The contribution of plant foods to the vitamin A supply of lactating women in Vietnam: a randomized controlled trial\textsuperscript{1–4}

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ABSTRACT

Background: More information is needed on the efficacy of carotenoids from plant foods in improving vitamin A status.

Objective: We aimed to quantify the efficacy of provitamin A–rich vegetables and fruit in improving vitamin A status.

Design: Breastfeeding women in 9 rural communes in Vietnam were randomly allocated to 1 of 4 groups: the vegetable group (n = 73), which ingested 5.6 mg β-carotene/d from green leafy vegetables; the fruit group (n = 69), which ingested 4.8 mg β-carotene/d from orange or yellow fruit; the retinol-rich group (n = 70), which ingested 610 μg retinol/d from animal foods and 0.6 mg β-carotene/d; and the control group (n = 68), which ingested 0.4 mg β-carotene/d. Meals of groups 1, 2, and 4 contained <30 μg retinol/d. Lunch and dinner were provided 6 d/wk for 10 wk.

Results: Mean (95% CI) changes in serum retinol concentrations of the vegetable, fruit, retinol-rich, and control groups were 0.09 (0.03, 0.16), 0.13 (0.07, 0.19), 0.25 (0.17, 0.33), and 0.00 (−0.06, 0.06) μmol/L, respectively. Mean (95% CI) changes in breast-milk retinol concentrations were 0.15 (0.04, 0.27), 0.15 (0.02, 0.28), 0.48 (0.32, 0.64), and −0.06 (−0.21, 0.09) μmol/L, respectively. According to these findings, the equivalent of 1 μg retinol would be 12 μg β-carotene (95% CI: 8, 22 μg) for fruit and 28 μg β-carotene (17, 84 μg) for green leafy vegetables. Thus, apparent mean vitamin A activity of carotenoids in fruit and in leafy vegetables was 50% (95% CI: 27%, 75%) and 21% (7%, 35%), respectively, of that assumed.

Conclusion: The bioavailability of carotenoids from vegetables and fruit is less than previously assumed. Am J Clin Nutr 2007;85:1112–20.

KEY WORDS Bioavailability, vitamin A, Vietnam, breastfeeding women, carotenoids, food-based approaches

INTRODUCTION

Vitamin A deficiency continues to be an important public health problem in many developing countries (1, 2). It may be prevented by strategies that increase vitamin A intake, including pharmaceutical approaches and dietary approaches (including foods naturally rich in vitamin A or food whose vitamin A content has been increased through fortification, breeding, or genetic modification), and by public health approaches to reduce the need for high utilization of vitamin A. In developing countries, vegetables and fruit are estimated to provide ∼70–80% of total vitamin A intake (3, 4). Consumption of vegetables and fruit is promoted because they contain provitamin A carotenoids and other nutrients. Apart from being precursors of retinol, carotenoids may have distinct functions of their own in humans and animals that are based on their antioxidant capacities (5).

One of the problems of relating the dietary intake of carotenoids to health status is that information on the bioavailability of carotenoids and their bioefficacy as a source of vitamin A is limited. Until the mid-1990s, it was assumed that consuming 6 μg dietary β-carotene was equivalent to consuming 1 μg retinol (3), and thus the conversion ratio was considered to be 6:1. However, a study of breastfeeding women in Indonesia found no improvement in serum and breast-milk concentrations of retinol after feeding dark-green leafy vegetables and carrots (6), and a subsequent study of anemic Indonesian schoolchildren found apparent β-carotene conversion factors of 12:1 and 26:1 in fruit and in leafy vegetables, respectively (7). In 2001, on the basis of data from developed countries, the US Institute of Medicine revised its recommended conversion factors to 12:1 for dietary β-carotene and 24:1 for other carotenoids (8). The International Vitamin A Consultative Group has adopted the Institute of Medicine’s conversion factors but suggested that those factors should be even lower (reflecting lower bioefficacy, which is defined as the efficiency with which ingested dietary provitamin A carotenoids are first absorbed and then converted to retinol) in malnourished populations (9). West et al (10) argued that the conversion factor for dietary β-carotene from a mixed diet (ratio of vegetables to fruit = 4:1) may be closer to 21:1. Other investigators, by identification of signs specific to vitamin A deficiency (eg, night blindness) or by using stable-isotope methods, confirmed that the bioavailability and hence the bioefficacy of carotenoids from fruit and vegetables were lower than previously thought (11–14).

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The study reported here was conducted in breastfeeding women in Vietnam; its design was similar to that of the study of Indonesian schoolchildren: to assess the bioefficacy of β-carotene provided by either fruit or vegetables and to compare it with the efficacy of β-carotene obtained from the consumption of retinol in retinol-rich foods. On the basis of this comparison, we estimated conversion factors.

SUBJECTS AND METHODS

Subjects

The study was carried out in 9 rural communes in the Pho Yen district, Thai Nguyen Province, Vietnam (=60 km from Hanoi) from April through August 1996. Communes are the lowest administrative unit in the country, and each consists of a few subcommunes or villages. By Vietnamese national standards, the inhabitants had a low or middle socioeconomic status. Rice is the staple food and the main constituent of the 3 meals that are commonly eaten each day (ie, breakfast, lunch, and dinner). Green vegetables are consumed frequently, but consumption of ripe yellow or orange fruit is low because those types of fruit are more expensive. At the time of the study, there was a local abundance of spring vegetables, but mangoes were imported because they were not yet in season. Breastfeeding is universally practiced, and the average duration of breastfeeding is 18–20 mo. Many women return to physical labor 1 or 2 mo after delivery.

Using a power calculation based on the study of breastfeeding women in Indonesia (8), we estimated that 61 women per group would yield a 90% probability % of detecting a between-group difference of change in serum retinol concentrations of ≥ 0.20 μmol/L (α = 0.05). We decided to enroll 75 mothers per group, assuming that data on ≥65 subjects would be available for analysis. Selection criteria for the study were anemia (a hemoglobin concentration < 120 g/L), breastfeeding an infant aged 5–14 mo, and no chronic illness. Anemia was a selection criterion because anemic subjects are more likely to have a low vitamin A status, as measured by serum retinol concentration (6, 15, 16), and hence their serum retinol concentration would be more likely to change in response to an increased dietary intake of vitamin A. Breastfeeding an infant aged >4 mo was a criterion because women in this area were eligible to receive a high-dose vitamin A capsule shortly (within 6 wk) after delivery, and a maximum age of 14 mo for breastfeeding was used to reduce the chance that these women would cease breastfeeding during the 10-wk intervention period.

The commune health centers had been asked to provide a list of all breastfeeding women in each commune and to specify the age of the infants. All women breastfeeding an infant aged 5–15 mo were invited to have their hemoglobin concentration measured by finger-prick (Hemocue, Angelhölm, Sweden). All those with a hemoglobin concentration < 120 g/L were included to obtain the 300 women required. The purpose and procedures of the study were explained to the women and their family members, and almost all of these women were willing to participate in the study.

All subjects provided written informed consent. The study was approved by the Ministry of Health and the Scientific and Medical Ethics Committee of The National Institute of Nutrition, Vietnam.

Assignment of treatment group

At each commune, selected participants were stratified by the age of their breastfed child and then randomly assigned to 1 of 4 experimental groups—vegetables (V), fruit (F), retinol-rich (R), and control (C)—by using alternating sequences (V, F, R, C, V, ... for one age group in a commune; F, R, C, V, F, ... for another age group in the commune; and so on). In that way, each commune also had nearly equal numbers of subjects in each treatment group. On each of 6 d/wk (Monday through Saturday) for 10 wk, the treatment groups received 2 meals (lunch and dinner) that differed in the amount and sources of retinol and provitamin A carotenoids. The vegetable group received provitamin A from leafy vegetables, the fruit group received provitamin A from yellow or orange fruit, the retinol-rich group received retinol-rich foods, and the control group received meals low in both provitamin A and retinol. To provide a complete meal to all groups, foods with high vitamin A content were replaced by foods with low vitamin A content. For example, the fruit group received carotene-rich fruit (eg, mango or papaya), low-carotene vegetables (eg, cabbage), and low-retinol foods (eg, pork). Meals contained sufficient fat to allow maximal absorption of carotenoids and retinol [minimum required amount is 3 g/meal (17)]. The size of the side dishes was fixed, whereas rice consumption was ad libitum (on average, 450 rice/d was provided per woman). The local custom is that lunch and dinner are main meals, whereas a small dish of rice with fish sauce is consumed for breakfast. Provision of lunch and dinner to the participants allowed control of most of the variability in their vitamin A intake.

Interventions

Meal preparation and eating

To standardize meals and supervise consumption, meals were provided from central kitchens, one per subcommune. A total of 29 kitchens were established, each of which served =10 women under the supervision of cooks and a field team. At each kitchen, the meals were prepared by 2–3 volunteers who were members of the Women’s Union (a structure that exists throughout Vietnam, through which women are organized at the local level to participate in community activities). Every 6 d, these volunteers were provided with recipes and lists of the amount of each food to be purchased, cleaned, and cooked per day. Foods not available at the local market, such as mangoes, were provided by the field team. After the foods had been cleaned, the cooks weighed and divided them in half; one half was to be prepared for lunch and the other half for dinner. Each woman was allocated a seat in the dining room to ensure that she received the correct meal. Lunch was served at 1200 and dinner was served between 1700 and 1900. Participants and family members were informed thoroughly about the importance of consuming all of the side dishes. The cooks kept a record of attendance and recorded the foods left over after each meal, excluding rice. The women were asked what they ate for breakfast each day and for their meals on Sunday.

Measurements

On the day before the first meal and on the day after the last meal (10 wk after start of the intervention), women were examined medically, a venous blood sample was drawn, and a breastmilk sample was collected. Blood (6 mL) was collected from an
The hexane layers were pooled and evaporated to dryness under water, the compounds were extracted twice with 3.0 mL hexane. Hydroxide in ethanol (96% by vol); after the addition of 1.5 mL potassium hydroxide in methanol. Dichloromethane (100 mL) and ammonia (25% wt:vol) and 25% by vol), and 25 μL was injected for HPLC analysis. Separations were monitored at 320 nm (retinol) and 450 nm (carotenoids). All sample preparations were done under subdued yellow light. Within- and between-run CVs were 1.6% and 1.9% for retinol, 3.4% and 8.2% for β-carotene, 4.6% and 7.0% for α-carotene, 3.6% and 11.4% for β-cryptoxanthin, 4.1% and 6.6% for lutein, and 9.6% and 9.3% for zeaxanthin, respectively.

Feces

Stool samples were examined for the presence of worm eggs by a parasitologist using the Ridley method. The load of worm eggs was quantified by using the Kato-Katz method (19).

Duplicate food portions

Duplicate samples of the meals, without rice, were collected on 2 occasions (week 2 and week 9 of the intervention). On each occasion, portions were collected for analysis over 3 consecutive days, which corresponded to the 3-d menu cycle. Duplicate samples of the meals were pooled per treatment group per 3 consecutive days, frozen at −20 °C, and later shipped (in dry ice) to the Division of Human Nutrition and Epidemiology for analyses of fat, protein, dietary fiber, ash, dry matter, iron, retinol, and carotenoids (20, 21). Carbohydrate content was calculated by the difference from the analyzed content of the above substances. For analysis of retinol and carotenoids, samples were homogenized and extracted with tetrahydrofuran, and the volume of the solvent was reduced to near-dryness. The residue was saponified overnight at room temperature with 25 mL of 5% (wt:vol) potassium hydroxide in methanol. Dichloromethane (100 mL) and water (75 mL) were added, and, after removal of the upper water layer, the lower dichloromethane layer was washed with water until the pH was <9.5. The dichloromethane layer was then evaporated to dryness, and the residue was dissolved in methanol:tetrahydrofuran (3:1, by vol) and analyzed by HPLC. The results were averaged per treatment group.

Nutrient intake

The Vietnamese food composition table was used to calculate the nutrient intake other than from the meals provided (except carotene content) on the basis of the 24-h recall data collected once a week (22). For some fruit and vegetables, the data on carotene content were taken from the results of analysis done at Wageningen University (20); for other fruit and vegetables, the data were taken from the table with the most recent data on the carotene content of food in developing countries (23). Nutrient intake was then calculated at the Institute of Nutrition (Hanoi, Vietnam) by using FOXPRO software (version 2.5; Cambria, Palo Alto, CA).

Statistical analysis and estimation of conversion factors

Descriptive data are reported as means and SDs for normally distributed parameters and as medians and 25th and 75th percentiles for nonnormally distributed parameters. Nutrient intake during the intervention period other than that from the foods provided was calculated from the median intake of each woman as assessed by weekly 24-h recall data. The effect of consumption

Analyses

Breast milk

Immediately upon arrival at the nearby laboratory, breast milk was stored at −20 °C for ≈3 wk, and then it was sent in dry ice to the laboratory of the Division of Human Nutrition and Epidemiology, Wageningen University, where the samples were stored at −80 °C until analysis of retinol and carotenoids. The samples were prepared for HPLC analysis under subdued yellow light. To 1000 μL milk, 125 μL ammonia (25% wt:vol) and 1000 μL ethanol (96% by vol) were added. The mixture was extracted twice with 4 mL of a mixture of petroleum ether (boiling point: 40–60 °C) and diethylether (1:1 by vol). The upper layers were removed, pooled, and evaporated under nitrogen at 35 °C until they reached near-dryness. The residue was saponified and extracted with tetrahydrofuran, and the volume of the solvent was reduced to near-dryness. The hexane layers were pooled and evaporated to dryness under nitrogen; the residue was dissolved in 250-μL mixture of methanol and tetrahydrofuran (3:1 by vol), and 25 μL was injected for HPLC analysis. Separations were monitored at 320 nm and 450 nm (carotenoids). Within- and between-run CVs were 6.0% and 1.5% for retinol and 3.8% and 5.4% for β-carotene, respectively. Mean (±SD) recoveries (n = 10) were 101 ± 6% for retinol and 103 ± 4% for β-carotene.

Blood

For baseline and evaluation assessment, hemoglobin concentrations were measured by using the cyanmethemoglobin method (Merkotest, Merck, Darmstadt, Germany), and hematocrit was measured by using a centrifuge method. The remaining blood was centrifuged (1200 × g for 15 min at room temperature), and serum was frozen for subsequent shipping in dry ice for analysis of retinol and carotenoid concentrations (18) at the laboratory of the Division of Human Nutrition and Epidemiology. To 500 μL serum, 500 μL sodium chloride (0.9% wt:vol in water) and 1000 μL ethanol (containing retinyl acetate as an internal standard) were added; next, they were extracted twice with 2.0 mL hexane. The hexane layers were pooled and evaporated to dryness under nitrogen at 35 °C. The residue was dissolved in a 250-μL mixture of methanol and tetrahydrofuran (3:1 by vol), and 25 μL was injected for HPLC analysis. Separations were monitored at 320 nm (retinol) and 450 nm (carotenoids). All sample preparations were done under subdued yellow light. Within- and between-run CVs were 1.6% and 1.9% for retinol, 3.4% and 8.2% for β-carotene, 4.6% and 7.0% for α-carotene, 3.6% and 11.4% for β-cryptoxanthin, 4.1% and 6.6% for lutein, and 9.6% and 9.3% for zeaxanthin, respectively.

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Statistical analysis and estimation of conversion factors

Descriptive data are reported as means and SDs for normally distributed parameters and as medians and 25th and 75th percentiles for nonnormally distributed parameters. Nutrient intake during the intervention period other than that from the foods provided was calculated from the median intake of each woman as assessed by weekly 24-h recall data. The effect of consumption
of a diet rich in vegetables, fruit, or retinol was assessed by comparing serum and breast-milk indicators of vitamin A and carotenoid status measured at 10 wk with the values measured at 10 wk in women consuming the control diet. Distributions of serum indicators were normalized by log transformation before analysis in a multivariate linear regression model. For each indicator, we used multivariate regression to adjust for stratification factors (ie, age of the child and community of residence) and to investigate potential confounding effects due to differences between groups in baseline characteristics (ie, serum indicator concentration at baseline or infection with *Ascaris lumbricoides*, *Trichuris trichiura*, or hookworm), whether significant or not. Because adjustment did not substantially affect the estimates of effect, we present changes and effects adjusted only for the baseline concentration of the indicator. Bonferroni’s adjustment was used for multiple comparisons, and the changes in normally distributed, log-transformed variables in serum and of breast-milk retinol are expressed relative to those value in the control group to ascertain the magnitude of the difference. When log transformation did not result in normally distributed values, we compared absolute within-group changes between baseline and 10 wk in serum and breast-milk indicators by using the Kruskal-Wallis test for 2 groups and then the Mann-Whitney U test (pairwise comparison) with Bonferroni correction. Wilcoxon’s signed-rank test was then used to examine the significance of within-group changes.

The conversion factor ($x$) for calculating the amount of retinol equivalents provided by provitamin A carotenoids from fruit was derived from the following formula (9):

$$\Delta \text{Serum retinol concentration}/(\text{retinol intake}) = \Delta \text{serum retinol concentration}/(\text{retinol intake})$$

where $\Delta$ serum retinol concentration was calculated by subtracting the values at the end of follow-up from those at baseline, by using the following equations:

$$\text{Retinol intake} = \mu g \text{ retinol/g food} \quad (2)$$

and

$$\beta-\text{Carotene equivalent intake} =$$

$$\left(\mu g \text{ all-trans-}\beta-\text{carotene} + 0.5 \times \mu g \alpha-\text{carotene} + 0.5 \times \mu g \text{ cis-isomers of } \beta-\text{carotene}/\mu g \text{ food} \right) \quad (3)$$

The conversion factor for vegetables was obtained similarly. Upper and lower limits of the 95% CI for conversion factor $x$ were estimated by using the limits of the 95% CIs for the changes in serum concentrations in the respective treatment groups. We used SPSS for WINDOWS software (version 11.5.1; SPSS Inc, Chicago, IL) for all statistical calculations.

**RESULTS**

**Subjects**

Of 1512 women who were screened, 298 met our inclusion criteria. Of the 298 breastfeeding women who were randomly assigned, 280 (93.9%) completed the 10-wk intervention. One woman refused further participation during the intervention, 2 women moved away from the study area, 6 women became pregnant, 2 women were sick when follow-up data were collected, 1 woman lost her child, and 6 women refused to give blood at follow-up. Further details are provided in Figure 1.
Food consumption and nutrient intake

The record of attendance and leftovers indicated that the proportion of the provided fruit consumed by the fruit group was 96% (95% CI: 92%, 100%), the proportion of the provided vegetables consumed by the vegetable group was 94% (91%, 97%), and the proportion of the provided retinol-rich foods consumed by the retinol-rich group was 98% (96%, 100%). Food eaten apart from that provided by the intervention was mainly breakfast, which generally contained 1–2 bowls of rice (60–120 g rice) with either fish sauce or tofu. The vitamin A content, largely from provitamin A carotenoids, of the foods eaten apart from those provided was very small and did not differ significantly between groups, and therefore it was not considered in subsequent analyses. Energy and nutrient intakes during the intervention, including the intakes from the food provided, are shown in Table 2. The high energy intake is largely due to high consumption of rice among breastfeeding women in the study area, which is related to the belief that greater consumption of rice increases breast-milk production. The meals provided 5.6 mg all-trans \( \beta \)-carotene equivalent plus 27 \( \mu \)g retinol for the vegetable group, 4.8 mg all-trans \( \beta \)-carotene equivalent plus 15 \( \mu \)g retinol for the fruit group, 0.6 mg \( \beta \)-carotene equivalent plus 610 \( \mu \)g retinol for the retinol-rich group, and 0.4 mg \( \beta \)-carotene equivalent plus 1 \( \mu \)g retinol for the control group. The dietary fiber content in food provided was 10 g in the vegetable and fruit groups and 7 g in the retinol-rich and control groups (data not shown). The intakes of fat, protein, and carbohydrates did not differ significantly between the 4 groups, but iron intake was slightly but significantly higher in the retinol-rich group. Fat intake contributed >16% of energy intake, and the intake of 50 g/d was sufficient to allow maximum absorption of carotenoids and retinol.

Effects of interventions on serum indicators of vitamin A status

Each group’s serum concentrations of retinol and carotenoids at baseline and after the intervention are shown in Table 3. The only significant difference in serum concentrations of retinol and carotenoids at baseline between groups was the significantly (\( P < 0.001 \)) higher concentration of \( \beta \)-cryptoxanthin in the vegetable group. At the end of follow-up, serum concentrations of retinol were 24% higher in the retinol-rich group and 10% and 12% higher in the vegetable and fruit group, respectively, than in the control group (see Table 3). The serum concentrations of \( \beta \)-cryptoxanthin increased significantly (\( P < 0.05 \)) more in the fruit group than in the control group; the difference between the increase in the retinol-rich and control groups was small but significant. Moreover, significantly (\( P < 0.05 \) for all) greater increases than in the control group were found in the serum concentrations at 10 wk and after correction for baseline values for all-trans-\( \beta \)-carotene in all 3 groups, for cis-\( \beta \)-carotene in the fruit group, for \( \alpha \)-carotene in the vegetable and fruit groups, for zeaxanthin in the fruit and retinol-rich groups, and for lutein in the vegetable group. It should be noted that most concentrations decreased during the intervention period, particularly in the control group.

Estimation of apparent conversion factors

The absolute change in serum retinol concentrations is shown in Table 3. To estimate the apparent conversion factor, this change and the intake of \( \beta \)-carotene equivalents and of retinol (see Table 2) were used. The estimation is based on the assumption that the ratio of the intake of retinol equivalents derived from different sources to the change in serum concentration of retinol is constant. Because the concentration of retinol had not changed...
that 28 vegetables or 12 significantly lower than at baseline. The breast-milk milk retinol concentrations were 1.4, 1.3, and nearly 2.0 times higher still in the vegetable group, and highest in the fruit group. Breast-milk lutein concentrations remained the same as they were in the control group. At 10 wk, the breast-

Effects of the intervention on breast-milk indicators of vitamin A status

Breast-milk concentrations of retinol and carotenoids in each group at baseline and 10 wk after the start of the interventions are shown in Table 4. After the intervention, breast-milk retinol concentrations were 1.4, 1.3, and nearly 2.0 times as high in the fruit, vegetable, and retinol-rich groups, respectively, as they were in the control group. At 10 wk, the breast-milk β-carotene concentrations in the control group were significantly lower than at baseline. The breast-milk β-carotene concentrations at 10 wk were lowest in the control group; and zeaxanthin decreased in all 4 groups.

TABLE 2
Nutrient intake during intervention, including foods provided

<table>
<thead>
<tr>
<th>Energy or nutrient consumed per day</th>
<th>Vegetable group</th>
<th>Fruit group</th>
<th>Retinol-rich group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2817 (2602–2950)</td>
<td>2781 (2565–2956)</td>
<td>2845 (2507–2956)</td>
<td>2755 (2510–2958)</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>49 (48–52)</td>
<td>52 (51–56)</td>
<td>50 (49–54)</td>
<td>50 (48–53)</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>92 (86–97)</td>
<td>94 (89–100)</td>
<td>94 (86–101)</td>
<td>95 (89–97)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>485 (438–513)</td>
<td>465 (421–501)</td>
<td>467 (426–508)</td>
<td>475 (438–507)</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>18 (17–19)</td>
<td>17 (16–18)</td>
<td>21 (20–22)</td>
<td>17 (16–18)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>120 (115–132)</td>
<td>173 (161–186)</td>
<td>48 (36–50)</td>
<td>49 (35–52)</td>
</tr>
</tbody>
</table>

Vitamin A and carotenoids

- Retinol (µg) 27 15 610 1
- all-trans-β-Carotene (µg) 5037 3443 436 239
- cis-β-Carotene (µg) 777 404 67 35
- α-Carotene (µg) 437 383 323 194
- β-Cryptoxanthin (µg) 18 2157 67 3
- Lutein (µg) 7590 1661 1022 709
- Zeaxanthin (µg) 320 348 103 35
- β-Carotene equivalent (µg) 5652 4792 644 355

1 Values were summed of nutrients from foods provided (analyzed) and intake of nutrients from foods besides foods provided, calculated from food-composition tables. Rice provided was estimated at 450 g/d for all subjects. Macronutrient intake did not differ significantly between the 4 groups. Micronutrient intakes (retinol, carotenoids, iron, and vitamin C) differed according to the design of the study. Values in a row with different superscript letters are significantly different (Kruskal-Wallis test for multiple comparisons); if P < 0.05, Mann-Whitney U test for 2 comparisons was also used, with Bonferroni correction for multiple comparisons.

2 Median; 25th–75th percentiles in parentheses (all such values).

3 P < 0.001 (Kruskal-Wallis test).

4 Mean (of 2 analyses of pooled samples) (all such values). Values obtained from analysis of the meal provided, because foods consumed besides food provided had very low retinol and carotenoid content. No range or significance of differences is reported.

5 β-Carotene equivalent (µg) = all-trans-β-carotene (µg) + 1/2 (cis-β-carotene + α-carotene + β-cryptoxanthin) (µg).

DISCUSSION

According to our findings, an appropriate estimation would be that 12 µg β-carotene (95% CI: 8.22 µg) from fruit and 28 µg β-carotene (95% CI: 17.84 µg) from dark-green leafy vegetables is equivalent to 1 µg retinol for ratios of 12:1 and 28:1, respectively, rather than the previously assumed ratio of 6:1 for all dietary β-carotene (3). The results of the current study agree highly with those of the study in Indonesian schoolchildren by de Pee et al (7), who found apparent onversion factors of 12:1 for fruit and 26:1 for vegetables, and were part of the evidence on which West et al (10) based their proposed conversion factor for dietary β-carotene from a mixed diet (ratio of vegetables to fruit = 4:1) of 21:1.

Our method of estimating the apparent conversion factors is based on changes in serum retinol concentration in humans after consumption of β-carotene and retinol-rich food, which appears appropriate, at least for the current study and that in Indonesia (7). The main reason that this method appears to be appropriate is that the largest change in serum retinol concentration occurred in the retinol-rich group (the positive control group), which indicated that the serum retinol concentration at baseline was suboptimal. Therefore, and because the increase in serum retinol in the vegetable group was smaller than that in the fruit group, that measurement proved to be a good quantitative indicator of improvement in vitamin A status. Another reason that this method appears to be appropriate is that the findings do not differ significantly between this study and the study from Indonesia (7) or from findings from more recent studies that used stable-isotope techniques. Tang et al reported conversion factors of 27:1 for dark-green leafy vegetables (11) and 20:9:1 for pureed spinach.
TABLE 3

Effects of interventions on serum indicators of vitamin A and carotenoid status

<table>
<thead>
<tr>
<th></th>
<th>Vegetable group (n = 73)</th>
<th>Fruit group (n = 69)</th>
<th>Retinol-rich group (n = 70)</th>
<th>Control group (n = 68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>1.15 (1.07, 1.24)</td>
<td>1.11 (1.03, 1.19)</td>
<td>1.12 (1.03, 1.21)</td>
<td>1.12 (1.04, 1.21)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>1.26 (1.19, 1.33)</td>
<td>1.25 (1.19, 1.32)</td>
<td>1.39 (1.31, 1.47)</td>
<td>1.12 (1.04, 1.21)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>1.10 (1.03, 1.18)</td>
<td>1.12 (1.05, 1.20)</td>
<td>1.24 (1.16, 1.32)</td>
<td>1.00</td>
</tr>
<tr>
<td>cis-β-Carotene (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.09 (0.03, 0.16)</td>
<td>0.13 (0.07, 0.19)</td>
<td>0.25 (0.17, 0.33)</td>
<td>0.00 (−0.06, 0.06)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.45 (0.41, 0.51)</td>
<td>0.52 (0.46, 0.60)</td>
<td>0.29 (0.25, 0.32)</td>
<td>0.21 (0.18, 0.24)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>1.98 (1.73, 2.28)</td>
<td>2.45 (2.13, 2.82)</td>
<td>1.32 (1.15, 1.52)</td>
<td>1.00</td>
</tr>
<tr>
<td>all-trans-β-Carotene (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.052 (0.029–0.075)</td>
<td>0.041 (0.028–0.072)</td>
<td>0.041 (0.024–0.058)</td>
<td>0.037 (0.019–0.066)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.039 (0.026–0.055)</td>
<td>0.052 (0.032–0.070)</td>
<td>0.025 (0.009–0.038)</td>
<td>0.037 (0.009–0.027)</td>
</tr>
<tr>
<td>α-Carotene (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.038 (0.031, 0.046)</td>
<td>0.050 (0.043, 0.058)</td>
<td>0.050 (0.043, 0.057)</td>
<td>0.046 (0.040, 0.054)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.090 (0.082, 0.098)</td>
<td>0.081 (0.071, 0.092)</td>
<td>0.064 (0.057, 0.073)</td>
<td>0.058 (0.050, 0.067)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>0.040 (0.026, 0.054)</td>
<td>0.034 (0.024, 0.044)</td>
<td>0.015 (0.007, 0.023)</td>
<td>0.014 (0.004, 0.023)</td>
</tr>
<tr>
<td>Lutein (μmol/L)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>At baseline</td>
<td>0.95 (0.81, 1.12)</td>
<td>0.84 (0.70, 1.01)</td>
<td>0.95 (0.85, 1.06)</td>
<td>0.91 (0.82, 1.01)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.91 (0.84, 0.99)</td>
<td>0.46 (0.41, 0.51)</td>
<td>0.53 (0.46, 0.60)</td>
<td>0.44 (0.39, 0.49)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>2.06 (1.79, 2.38)</td>
<td>1.06 (0.92, 1.23)</td>
<td>1.19 (1.03, 1.37)</td>
<td>1.00</td>
</tr>
<tr>
<td>β-Cryptoxanthin (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.059 (0.039–0.078)</td>
<td>0.031 (0.020–0.048)</td>
<td>0.032 (0.020–0.046)</td>
<td>0.029 (0.019–0.051)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.032 (0.023–0.060)</td>
<td>0.409 (0.222–0.701)</td>
<td>0.036 (0.025–0.047)</td>
<td>0.020 (0.009–0.032)</td>
</tr>
<tr>
<td>Zeaxanthin (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.036 (0.023–0.059)</td>
<td>0.027 (0.009–0.045)</td>
<td>0.034 (0.020–0.052)</td>
<td>0.034 (0.024–0.046)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.018 (0.009–0.027)</td>
<td>0.026 (0.009–0.039)</td>
<td>0.027 (0.016–0.036)</td>
<td>0.009 (0.009–0.023)</td>
</tr>
</tbody>
</table>

1 Δ change. Values in a row with different superscript letters are significantly different, P < 0.05. For normally distributed parameters, which had been log transformed, linear regression was used after control for baseline values, and a Bonferroni adjustment was made because of multiple comparisons. For nonnormally distributed variables, a comparison among groups of the absolute difference between baseline and 10-wk values used the Kruskal-Wallis test (multiple comparisons); if P < 0.05, the Mann-Whitney U test (2 comparisons) with Bonferroni correction for multiple comparisons was also used.

2 Geometric; 95% CI in parentheses (all such values).

3 P < 0.001 (overall F test for normally distributed parameters and Kruskall-Wallis test for nonnormally distributed parameters).

4 Differences between groups were not tested, because 10-wk values were compared after control for baseline values (see the 10-wk values above).

5 Proportional difference in values measured at 10 wk in each group relative to values measured at 10 wk in the control group, as derived by exponentiation of effect estimates in a linear regression model with log-transformed values of the serum indicator. Thus, 1.10 represents a 10% greater increase and 2.00 a 100% greater increase in the particular serum concentration than was observed in the control group.

6 Median; 25th–75th percentiles in parentheses (all such values).

7 Significant difference between baseline and 10-wk concentrations within the treatment group for nonnormally distributed parameters (Wilcoxon signed-rank test); 1P < 0.01, 5P < 0.001, 8P < 0.05.

and 14.8:1 for carrots (14). The first study was conducted among Chinese children and the latter among healthy adults in the United States. A study by Haskell et al (12) in Bangladeshi men used the deuterated-retinol-dilution (DRD) technique and found factors of 13:1 for fruit and 10:1 for spinach. The difference between the conversion factor found in the latter study and the conversion factors in studies in Indonesia and Vietnam or those reported by Tang et al may be related to one of several factors. Those factors include the food preparation techniques used—ie, variations in the extent of matrix destruction—(24–26), variations in the presence and intensity of helminthes infestation—because the Bangladeshi men underwent deworming, but the Indonesian children and the Vietnamese women did not—(27, 28), the other foods in the meal that may contain fewer or more enhancers or inhibitors of absorption—eg, fat, cellulose, and pectin—(17, 29, 30), and, probably, the particular variety of vegetable—ie, the kind of matrix.

Although we used changes in the serum retinol concentration for our comparisons, the question could be asked as to whether changes in the breast-milk retinol concentration could also be used to compare the bioefficacy of carotenoids from vegetables or fruit with that of carotenoids from retinol-rich foods. The relative changes in the retinol concentration in breast milk were much greater than those in all 3 groups compared with the control group (12). The large breast-milk retinol response suggests an important benefit for the breastfed child, especially when the mother increases her consumption of retinol-rich food. However, less is known about how the retinol concentration in breast milk is regulated, and the findings of the present study in Vietnam and the study in Indonesia also do not identify the breast-milk retinol concentration as a better quantitative indicator of changes of a woman’s vitamin A status than her serum retinol concentration. The changes of breast milk carotenoid concentrations also are not parallel to those in serum. The concentration of lutein remained stable in the vegetable group, whereas it decreased in the other groups, and that of β-cryptoxanthin increased in the fruit group; however, the change in the β-carotene concentrations in breast
milk in both groups, as compared with that in the control group, was much smaller than that observed in serum.

Another functional indicator of vitamin A status is night blindness, which is the first sign of vitamin A deficiency. Haskell et al (13) found that changes in serum retinol concentrations in pregnant Nepali women with night blindness differed by group, depending on the specific dietary or pharmaceutical source of the vitamin A the group received. However, the reduction in the prevalence of night blindness was substantial (and not significantly different) in all groups that received a form of vitamin A (95% CI: 17, 84) for green leafy vegetables, a finding that is similar to findings of other recent studies and that supports the inherent Nepali women with night blindness differed by group, depending on the specific dietary or pharmaceutical source of the vitamin A the group received. However, the reduction in the prevalence of night blindness was substantial (and not significantly different) in all groups that received a form of vitamin A.

In conclusion, this study found apparent conversion factors for β-carotene to retinol of 12:1 (95% CI: 8, 22) for fruit and 28:1 (95% CI: 17, 84) for green leafy vegetables, a finding that is similar to findings of other recent studies and that supports the conversion factor of 21:1 for dietary β-carotene from a mixed diet that was proposed by West et al (10). Further research should focus on quantifying the effects of parasitic infection and various food matrices on the bioefficacy of β-carotene, on assessing interindividual variations in response to intake of dietary provitamin A carotenoids, and on estimating the absorption of different carotenoids.

We thank the health workers, Women’s Union members, the community leaders of 10 communes, the staff of district health service of the Pho Yen district, Thai Nguyen province—Le Bach Mai, Nguyen Kim Canh, Le Danh Tuyen, and Nguyen Lan—and other researchers of Institute of Nutrition. We also thank the late Peter van de Bovenkamp and coworkers for laboratory

### TABLE 4

**Effects of interventions on breast-milk indicators of vitamin A and carotenoid status**

<table>
<thead>
<tr>
<th></th>
<th>Vegetable group (n = 71)</th>
<th>Fruit group (n = 65)</th>
<th>Retinol-rich group (n = 69)</th>
<th>Control group (n = 63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.74 (0.66, 0.83)</td>
<td>0.74 (0.66, 0.83)</td>
<td>0.72 (0.63, 0.83)</td>
<td>0.69 (0.59, 0.80)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.86 (0.76, 0.97)</td>
<td>0.90 (0.81, 0.99)</td>
<td>1.23 (1.12, 1.35)</td>
<td>0.63 (0.55, 0.73)</td>
</tr>
<tr>
<td>ΔBaseline – 10 wk</td>
<td>0.15 (0.04, 0.27)</td>
<td>0.15 (0.02, 0.28)</td>
<td>0.48 (0.32, 0.64)</td>
<td>−0.06 (−0.21, 0.09)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>1.33 (1.14, 1.56)</td>
<td>1.39 (1.19, 1.63)</td>
<td>1.92 (1.64, 2.24)</td>
<td>1.00</td>
</tr>
<tr>
<td>Lutein (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.029 (0.025, 0.035)</td>
<td>0.039 (0.032, 0.047)</td>
<td>0.020 (0.016, 0.026)</td>
<td>0.013 (0.010, 0.015)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.135 (0.119, 0.153)</td>
<td>0.122 (0.105, 0.143)</td>
<td>0.134 (0.118, 0.151)</td>
<td>0.116 (0.102, 0.133)</td>
</tr>
<tr>
<td>ΔBaseline – 10 wk</td>
<td>0.05 (0.027)</td>
<td>0.05 (0.04, 0.07)</td>
<td>0.07 (0.06, 0.081)</td>
<td>0.055 (0.049, 0.061)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>1.10</td>
<td>1.10</td>
<td>1.33</td>
<td>1.33</td>
</tr>
<tr>
<td>β-Cryptoxanthin (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At baseline</td>
<td>0.001 (0.000, 0.007)</td>
<td>0.003 (0.001, 0.008)</td>
<td>0.005 (0.001, 0.007)</td>
<td>0.005 (0.004, 0.006)</td>
</tr>
<tr>
<td>At 10 wk</td>
<td>0.005 (0.005, 0.013)</td>
<td>0.005 (0.004, 0.007)</td>
<td>0.003 (0.002, 0.004)</td>
<td>0.004 (0.003–0.006)</td>
</tr>
<tr>
<td>ΔBaseline – 10 wk</td>
<td>0.003 (0.002, 0.007)</td>
<td>0.005 (0.004, 0.007)</td>
<td>0.005 (0.002, 0.004)</td>
<td>0.005 (0.004, 0.006)</td>
</tr>
<tr>
<td>Δ Relative to control group</td>
<td>1.10</td>
<td>1.10</td>
<td>1.33</td>
<td>1.33</td>
</tr>
</tbody>
</table>

1. Δ, change. Values in a row with different superscript letters are significantly different, P < 0.05. For normally distributed, log-transformed parameters, linear regression controlled for baseline values and with Bonferroni adjustment for multiple comparisons. For nonnormally distributed parameters, Kruskal-Wallis test (multiple comparisons) was used for comparison between groups of mean differences between baseline and 10-wk values.

2. Geometric mean; 95% CI in parentheses (all such values).

3. P < 0.001 (overall F test for normally distributed parameters and Kruskal-Wallis test for nonnormally distributed parameters).

4. Median; 25th–75th percentiles in parentheses (all such values).

5. Proportional difference in values measured at 10 wk in each group relative to values measured at 10 wk in the control group, as derived by exponentiation of effect estimates in a linear regression model with log-transformed values of the serum indicator. Thus, 1.10 represents a 10% greater increase and 2.00 represents a 100% greater increase in the particular serum concentration than was observed in the control group.

6. Significant difference between baseline and 10-wk concentrations within treatment group, P < 0.001 (Wilcoxon’s signed-rank test for nonnormally distributed parameters).
analysis at the Division of Human Nutrition and Epidemiology, Wageningen University.

The authors’ contributions are as follows—NCK, CEW, SdP, JGAJH, and HHK: study design and interpretation of results; NCK, DB, and HDP: field work; NCK: analysis of the data and writing of the first draft of the manuscript; SdP and HV: assisted with writing of the manuscript; PJMH: laboratory analysis of serum and breast milk and milk samples; and all authors: revisions of the manuscript and approval of the final version. None of the authors had a personal or financial conflict of interest.

REFERENCES