the 9-wk food-intervention period

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 37)</th>
<th>Group B (n = 36)</th>
<th>Group C (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total-body vitamin A pool size (mmol retinol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.089 ± 0.044 (0.020–0.219)</td>
<td>0.084 ± 0.047 (0.012–0.229)</td>
<td>0.078 ± 0.061 (0.019–0.328)</td>
</tr>
<tr>
<td>After intervention²</td>
<td>0.175 ± 0.095 (0.075–0.490)</td>
<td>0.178 ± 0.121 (0.038–0.511)</td>
<td>0.167 ± 0.109 (0.050–0.451)</td>
</tr>
<tr>
<td>Liver vitamin A (μmol/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.105 ± 0.054 (0.020–0.277)</td>
<td>0.094 ± 0.045 (0.014–0.199)</td>
<td>0.094 ± 0.079 (0.022–0.443)</td>
</tr>
<tr>
<td>After intervention²</td>
<td>0.196 ± 0.108 (0.064–0.603)</td>
<td>0.188 ± 0.116 (0.042–0.633)</td>
<td>0.190 ± 0.123 (0.043–0.448)</td>
</tr>
<tr>
<td>Serum retinol (μmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.91 ± 0.18 (0.66–1.50)</td>
<td>0.84 ± 0.20 (0.47–1.26)</td>
<td>0.85 ± 0.18 (0.38–1.22)</td>
</tr>
<tr>
<td>After intervention²</td>
<td>0.91 ± 0.18 (0.50–1.34)</td>
<td>0.89 ± 0.22 (0.45–1.55)</td>
<td>0.89 ± 0.17 (0.36–1.32)</td>
</tr>
</tbody>
</table>

All values are x ± SD; range in parentheses. Groups A, B, and C were fed standardized carotene-rich meals containing 7, 15, and 29 g fat/d, respectively, 5 d/wk, for 9 wk at school. Including self-selected foods, the total daily fat intakes were 21, 29, and 45 g/d, respectively.

² Significant difference from baseline (2-factor ANOVA): P = 0.0001 for repeated measures, P > 0.05 for treatment group, and P > 0.05 for interaction of treatment group X repeated measures.

Helminthic infections

At baseline, the cumulative prevalence of helminths was 48%. *A. lumbricoides* was present in 9% of children, *T. trichiura*, in 43% of the children, and hookworm in 3% of the children. The intensity of the infections at baseline was mostly light (83%); the remaining intensities were moderate (14%) or heavy (3%). After treatment with albendazole, all infections were of light intensity at 2 mo, and only 4 children had moderate infections at 3 mo (all with *T. trichiura*). The cumulative prevalence of helminths at 2 and 3 mo were 20% and 33%, respectively, and were distributed in the 3 groups. Helminthic infections, particularly ascariasis, have been reported to affect the utilization of plant sources of β-carotene (6). In the present study, the presence of helminths midway through or at the end of the dietary intervention was probably not a confounding factor because the worm load was mostly light.

Serum C-reactive protein

Serum C-reactive protein concentrations ≥10 mg/L indicate an acute phase response to infection during which serum retinol may decrease transiently (42). None of the subjects had an abnormal C-reactive protein value at baseline or at postintervention.

TABLE 5

Prevalence of poor vitamin A status at baseline and after the 9-wk food-intervention period

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low liver vitamin A concentration, &lt;0.07 μmol/g²</td>
<td>36 (34.6)</td>
<td>7 (6.7)³</td>
</tr>
<tr>
<td>Low serum retinol concentration, &lt;0.70 μmol/L⁴</td>
<td>20 (19.2)</td>
<td>11 (10.6)⁵</td>
</tr>
</tbody>
</table>

¹ n = 104 children.
² Cutoff based on data from Olson (9, 33).
³ Significantly different from baseline, P = 0.0001 (McNemar’s test).
⁴ Cutoff based on data from the World Health Organization (41).
⁵ Not significantly different from baseline, P = 0.08 (McNemar’s test).

DISCUSSION

This study showed that the ingestion of carotene-rich yellow and green leafy vegetables with 2.4, 5, or 10 g fat/meal can improve the vitamin A status of school-age children. Using the DRD method, we observed 2-fold improvements in mean total-body vitamin A pool sizes and liver vitamin A concentrations in study participants after the ingestion of vegetable meals for 9 wk. Thus, carotene-rich vegetables are important bioavailable sources of vitamin A.

A liver vitamin A concentration <0.07 μmol/g is inadequate and may not protect against clinical signs of vitamin A deficiency (9, 33). In the present study, the number of children with inadequate liver vitamin A values decreased from 36 (34.6%) to 7 (6.7%), but even the 7 children whose liver vitamin A concentrations remained below normal concentrations responded positively to the dietary intervention with 1.4- to 3.7-fold increases in liver vitamin A values. It is probable that in these 7 children, who were among those with poorest vitamin A status at baseline, much of the incoming vitamin A molecules were transported to nonhepatic target tissues for maintenance of normal physiologic processes requiring vitamin A; thus, the fraction stored in liver was minimal.

The improvement in total-body vitamin A pool size observed in the Filipino schoolboys and girls in this study was greater than that observed by Haskell et al (14) in Bangladeshi men who were fed 4500 μg β-carotene from Indian spinach or sweet potato daily for 60 d. In the present study, the mean increases in total-body vitamin A pool sizes in the groups fed 2.4, 5, or 10 g fat/meal were 0.086, 0.094, and 0.089 mmol retinol, respectively. In the Bangladeshi study, the unadjusted mean increases in total-body vitamin A pool sizes in men fed Indian spinach or sweet potato were 0.023 and 0.011 mmol, respectively; the adjusted mean increases were 0.041 and 0.029 mmol, respectively, relative to that in a negative control group in which a mean decrease in total-body vitamin A pool size of 0.018 mmol was observed. The present study and the Bangladeshi study differed not only in the study participants’ ages, but also in their initial vitamin A status.

In the present study, the mean total-body vitamin A pool sizes of children in the 3 study groups at baseline were 0.089, 0.084, and 0.078 mmol; whereas those of the Bangladeshi men in the 2
vegetable groups were 0.115 and 0.095 mmol. Furthermore, in the present study, the mean serum retinol concentrations of children in the 3 study groups at baseline were 0.91, 0.84, and 0.85 μmol/L, whereas the mean plasma retinol concentrations at baseline in the Bangladeshi men in the 2 vegetable groups were 1.32 and 1.24 μmol/L. Animal (43, 44) and human (8) studies have indicated that the absorption and bioconversion of plant carotenoids to vitamin A are enhanced when vitamin A status is low.

Tang et al (13) observed no change in the total-body vitamin A pool size of 5–6-yr-old Chinese children who were fed green and yellow vegetables containing 4670 μg β-carotene/d for 5 d/wk for 10 wk. The children’s initial mean vitamin A pool size was 0.092 mmol, and their mean initial serum retinol concentration was 1.05 μmol/L. In a second group of children who were fed light-colored vegetables, a decrease in mean total-body vitamin A pool size of 0.027 mmol was observed at postintervention; thus, these investigators concluded that green and yellow vegetables can maintain body stores of vitamin A in Chinese children.

In the present study, serum β-carotene, α-carotene, and β-cryptoxanthin increased 5-fold, 19-fold, and 2-fold, respectively, at postintervention in all 3 study groups. Thus, these carotenoids were highly bioavailable from their vegetable sources. The difference in serum carotenoid responses could be related to the observation that the children’s usual intakes of β-carotene, α-carotene, and β-cryptoxanthin increased 14%, 95%, and 2-fold, respectively, during the intervention.

In 2001, the US Institute of Medicine (45) changed the equivalency of plant β-carotene:retinol from 6:1 to 12:1 and the equivalency of other plant provitamin A carotenoids:retinol from 12:1 to 24:1. Applying the recommended equivalency factors to the present study resulted in a total vitamin A intake of ≈21 299 μg retinol activity equivalents (RAEs) during the 9-wk intervention period—an amount that is less than the observed increase of ≈25 689 μg (≈0.0897 mmol) in the total-body vitamin A pool size during the same period. Because the vitamin A intake cannot be less than the increase in the amount of vitamin A stored, it is possible that the dietary intakes of provitamin A carotenoids and retinol were underestimated or that the bioconversion of plant provitamin A carotenoids to retinol was better than predicted when the recommended 12:1 and 24:1 conversion factors were used. This study was not designed to estimate the vitamin A equivalency of β-carotene in the mixed vegetables that were provided; thus, a conversion factor for β-carotene:retinol could not be determined. It should be noted that the recommended 12:1 conversion factor for β-carotene:retinol is an average estimate and that conversion factors could be higher or lower than 12:1.

Using the DRD technique and an optimal method for estimating vitamin A equivalency in selected vegetables (ie, inclusion of a positive control group fed preformed retinol and a negative control group not fed vegetables), Haskell et al (14) reported β-carotene:retinol conversion factors of ≈10:1 for Indian spinach and of ≈13:1 for sweet potato.

Other possible explanations for the discrepancy between the mean total vitamin A intake (when expressed as RAEs using the recommended 12:1 and 24:1 conversion factors) and the mean increase in vitamin A pool size are that the total-body vitamin A pool sizes were underestimated at baseline or were overestimated at postintervention. However, these explanations are unlikely because the paired serum samples at baseline and at postintervention for each subject were analyzed together. The values obtained were well within the range of values that have been reported for some children in developing nations (13, 26), which are lower than the values for US children (20) and Nicaraguan children (27), where a national program of sugar fortification with vitamin A is in place.

Dietary fat is needed for the absorption of carotenoids from plant foods (2). Absorption requires the release of carotenoids from the food matrix, formation of lipid micelles in the small intestine, uptake of carotenoids by intestinal mucosal cells, and transport of carotenoids or their metabolic products (eg, vitamin A) to the lymphatic or portal circulation (46). Although the standardized carotene-rich meals contained only 2.4, 5, or 10 g fat/meal (or 7, 15, or 29 g fat/d), self-selected snacks contributed additional dietary fat, so that the total fat intakes by the 3 study groups were 21, 29, and 45 g/d; these amounts were equivalent to 0.9, 1.4, and 2 times the children’s usual daily fat intakes, respectively. Although the sources of additional dietary fat were not eaten with the carotene-rich meals, delayed release of carotenoids from enterocytes occur when intraluminal lipids become available from the next foods to allow packaging of carotenoids into chylomicrons (47). During the intervention days, the total daily fat intake of the 3 groups provided 12%, 17%, and 24% of their total energy intake. On weekends, when the subjects consumed their usual diets, the children’s fat intake provided 18% of their total energy intake. In comparison, US children consume an average of 34% of total energy as fat (48).

Regardless of the amount of fat ingested by the Filipino children per meal or the total amount of fat they ingested per day, their mean serum provitamin A carotenoid concentrations, total-body vitamin A pool sizes, and liver vitamin A concentrations were increased similarly. Thus, the dietary fat requirement for optimal bioavailability and effectiveness of plant carotenoids is minimal. Brown et al (49) reported that, in young adults, the appearance of α- and β-carotene in plasma chylomicrons was higher after the ingestion of fresh salad with full-fat (28 g fat/meal) than with reduced-fat (6 g fat/meal) salad dressing. The present study differs from that of Brown et al (49) in that they fed raw vegetables in a salad mix, whereas we fed cooked vegetables in a meal. Conceivably, more dietary fat may be needed for the optimal bioavailability of carotenoids in raw than in cooked vegetables because food processing and heating disrupts the plant matrix and promotes the release of carotenoids (46). Roodenburg et al (50) reported that as little as 3 g fat is needed for the plasma uptake of α- and β-carotene, although these carotenoids were provided in purified form solubilized in fat and not as plant foods. In malnourished children aged 2–6 yr in India, Jayarajan et al (3) reported that a supplement of 40 g cooked spinach once daily with either 5 or 10 g groundnut oil for 4 wk resulted in similar enhancement of serum retinol.

We did not observe a change in serum retinol in this study. Because serum retinol is subject to homeostatic control over a wide physiologic range of liver vitamin A concentrations (9), it is not a good measure for assessing the effectiveness of interventions aimed at improving the vitamin A status of populations. In general, studies that showed no improvement in serum retinol with increased intakes of colored vegetables, fruit, or both, the mean serum retinol concentration of subjects at baseline was higher (ie, 0.89–2.31 μmol/L) (10–14) than that at baseline in studies in which improvements in serum retinol were found (ie, 0.57–0.76 μmol/L) (3–8). In the present study, the mean serum retinol concentrations at baseline in the 3 groups were 0.91, 0.84,
and 0.85 μmol/L, and no significant changes were noted at postintervention.

In summary, only a small amount of dietary fat (2.4 g/meal, or 21 g/d) is needed for optimal utilization of plant provitamin A carotenoids. The poor or marginal vitamin A status observed in the study participants at baseline cannot be attributed to insufficient fat intakes, but rather to insufficient intakes of food sources of vitamin A. Stable-isotope-dilution methods are a powerful tool for assessing vitamin A status and for evaluating the effectiveness of interventions aimed at improving the vitamin A status of populations. On the basis of data obtained with the use of other vitamin A assessment methods, the effectiveness of plant carotenoids in combating vitamin A deficiency has been questioned. Data from the present study indicate that it is possible to improve the total-body vitamin A pool size and restore low liver vitamin A concentrations to normal concentrations by eating sufficient amounts of carotenone-rich yellow and green leafy vegetables and minimal amounts of dietary fat. Thus, carotenone-rich plant foods can effectively meet vitamin A needs. This finding is of public health importance, especially in developing nations, where health professionals and policymakers can promote the use of yellow and green leafy vegetables for combating vitamin A deficiency in vulnerable groups. In the United States and other countries, where school-feeding and other food programs are in place, the information is useful for formulating dietary guidelines for inclusion of carotenone-rich vegetables in meals.

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