Relationships of body mass index with serum carotenoids, tocopherols and retinol at steady-state and in response to a carotenoid-rich vegetable diet intervention in Filipino schoolchildren

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Synopsis

In marginally nourished children, information is scarce regarding the circulating concentrations of carotenoids and tocopherols, and physiological factors influencing their circulating levels. We determined the serum concentrations of carotenoids, tocopherols and retinol at steady state and in response to a 9-week vegetable diet intervention in 9–12-year-old girls (n = 54) and boys (n = 65) in rural Philippines. We determined cross-sectional relationships of BMI (body mass index) with serum micronutrients level, and whether BMI is a determinant of serum carotenoid responses to the ingestion of carotenoid-rich vegetables. We measured dietary nutrient intakes and assessed inflammation by measurement of serum C-reactive protein levels. The children had low serum concentrations of carotenoids, tocopherols and retinol as compared with published values for similar-aged children in the U.S.A. The low serum retinol levels can be ascribed to inadequate diets and were not the result of confounding due to inflammation. Significant inverse correlations of BMI and serum all-trans-β-carotene, 13-cis-β-carotene, α-carotene, lutein, zeaxanthin and α-tocopherol (but not β-cryptoxanthin, lycopene and retinol) were observed among girls at baseline. The dietary intervention markedly enhanced the serum concentrations of all carotenoids. Changes in serum all-trans-β-carotene and α-carotene (but not changes in lutein, zeaxanthin and β-cryptoxanthin) in response to the dietary intervention were inversely associated with BMI in girls and boys. Thus, in Filipino school-aged children, BMI is inversely related to the steady-state serum concentrations of certain carotenoids and vitamin E, but not vitamin A, and is a determinant of serum β- and α-carotene responses, but not xanthophyll responses, to the ingestion of carotenoid-rich vegetable meals.

Key words: body mass index (BMI), carotenoid, retinol, tocopherol, vitamin A, vitamin E

INTRODUCTION

Carotenoids are fat-soluble compounds present in many coloured vegetables and fruits. In human tissues, the most common carotenoids present are lutein, β-carotene, lycopene, zeaxanthin, β-cryptoxanthin and α-carotene, but differences between individuals and population groups are substantial. Among the carotenoids, β-carotene, α-carotene and β-cryptoxanthin can be converted into vitamin A in the body. Pre-formed vitamin A (retinol) is found in animal foods, such as liver and dairy products, as well as in fortified foods. Vitamin A deficiency affects millions of children in developing nations with severe consequences, including infectious morbidity, blindness and death [1,2].

Vitamin E is an essential fat-soluble antioxidant. Naturally occurring compounds with vitamin E activity include four tocopherols and four tocotrienols [3]. The primary sources of...
dietary vitamin E are grains, seeds, nuts, vegetables and vegetable oils [4]. In human blood, vitamin E circulates predominantly as α-tocopherol (the most biologically active form), with lesser amounts of γ-tocopherol; this is due to the preferential binding of α-tocopherol to hepatic α-tocopherol-transfer protein compared with γ-tocopherol, and its preferential incorporation into lipoproteins for delivery to peripheral tissues [5,6].

Some possible health benefits of carotenoids and vitamin E that have been suggested include protection against various kinds of cancers, heart disease, eye disease, diabetes and other chronic illnesses [7–10]. Low blood concentrations of carotenoids and tocopherols could be markers of inadequate intake and disease risk, and dietary guidelines recommend the consumption of foods rich in these micronutrients [11].

Although serum retinol level data are available in marginally nourished children, information regarding their circulating concentrations of carotenoids and vitamin E are sparse. Thus in the present study on Filipino school-aged girls and boys, we have: (i) documented the circulating steady-state concentrations of carotenoids, tocopherols and retinol; (ii) evaluated the cross-sectional relationships of BMI (body mass index) and the serum concentrations of these micronutrients; (iii) determined serum micronutrient responses to a dietary intervention with carotenoid-rich vegetable meals; and (iv) investigated whether BMI is a determinant of serum micronutrient responses to the vegetable intervention.

**EXPERIMENTAL**

**Subjects**
The study participants were 9–12-year-old girls (n = 54) and boys (n = 65) enrolled in the elementary schools of Banawang and Overland, located in the adjacent rural communities of Banawang and Atillano Ricardo, in Bagac, Bataan province, Philippines. The children were in general good health, with no chronic or acute illnesses, febrile conditions or gastrointestinal problems. They had no clinical signs of vitamin A deficiency, did not take any nutritional supplements and were enrolled in a food intervention study to investigate the influence of amounts of dietary fat on the bio-availability and bioconversion of plant provitamin A carotenoids [12]. Written informed consent was obtained from the children and their carers. 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.

**Dietary intervention**
The data in the cross-sectional analyses comprised BMI and serum concentrations of carotenoids, tocopherols and retinol obtained from the study participants during the baseline phase of a food-intervention study, the details of which have already been described previously [12]. Briefly, standardized carotenoid-rich vegetable meals containing similar amounts of β-carotene (3368 μg/day), α-carotene (813 μg/day), lutein and zeaxanthin (1664 μg/day) and β-cryptoxanthin (43 μg/day), but differing in amounts of dietary fat (i.e. 7, 15 or 29 g/day) were provided to study participants for 5 days/week for 9 weeks at their schools. The vegetables included carrots, pechay (bok choy; Brassica chinensis), squash and kangkong (swamp cabbage; Ipomea batatas aquatica), which were incorporated into traditionally accepted recipes. Vegetables and other ingredients in the menu were boiled separately, and predetermined amounts were weighed and placed in individual food containers for each child. Refined coconut oil, the most common source of dietary fat in rural Philippine communities, was added by using custom-made ladles of different sizes that delivered the desired amounts. The food containers were colour-coded depending on the child’s study group, and participants in the same group ate together, separately from the other groups. Dietitians from the Nutrition Center of the Philippines 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. Because similar increases in serum carotenoid concentrations and similar improvements in the total-body vitamin A pool size (as assessed by stable-isotope-dilution techniques) were observed after 9 weeks in the groups fed the three different amounts of dietary fat [12], in the present study the data from all study participants were pooled.

**BMI**
BMI is calculated from the following equation:

\[
\text{BMI (kg/m}^2\text{)} = \frac{\text{body weight}}{\text{height}^2}
\]

The US Centers for Disease Control and Prevention (CDC) sex-specific BMI-for-age percentiles for children were used to classify the study participants as underweight (<5th percentile), healthy weight (5th to 85th percentile), at risk of overweight (85th to <95th percentile) and overweight (≥95th percentile) [13].

**Helminthic infections**
Fecal samples were analysed at baseline for helminths (Ascaris lumbricoides, Trichuris trichiura and hookworm) by using the Kato–Katz procedure [14]. Those found positive for any of these intestinal parasites at baseline were treated with 400 mg of chewable albendazole (Kopran Ltd, Mumbai, India) 1 week before the biochemical tests were initiated. The 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 [15] was used to classify the intensity of infection as light, moderate or heavy for each helminth.

**Blood handling and tests**
To prevent the degradation of light-sensitive compounds, venous blood was extracted into aluminum-wrapped evacuated tubes, and all subsequent procedures were carried out in a darkened environment. Blood samples were centrifuged immediately at 2000 g for 10 min, and serum was collected for analysis of carotenoid, tocopherol and retinol concentrations.
room. Blood was allowed to clot and then centrifuged at 2800 g for 30 min at room temperature (25°C). Aliquots (0.5 ml) of serum were transferred into aluminum-wrapped cryovials, frozen at −20°C and transported within 24 h on dry ice to a freezer (−70°C) in Manila (Philippines), where they were kept until hand-carried on dry ice to Tufts University (Boston, MA, U.S.A.). At Tufts University, the samples were stored at −70°C until analysed within 4 months.

For analyses of serum carotenoids, tocopherols and retinol, a 150 μl mixture of internal standards (i.e. echineneone, α-tocopheryl acetate and retinyl acetate respectively) in 100% ethanol was added to 200 μl of serum samples, followed by 0.5 ml of saline (8.5 g of sodium chloride per l of water). The concentrations of internal standards added were adjusted so that the HPLC chromatogram peak heights or areas were similar to those of the serum lutein (found to be the most abundant carotenoid), retinol and α-tocopherol peaks of the subjects. Serum extraction involved a two-step procedure using 2 ml of chloroform/methanol (2:1, v/v) and then 3 ml of hexane. After each extraction step, the tube was vortex-mixed and centrifuged at 684 g for 10 min at 4°C. The hexane layer was combined with the chloroform extract and the solvents were evaporated to dryness under nitrogen in a water bath at 37°C. The residue was redissolved in 150 μl of 100% ethanol; the solution was vortexed, sonicated for 30 s and 50 μl was injected on to the HPLC column. Carotenoids (lutein, zeaxanthin, β-cryptoxanthin, α-carotene, 13-cis-β-carotene, all-trans-β-carotene, cis- and trans-lycopene isomers), tocopherols (α-tocopherol and γ-tocopherol) and retinol were analysed simultaneously by a gradient reversed-phase HPLC procedure with a YMC30 carotenoid column (3-μm particle size; internal diameter × length: 4.6 mm × 150 mm) and a photodiode array detector (Waters Corp., Milford, MA, U.S.A.), which was set to monitor the absorbance of these groups of compounds at 450, 292 and 325 nm respectively. The HPLC mobile phase was methanol/methyl t-butyl ether/water (83:15:2, by vol.) with 1.5% ammonium acetate in the water; solvent A) and methanol/methyl t-butyl ether/water (89:0:2, by vol.) with 1% ammonium acetate in the water; solvent B). Prior to HPLC, the solvents were filtered through a 0.2-μm nylon membrane filter (Whatman, Maidstone, Kent, U.K.) and degassed by using a vacuum pump. The details of the gradient reversed-phase procedure at a flow rate of 1 ml/min, and the lower limits of detection for carotenoids, retinol and tocopherols using this HPLC procedure has been described previously [16]. The peak areas were calibrated against known amounts of standards, and concentrations were corrected for extraction and handling losses by determining the percentage recoveries of the internal standards (which ranged from 94 to 100%). The interassay coefficient of variations (cv) (%) were as follows: retinol, 3%; β-carotene, 3%; α-carotene, 3%; γ-tocopherol, 4%; α-tocopherol, 5%; β-cryptoxanthin, 5%; lutein, 5%; zeaxanthin, 6%; lycopene, 6%; 13-cis-β-carotene, 11. All solvents were purchased from Sigma–Aldrich (St Louis, MO, U.S.A.). The sources of internal standards and standard compounds were: Sigma–Aldrich for β-carotene, lutein, lycopene, α-tocopherol, γ-tocopherol, α-tocopheryl acetate, retinol and retinyl acetate; CaroTcNature GmbH (Lupsingen, Switzerland) for echinone, α-carotene and 13-cis-β-carotene; and ChromaDex (Santa Ana, CA, U.S.A.) for β-cryptoxanthin and zeaxanthin.

Because inflammation could result in a transient decrease in the serum concentrations of retinol [17] and carotenoids [18], we measured the serum concentration of CRP (C-reactive protein), an acute-phase protein the level of which is increased following an inflammatory stimulus. CRP was analysed at the Bureau of Research and Laboratories, Department of Health (BRL-DOH), Manila, Philippines, with the NycoCard CRP single-test kit, a solid-phase sandwich immunometric procedure which uses a NycoCard READER II System (Axis-Shield Group, Oslo, Norway). This test has a cv of 5%, and a range of measurement of 5–150 mg/l in serum samples; the measuring interval is 1 mg/l.

Serum albumin, often used to assess general malnutrition, was analysed at the Bureau of Research and Laboratories, Department of Health (BRL-DOH) with the albumin liquiscolor test kit, which utilizes a photometric colorimetric procedure using the Bromocresol Green method (Human Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). The test is linear up to an albumin concentration of 70 g/l; the sensitivity is <1 g/l and the inter-assay cv is 1.5%.

Serum cholesterol, used to adjust for serum tocopherol, was analysed at the Bureau of Research and Laboratories, Department of Health (BRL-DOH) with the cholesterol liquiscolor test kit, which utilizes an enzymatic colorimetric test with lipid-clearing factor (Human Gesellschaft fur Biochemica und Diagnostica mbH). The test is linear up to a cholesterol concentration of 750 mg/dl (19.3 mmol/l); the sensitivity is 1.7 mg/dl, the recovery in control sera is 97%, and the inter-assay cv is 2.9%.

Dietary assessments

The usual dietary intakes of the study participants were assessed at baseline with the use of 24 h food-recall interviews conducted by study dietitians on 3 non-consecutive days. The Philippine Food Composition Tables [19] and the US Department of Agriculture National Nutrient Database [4] were used to assess intakes of carotenoids, retinol and vitamin E. The contribution of provitamin A carotenoids to the total vitamin A intake was estimated as RAEs (retinol activity equivalents) using a conversion factor of 12:1 for β-carotene and 24:1 for α-carotene and β-cryptoxanthin [20].

Statistical analyses

Age, anthropometric and biochemical data for girls and boys were compared using Student’s unpaired t test. The habitual dietary intakes of carotenoids, vitamin A and vitamin E were correlated with the steady-state (baseline) serum concentrations of these micronutrients. The relationships of baseline BMI with serum carotenoids, tocopherols and retinol under steady-state conditions and in response to the vegetable diet intervention were investigated using simple linear regression. Variables that were not normally distributed were logarithmically transformed prior to analyses. r (Pearson product–moment correlation coefficient) or ρ (Spearman rank-order correlation coefficient)
correlation coefficients were obtained. For serum measurements at baseline and after the dietary intervention, two-factor ANOVA was used to study the main effects of gender and repeated measures, and the interaction of these two main effects. All statistical analyses were performed by using STATVIEW SE + GRAPHICS software (Abacus Concepts, Berkeley, CA, U.S.A.). A value of $P < 0.05$ was considered to be statistically significant.

**RESULTS**

Table 1 shows the characteristics of the study participants at baseline. Girls were taller than boys; there were no differences in age, body weight and BMI, although the girls had a wider range of BMI values than boys. On the basis of the US Centers for Disease Control and Prevention (CDC) sex-specific BMI-for-age percentiles for children [13], 29.6% of girls and 30.8% of boys were underweight; 1.9% and 1.5% were at risk of overweight respectively; and 1.9% and 0% were overweight respectively.

There were no differences in serum albumin concentrations between boys and girls (Table 1). The normal range of serum albumin in children < 14 years old is 38–54 g/l [21]. At baseline, 16.7% of girls and 9.2% of boys had serum albumin values < 38 g/l; at post-intervention, 5.6% and 1.5% had low serum albumin values respectively.

The thresholds used to define an abnormal serum CRP value varied from > 5 mg/l [17] to > 10 mg/l [22]. There were no subjects with CRP values > 10 mg/l. Four children had CRP concentrations of 6–7 mg/l at baseline, and three had CRP values of 6–9 mg/l at post-intervention. However, none of them had serum retinol levels that are considered low, i.e. < 0.70 μmol/l [23], and except for one subject (whose diet was poor) their serum retinol and β-carotene concentrations were above the mean serum values of these compounds for the entire cohort. Table 1 shows that the percentage of subjects with low serum retinol (18.5% in girls and 9.2% in boys) far exceed those with abnormal CRP values (5.6% in girls and 1.5% in boys). Thus the poor serum retinol concentrations observed in this population cannot be ascribed to inflammation, but rather to poor dietary intakes (Table 2). The exclusion of the children with normal CRP values from data analyses did not alter the interpretation of the study results for serum retinol and carotenoids. Serum albumin values could also be decreased by inflammation [21]. However, only one child with an elevated serum CRP (9 mg/l) had a low albumin value (34 g/l).

Helminthic infections, particularly ascariasis, have been reported to affect the utilization of plant sources of β-carotene [24]. At baseline, before anti-helminthic treatment, the cumulative prevalence of helminths (mostly T. trichiura) in the subjects was 48%, and the intensity of the infections was mostly light. Repeat tests carried out midway and at the end of the 9-week intervention period showed a recurrence of light helminthic infections; the cumulative prevalence was 20% and 30% respectively. The recurrence of helminths midway through the study was most probably not a confounding factor, because the worm load was light.

Cut-off serum α-tocopherol values ranging from 7–28 μmol/l have been used by various investigators for estimating vitamin E deficiency [25]. On the basis of the most common cut-off deficiency value of < 11.6 μmol/l [25], the prevalence of vitamin E deficiency among the children who participated in this study was 7.4% in girls and 9.2% in boys (Table 1).

Table 2 shows the habitual dietary intakes of the study participants. The mean total vitamin A intakes from retinol plus provitamin A carotenoids by girls and boys were 199 and 189 μg of RAE/day respectively. These amounts are only 49.8% and 47.3% respectively of the recommended vitamin A intake of 400 μg/day for girls and boys in this age group for the Philippines [26]. The usual vitamin E intakes of girls and boys were 1.01 and 0.91 mg/day respectively. These amounts are only 9.2% and 9.1% of the recommended vitamin E intakes of 11 and

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>Girls (n = 54)</th>
<th>Boys (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>10.6 ± 0.8 (8.9–11.9)</td>
<td>10.7 ± 0.9 (8.9–12.0)</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>27.0 ± 6.8 (18.4–54.2)</td>
<td>25.6 ± 4.5 (17.4–37.8)</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.32 ± 0.08 (1.19–1.53)</td>
<td>1.29 ± 0.07 (1.12–1.49)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.3 ± 2.2 (13.1–25.5)</td>
<td>15.2 ± 1.3 (12.8–20.1)</td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>3.6 ± 1.1 (1.9–6.3)</td>
<td>3.3 ± 1.0 (1.9–7.5)</td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>42.5 ± 4.7 (28–53)</td>
<td>42.3 ± 3.8 (31–48)</td>
<td></td>
</tr>
<tr>
<td>Low serum albumin (%)†</td>
<td>16.7</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>High serum C-reactive protein (%)†</td>
<td>5.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Low serum retinol (%)§</td>
<td>18.5</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Low serum α-tocopherol (%)∥</td>
<td>7.4</td>
<td>9.2</td>
<td></td>
</tr>
</tbody>
</table>

* Girls versus boys, $P = 0.04$ (Student's unpaired t test).
† $\geq 38$ g/l [21].
‡ > 5 mg/l [17].
§ < 0.70 μmol/l [23].
∥ < 11.6 μmol/l [25].
Compared with boys, girls had higher mean serum concentrations of carotenoids, tocopherols and retinol at baseline. The differences observed between girls and boys may be due to the wider range of BMI values in girls than boys. In both genders, BMI was unrelated to serum retinol concentrations.

Table 3 shows the means ± S.D. (range) for the serum concentrations of carotenoids, tocopherols and retinol at baseline. Compared with boys, girls had higher mean serum concentrations of all the carotenoids; however, significant differences were observed only for β-cryptoxanthin ($P = 0.01$) and 13-cis-β-carotene ($P = 0.02$). No gender differences were observed in the serum concentrations of retinol, α-tocopherol and γ-tocopherol; however, when serum vitamin E was expressed in terms of serum total cholesterol, girls tended to have a lower mean α-tocopherol/cholesterol value than boys ($P = 0.054$). The serum cholesterol concentration (means ± S.D.) in girls and boys were 3.6 ± 1.1 and 3.3 ± 1.0 mmol/l respectively, and these values were not significantly different ($P = 0.15$). In the girls, dietary β-cryptoxanthin correlated with serum β-cryptoxanthin at baseline ($r = 0.30, P = 0.03$). Dietary lycopene correlated with serum lycopene in the boys ($r = 0.25, P = 0.04$), and these variables also tended to correlate among girls ($r = 0.26, P = 0.06$). For other carotenoids, vitamin A and vitamin E, no correlations between dietary intakes and serum concentrations were observed.

Table 4 shows that, in cross-sectional analyses, BMI correlated inversely with serum levels of all-trans-β-carotene, 13-cis-β-carotene, α-carotene, lutein and zeaxanthin, but not with lycopene or β-cryptoxanthin among girls. In the boys, the only carotenoid that showed such an association with BMI was 13-cis-β-carotene. In girls, BMI also correlated inversely with α-tocopherol and tended to correlate inversely with γ-tocopherol. The differences observed between girls and boys may be due to the wider range of BMI values in girls than boys. In both genders, BMI was unrelated to serum retinol concentrations.

Complete serum biochemical analyses was performed on all of the girls ($n = 54$) and 62 of 65 boys after the dietary intervention. Statistically significant increases in serum concentrations of all-trans-β-carotene (5-fold), α-carotene (19-fold), 13-cis-β-carotene (3-fold), lutein (6-fold), zeaxanthin (2-fold), and β-cryptoxanthin (2-fold) were observed, and the serum carotenoid responses were not significantly different in girls and boys (Figure 1).
Table 4 Cross-sectional correlations of BMI with serum carotenoids, tocopherols and retinol at baseline

<table>
<thead>
<tr>
<th>Carotenoid, tocopherol or retinol</th>
<th>Correlation coefficients</th>
<th>Girls (n = 54)</th>
<th>Boys (n = 65)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ρ or r</td>
<td>P</td>
<td>ρ or r</td>
</tr>
<tr>
<td>All-trans-β-carotene</td>
<td>−0.35</td>
<td>0.01</td>
<td>−0.16</td>
</tr>
<tr>
<td>13-cis-β-Carotene</td>
<td>−0.32</td>
<td>0.02</td>
<td>−0.27</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>−0.39</td>
<td>0.004</td>
<td>0.07</td>
</tr>
<tr>
<td>Lycopene</td>
<td>−0.09</td>
<td>0.50</td>
<td>0.21</td>
</tr>
<tr>
<td>Lutein</td>
<td>−0.30</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>−0.39</td>
<td>0.005</td>
<td>−0.10</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.21</td>
<td>0.13</td>
<td>−0.08</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>−0.33</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>−0.25</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Retinol</td>
<td>0.23</td>
<td>0.10</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Mean serum retinol tended to increase by 0.056 μmol/l (from 0.871 to 0.927 μmol/l) among girls, and by 0.021 μmol/l (from 0.878 to 0.899 μmol/l) among boys (two-factor ANOVA: P = 0.052 for repeated measures, with no interaction of gender x repeated measures). Although the present study was primarily designed as a carotenoid-rich vegetable diet intervention, significant increases in mean serum α-tocopherol were also observed, from 16.40 to 18.54 μmol/l in girls, and from 16.87 to 17.77 μmol/l in boys (two-factor ANOVA: P = 0.0001 for repeated measures), and these responses tended to be significantly different (P = 0.056 for gender x repeated measures). Significant reductions in mean serum γ-tocopherol were observed: from 0.758 to 0.704 μmol/l in girls, and from 0.760 to 0.683 μmol/l in boys (two-factor ANOVA: P = 0.0001 for repeated measures, with no interaction of gender x repeated measures).

DISCUSSION

Data for serum concentrations of carotenoids and tocopherols in marginally nourished children are sparse. Guidelines regarding cut-off serum values for carotenoid adequacy have not been

Table 5 Correlations of baseline BMI with changes in serum carotenoid concentrations after 9 weeks of dietary intervention with carotenoid-rich vegetable meals

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Correlation coefficients</th>
<th>Girls (n = 54)</th>
<th>Boys (n = 62)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ρ or r</td>
<td>P</td>
<td>ρ or r</td>
</tr>
<tr>
<td>All-trans-β-carotene</td>
<td>−0.281</td>
<td>0.04</td>
<td>−0.278</td>
</tr>
<tr>
<td>13-cis-β-Carotene</td>
<td>−0.371</td>
<td>0.01</td>
<td>−0.183</td>
</tr>
<tr>
<td>α-Carotene</td>
<td>−0.370</td>
<td>0.01</td>
<td>−0.297</td>
</tr>
<tr>
<td>Lutein</td>
<td>−0.172</td>
<td>0.21</td>
<td>−0.099</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>0.014</td>
<td>0.92</td>
<td>−0.049</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>0.140</td>
<td>0.31</td>
<td>−0.189</td>
</tr>
</tbody>
</table>

Table 5 shows that, in girls and boys, baseline BMI was inversely and significantly correlated with the changes in the serum concentrations of all-trans-β-carotene and α-carotene following 9 weeks of dietary intervention; in girls, a significant inverse correlation of BMI with change in serum 13-cis-β-carotene was also observed. In girls and boys, BMI was unrelated to the changes in serum concentrations of lutein, zeaxanthin, β-cryptoxanthin, tocopherols and retinol.

![Figure 1](image-url)
established; however, the serum carotenoid concentrations of the Filipino children in this study were much lower than those that have been reported for children in the U.S.A. For example, compared to data from the third National Health and Nutrition Examination Survey (NHANES III) [27], serum β-carotene in Filipino children was 65% of that found in children in U.S.A.; α-carotene, 35%; β-cryptoxanthin, 33%; lutein plus zeaxanthin, 87%; lycopene, 21%. In the present study, girls had significantly greater mean serum concentrations of β-cryptoxanthin and 13-cis-β-carotene than boys; there were no significant differences between girls and boys in serum concentrations of other carotenoids. In NHANES III, it was found that serum lycopene, but not other carotenoids, was significantly higher in boys than girls [27]. Differences between sexes in their circulating carotenoid concentrations most probably reflect dietary intakes and not physiologic differences in carotenoid metabolism. In the present study, girls had higher intakes of β-cryptoxanthin, β-carotene and other carotenoids than boys, although the differences were not statistically significant.

No differences between girls and boys were observed in their circulating concentrations of α- or γ-tocopherol per unit volume of serum. Their baseline mean α-tocopherol values (16.4 and 16.8 μmol/l respectively) were lower compared with those of 9–13-year-old children in the U.S.A. (18.4 μmol/l) who participated in NHANES III and among whom no gender difference in circulating vitamin E was also observed [11]. Among girls, but not boys, there was a significant correlation between serum α-tocopherol and total cholesterol ($\rho = 0.35$, $P = 0.01$). When serum α-tocopherol values were expressed in terms of serum cholesterol, girls tended to have lower values than boys ($P = 0.054$), possibly due to the higher (although non-statistically significant) mean serum cholesterol concentration in girls than boys.

On the basis of the most common cut-off deficiency value for serum α-tocopherol of <11.6 μmol/l [25], the prevalence of vitamin E deficiency among the children who participated in the present study was 7.4% in girls and 9.2% in boys. Clinical signs of vitamin E deficiency are rare, but can occur as a result of genetic defects in the α-tocopherol transfer protein and in disorders of fat metabolism [3,26,27]. Vitamin E deficiency has been described in protein-energy malnutrition, and administration of this vitamin has been reported to reduce the neurologic dysfunction in children suffering from this condition [30].

In the present study, the mean ratio of α-tocopherol/γ-tocopherol in serum was 22 (range 10–31). Among adults in the U.S.A. (> 20 years of age) who participated in NHANES III, serum α- to γ-tocopherol ratios increased significantly from 4 to 8 with increasing age, and was higher in Caucasians compared with Mexican Americans and African Americans [25]. Among Costa Rican adults, mean triacylglycerol-adjusted plasma α- to γ-tocopherol ratios in the range 9 to 14 have been reported, depending on the major oil or fat used for cooking [31].

We found a significant positive correlation between serum α- and γ-tocopherol ($\rho = 0.66$, $P = 0.0001$). Other studies, however, found a negative correlation between the circulating concentrations of these vitamin E compounds [32,33]. It is possible that differences in the circulating α- and γ-tocopherol concentrations among populations could be related to race, age, dietary factors, use of supplements, serum lipid concentrations and interaction of vitamin E with other micronutrients. There was no difference in serum retinol concentrations between Filipino girls and boys. Similarly, in NHANES III no gender difference in serum retinol concentration was observed among children < 14 years of age, although among adults, men had significantly higher serum retinol concentrations than women [34]. The serum retinol values in the Filipino study participants were 63% of values that have been reported for 9–13-year-old children in U.S.A. in the NHANES III study [34]. 19% of girls and 9% of boys had serum retinol concentrations that are considered low, i.e. < 0.70 μmol/L [23]. Subclinical vitamin A deficiency remains a public health problem among children and pregnant and lactating women in the Philippines [35].

We did not observe a difference in mean BMI between girls and boys in the present study, although a wider range of values was seen among girls. It has been reported that in 5–17-year-old children in U.S.A., BMI increases with age and is slightly higher for girls than boys [36].

In cross-sectional analyses, we observed that in girls, BMI correlated inversely with serum carotenoids, except for lycopene and β-cryptoxanthin. Among children in U.S.A. who participated in NHANES III, Ford et al. [27] reported that BMI was inversely related to all serum carotenoids, except for lycopene, and that overweight children had the lowest serum carotenoid concentrations.

In response to 9 weeks of carotenoid-rich vegetable meals, there were marked increases in the serum concentrations of all carotenoids. Significant inverse correlations between BMI and changes in serum all-trans-β-carotene and α-carotene concentrations in response to the dietary intervention were observed in girls and boys, but no association was observed between BMI and changes in the serum concentrations of the xanthophyll carotenoids. These results are similar to those obtained by Yeum et al. [37] on older women fed high-carotenoid diets for 15 days, in which a significant inverse correlation was observed between BMI and change in plasma all-trans-β-carotene, but not between BMI and change in plasma lutein or β-cryptoxanthin.

It is not surprising that we did not observe a relationship between BMI and serum retinol concentration, because the circulating retinol levels are homeostatically controlled over a wide physiological range of liver vitamin A concentrations [38]; although a positive correlation has been reported between BMI and serum retinol values among children in U.S.A. in NHANES III by Ballew et al. [34]. The vitamin A status of the children improved markedly with the 9-week vegetable intervention, and the results have been reported previously [12]. There were 2-fold increases in the total-body vitamin A pool size and in liver vitamin A concentration (as assessed by stable-isotope-dilution methodology), with no significant change in the serum retinol concentration in groups fed standardized meals with different
amounts of dietary fat [12]. In the present study, in which the data were pooled and stratified according to gender, the resulting increase in the number of subjects per group may have accounted for the borderline statistical significance in the small increase in serum retinol observed. The baseline BMI was unrelated to these changes in serum retinol concentrations.

In girls, but not boys, we found that BMI was inversely correlated with the steady-state serum concentrations of α-tocopherol and γ-tocopherol, which could be related to the wider range in BMI values in girls than boys. An inverse correlation between BMI and plasma α-tocopherol concentration has been reported by Sinha et al. [39] in healthy men in U.S.A. who were non-supplement users. Among post-menopausal women in the U.S.A., White et al. [32] reported that higher BMI was associated with lower serum α-tocopherol and higher γ-tocopherol concentrations.

Studies have suggested that BMI is a good predictor of the percentage of body fat in adults [40,41] and in school-aged children [42–44]. Carotenoids and tocopherols are lipophilic compounds and are deposited in fatty tissues of the body [31,45]. Thus it is probable that body fat could influence the concentrations of circulating carotenoids and tocopherols. Kimmons et al. [46] analysed BMI and serum data from NHANES III, and found a higher prevalence of low serum carotenoid concentrations among overweight and obese persons of both sexes and a higher prevalence of low serum vitamin E among women. Zhu et al. [47], however, have suggested that BMI is a relatively inaccurate measure of adiposity because it does not distinguish between body mass contributed by fat and by lean body mass. In a study by Zhu et al. [47], BMI and hydrodensitometry were used to assess body composition in adult men, and plasma β-carotene concentration was inversely correlated, not only with BMI and the body’s fat mass, but also with the fat-free mass. Furthermore, plasma total carotenoids inversely correlated with BMI and fat-free mass, but not fat mass. Thus, according to Zhu et al. [47], the fat-free mass which includes non-adipose tissues may play important roles in determining steady-state plasma carotenoid levels by serving as dynamic reservoirs that actively take up lipoprotein-associated carotenoids from plasma. In addition to adipose tissue, carotenoids and tocopherols are found in liver, kidney, adrenal, lung, testis, pancreas and other tissues [48–50]. Inverse associations of BMI or abdominal obesity and adipose tissue concentrations of carotenoids or tocopherols have been reported in adults [51,52].

In summary, we have reported that Filipino school-aged children in this study have low circulating concentrations of carotenoids, tocopherols and retinol, as compared with published values for similar-aged children in the U.S.A. The low levels of these micronutrients cannot be ascribed to inflammation, but rather to poor dietary intakes. In girls, but not boys, the steady-state serum concentrations of carotenoids (except lycopene and β-cryptoxanthin) and tocopherols were inversely correlated with BMI. In girls and boys, BMI is a predictor of the serum all-trans-β-carotene and α-carotene responses, but not the serum xanthophyll responses, to ingestion of vegetable meals that are rich in these carotenoids.

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