

# Bioconversion of plant carotenoids to vitamin A in Filipino school-aged children varies inversely with vitamin A status<sup>1-4</sup>

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## ABSTRACT

**Background:** It is important to understand the factors affecting strategies to improve the vitamin A status of populations. We reported previously that a 3-d deuterated-retinol-dilution (DRD) procedure might be used to indicate total body stores of vitamin A.

**Objective:** We studied the ability of 3-d DRD to detect changes in the body pool size of vitamin A and the effect of vitamin A status on the bioconversion of plant carotenoids to vitamin A.

**Design:** Two separate, unrelated studies were conducted in 7–13-y-old children with poor or marginal serum retinol concentrations (0.32–0.93  $\mu\text{mol/L}$ ) by feeding them controlled diets daily for 5 d/wk for 12 wk, after treatment with an anthelmintic drug. In school 1 ( $n = 27$ ), lunch and 2 snacks that were provided at school contained 2258 retinol equivalents/d (mostly from orange fruit and vegetables) and 5.3 MJ/d from 33 g fat, 37 g protein, and 209 g carbohydrates; in school 2 ( $n = 25$ ), 2 snacks provided 2.5 MJ/d from 9.4 g fat, 9.6 g protein, and 119 g carbohydrates, but no carotenes.

**Results:** In school 1, mean serum  $\beta$ -carotene increased from 0.12 to 0.62  $\mu\text{mol/L}$  ( $P = 0.0001$ ) and serum retinol increased from 0.68 to 1.06  $\mu\text{mol/L}$  ( $P = 0.0001$ ). In school 2, serum  $\beta$ -carotene increased from 0.06 to 0.11  $\mu\text{mol/L}$  ( $P = 0.0001$ ) and serum retinol increased from 0.66 to 0.86  $\mu\text{mol/L}$  ( $P = 0.0001$ ). In school 1, but not school 2, improvement in serum retinol varied inversely with baseline retinol ( $r = -0.38$ ,  $P = 0.048$ ). In both schools, 3-d DRD showed reductions in the ratio of serum deuterated to nondeuterated retinol (D:H retinol) postintervention, denoting improvements in vitamin A status; the higher D:H retinol (ie, the poorer the status) at baseline, the greater the reduction in D:H retinol postintervention (school 1:  $r = -0.99$ ,  $P = 0.0001$ ; school 2:  $r = -0.89$ ,  $P = 0.0001$ ).

**Conclusions:** Three-day DRD can detect changes in the body pool size of vitamin A, although a predictive equation to quantify total body stores of vitamin A with the use of 3-d data needs to be developed. Bioconversion of plant carotenoids to vitamin A varies inversely with vitamin A status; improvement in status after dietary interventions is strongly influenced by total body stores of vitamin A and is influenced little or not at all by serum retinol. *Am J Clin Nutr* 2000;72:455–65.

**KEY WORDS** Vitamin A, retinol,  $\beta$ -carotene bioconversion, carotenoids, fruit, vegetables, anthelmintic drugs, deuterated retinol dilution, stable isotope dilution, school-age children

## INTRODUCTION

Factors that influence the bioavailability of vitamin A from provitamin A carotenoids include the food matrix (1), food processing (2), fat intake (3–6), protein intake (7–9), and the presence of intestinal parasites (5). Moreover, in 1961 Olson (10) reported that injection of vitamin A together with [<sup>14</sup>C]- $\beta$ -carotene into ligated loops of rat intestine inhibited the formation of [<sup>14</sup>C]-retinyl ester, and formation of retinyl ester in intestinal mucosa increased linearly at low but not at high doses of  $\beta$ -carotene. Since then, some animal studies (11–14) but not others (14, 15) showed that bioconversion of  $\beta$ -carotene to vitamin A is influenced by intakes of vitamin A or  $\beta$ -carotene, or both. In chicks, decreased carotene absorption and decreased carotene concentrations in serum, liver, and toe-web skin were found after dietary vitamin A was increased (11). In rats, intestinal  $\beta$ -carotene 15,15'-dioxygenase activity was higher in those fed low amounts than in those fed high amounts of vitamin A (12, 13) or  $\beta$ -carotene (13). However, other researchers reported that neither  $\beta$ -carotene nor retinol depletion or excess feeding of these 2 substances to rats greatly affected the rats' intestinal  $\beta$ -carotene 15,15'-dioxygenase activity (15). In hamsters, intestinal enzyme activity was enhanced when the animals were fed a vitamin A-deficient diet; however, no effect on enzyme activity of feeding high amounts of  $\beta$ -carotene was found, and no relation was noted between the vitamin A or  $\beta$ -carotene content of the liver and intestinal enzyme activity (14). In humans, the limited data on this subject are likewise equivocal. In undernourished children in India, it was reported that consumption of

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green, leafy vegetables (spinach or amaranth) resulted in a rise in serum retinol and that the rise was greater in children whose initial serum retinol concentration was  $\leq 0.70$  (4) or  $<0.87$   $\mu\text{mol/L}$  (16) than in those with higher initial serum retinol concentrations. In contrast, in Guatemalan schoolchildren, no significant association was found between baseline plasma retinol (range: 0.33–1.41  $\mu\text{mol/L}$ ) and postprandial retinyl palmitate in plasma 2 h after ingestion of  $\beta$ -carotene capsules (17).

It is well known that circulating retinol is under homeostatic control over the physiologic range of liver vitamin A concentrations (18); thus, the usefulness of serum or plasma retinol as a measure of vitamin A status is limited. The use of stable isotopes for assessment of total-body vitamin A stores is more promising. A deuterated-retinol-dilution (DRD) procedure described by Furr et al (19), which is a modification of the procedure of Bausch and Rietz (20), requires the equilibration of an oral dose of deuterated vitamin A with the body pool of vitamin A; equilibration time is  $\approx 17$ –20 d in adults (21, 22). From studies in older adults with adequate vitamin A status, we reported that a shortened DRD procedure (3 d) is a promising tool for the assessment of total-body vitamin A stores (22); we found that the ratio of deuterated to nondeuterated retinol in serum (D:H retinol) 3 d after an oral dose of [ $^2\text{H}_4$ ]retinyl acetate correlated well with the calculated values for total-body stores of vitamin A when the procedure of Furr et al (19) was used.

The aims of these studies were to investigate the ability of 3-d DRD to detect changes in body pool size of vitamin A after dietary intervention in dewormed schoolchildren and to study the effect of vitamin A status on the bioconversion of plant carotenoids to vitamin A. We hypothesized that 3-d DRD could detect changes in the body pool size of vitamin A and would prove to be a good measure of vitamin A status.

## SUBJECTS AND METHODS

### Subjects

The study sites were 2 elementary schools in 2 rural villages: Santa Elena (school 1), in the municipality of Santo Tomas, Batangas province; and Hukay (school 2), in the municipality of Silang, Cavite province. These villages are  $\approx 70$  and 50 km south of Manila, respectively. Santa Elena was chosen on the basis of a 1993 survey done by the Nutrition Center of the Philippines that showed a 30% prevalence of low serum retinol concentrations ( $<0.70$   $\mu\text{mol/L}$ ) in schoolchildren in Santo Tomas (23). Hukay was chosen on the basis of 1995 school health records that showed a 27% prevalence of moderately and severely underweight schoolchildren. Children of both sexes who were enrolled in grades 1–6 were eligible to participate in the screening procedures. Informed consent was obtained from parents or guardians. Approval to conduct these studies was obtained from the Ethical Review Board of the Philippine Council for Health Research and Development and the Tufts University–New England Medical Center Human Investigation Review Committee.

The screening procedures took place in July and August 1996 and consisted of a physical examination, including an eye examination for xerophthalmia; anthropometric measurements (weight, height, and midupper arm circumference); conjunctival impression cytology (CIC) for vitamin A status (24); stool collection for examination of intestinal parasites by using the Kato Katz method (25); and a blood draw for determination of serum

retinol, carotenoids, and iron status. Only the results of the intervention study are presented in this article; other data obtained at screening will be presented in separate articles.

In school 1, 6 (3.2%) of the 186 children who were eligible to participate declined. Those who agreed to participate went through one or more of the above-mentioned screening procedures: 93% completed the physical examination, 92% completed the anthropometric measurements, 91% completed the CIC procedure, and 83% agreed to a blood draw. In school 2, 7 (3.1%) of the 226 children who were eligible to participate declined. Those who agreed to participate went through one or more of the above-mentioned screening procedures: 95% completed the physical examination, 99% completed the anthropometric measurements, and 98% completed the CIC procedure; however, only 60% agreed to a blood draw.

Children with the lowest serum retinol concentrations but without xerophthalmia and who were generally in good health with no chronic, febrile, or infectious illness and no prolonged gastrointestinal disorders were invited to participate in the intervention studies; an abnormal CIC result without xerophthalmia was not considered as an exclusion criteria: 27 children from school 1 and 25 children from school 2 were enrolled in these studies.

### Dietary assessments

The food intake in the participants' homes at baseline (preintervention) was recorded by the caregivers or by the study participants themselves (ie, by those aged 10–13 y) by using the 24-h recall method. During the intervention period, all food intakes at the schools were recorded, as were absences; dietary intakes of foods eaten at home and on weekends were not assessed, and no specific dietary instructions were given to the caregivers or the children. Philippine food tables (26) were used to assess dietary intakes at baseline and at the schools; these tables provide data for total carotenoids and not for individual provitamin A carotenoids. Vitamin A intakes from plant and animal sources were estimated and a 6:1 equivalence was used for bioconversion of plant carotenoids (assumed to be mostly  $\beta$ -carotene) to retinol, a ratio that has been questioned as new data have emerged (1).

### Intervention studies

To ensure compliance during the feeding period, initial interviews with mothers and children were conducted to determine the children's preferred fruit and vegetables and their preferred methods of preparation or cooking. This information was used in the choice of foods and menus provided during the intervention studies. From late November 1996 to mid February 1997, for 5 d/wk for 12 wk, the participants were fed controlled diets in their schools. One to 2 wk before the dietary intervention, the subjects were treated for intestinal parasites with 100 mg of chewable albendazole tablets (SmithKline Beecham Pharmaceuticals, Philadelphia) because infestations with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm were found.

Two intervention studies were conducted; the studies in school 1 and school 2 were unrelated, and one study was not considered to be a control for the other. The characteristics of the children were not similar at baseline. In school 2, the subjects were more undernourished and there were more underweight and stunted children than there were in school 1; the home dietary intakes of the children in school 2 were poorer than those of the children in school 1. In school 1, the intervention consisted of albendazole treatment and daily feeding of a midmorning snack, lunch, and a midafternoon snack with 5-d rotating menus containing orange

fruit and vegetables (mango, melon, papaya, squash, sweet potato, and carrots) prepared in preferred traditional ways. Fat was added to vegetable dishes (by frying or sautéing in oil or boiling in coconut milk); fruit eaten raw was eaten with a fat-containing dish. For example, a snack could consist of a mango shake (a blend of ripe mango, sugar, and water) and fried sweet potato; lunch could contain ripe papaya fruit and sautéed squash or carrots. The foods provided on average of  $\approx 13$  mg  $\beta$ -carotene [2192 retinol equivalents (RE)] per child per day, an amount that is  $\approx 5$  times the Philippine recommended dietary allowance (RDA) for vitamin A for these age groups, the RDA being 400 and 425 RE, respectively, for children aged 7–9 y and 10–12 y (26). Note that the Philippine RDA for vitamin A is much less than the 1989 US RDA of 700 RE for children aged 7–10 y and 800 and 1000 RE, respectively, for females and males aged 11–14 y (27).

In school 2, the intervention consisted of albendazole treatment and daily feeding of midmorning and midafternoon snacks that provided no sources of vitamin A or carotenoids, eg, a variety of rice cakes and cereals, bread, biscuits, French fries, coconut, peanut butter, sugar-sweetened juice of pineapple, guyabano (soursoy), and sago (tapioca).

In both schools, the children were allowed to eat and drink as much as they wanted and the amounts consumed were recorded. Any foods left unconsumed were also recorded. Nutritionists and dietitians from the Nutrition Center of the Philippines purchased, prepared, and served the foods to the children; measured plate waste; and calculated the nutrient intakes of each child from the food eaten at school.

#### Blood handling and tests

Biochemical analyses before and after the 12-wk food-intervention period included serum retinol, carotenoids, albumin, transthyretin (prealbumin), C-reactive protein,  $\alpha_1$ -antitrypsin, ferritin, transferrin receptor, and blood hemoglobin. Hemoglobin measurements were done in the field with a standard cyanomethemoglobin method immediately after blood drawing. A centrifuge and refrigerator with freezer were transported from Manila to the rural study sites for preparation and storage of serum samples. Nonfasting blood was drawn at school in the morning; in a study in 5–8-y-old children, it was reported that blood samples can be obtained either fasting or within 4 h after breakfast without altering the results for serum concentrations of retinol or carotenoids (28). To protect light-sensitive retinoids and carotenoids from photodegradation, blood was drawn into aluminum-wrapped evacuated tubes, allowed to clot, and centrifuged at  $2800 \times g$  for 10 min at room temperature in a dark room. Serum (0.5–1-mL aliquots) was transferred with a pipette into cryovials, which were immediately stored at  $-20^\circ\text{C}$ . At the end of each day, the samples were transported to Manila under ice and stored at  $-70^\circ\text{C}$  until shipped frozen under dry ice to the Human Nutrition Research Center at Tufts University in Boston, where they were kept at  $-70^\circ\text{C}$  until analyzed.

Serum retinol and carotenoids were analyzed under red light by using gradient reversed-phase HPLC (29, 30) with retinyl acetate and echinenone as internal standards. C-reactive protein,  $\alpha_1$ -antitrypsin, and transthyretin were assayed with immunoprecipitation analysis by using the SPQ Antibody Reagent Set II (Atlantic Antibodies, Stillwater, MN) and albumin was assayed by using the Roche Reagent for Albumin (Roche Diagnostic Systems, Inc, Somerville, NJ). Serum ferritin was assayed by using the MAGIC Ferritin [ $^{125}\text{I}$ ] radioimmunoassay kit (Ciba Corning Diagnostics Corp, Medfield, MA). Serum transferrin receptor

was analyzed with the Ramco TFR test kit (Ramco Laboratories, Inc, Houston), an enzyme immunoassay procedure.

#### Three-day deuterated-retinol-dilution procedure

To assess total body stores of vitamin A, a 3-d isotope-dilution procedure using deuterated vitamin A was done. Cambridge Isotope Laboratories (Andover, MA) synthesized tetradeuterated retinyl acetate (*all-trans*-retinyl-10,19,19,19- $^{2}\text{H}_4$ acetate) and octadeuterated retinyl acetate (*all-trans*-retinyl 10,14,19,19,19,20,20,20- $^{2}\text{H}_8$ acetate). Capsules containing 5.0-mg amounts of these isotopes dissolved in corn oil were prepared in Boston as described previously (22).

At baseline, a 5-mg capsule of [ $^{2}\text{H}_4$ ]retinyl acetate was administered orally with food to each child by a dietitian from the Nutrition Center of the Philippines and blood was drawn 3 d after the isotope was administered for determination of D:H retinol. The day immediately after the 12-wk food intervention ended, the isotope-dilution test was repeated by orally administering a 5-mg capsule of [ $^{2}\text{H}_8$ ]retinyl acetate; blood was drawn 3 d later. [ $^{2}\text{H}_8$ ]retinyl acetate was used postintervention to distinguish serum [ $^{2}\text{H}_8$ ]retinol from any residual [ $^{2}\text{H}_4$ ]retinol. During the 3-d test period, at baseline and postintervention, the subjects ate their usual diets at home. To analyze for retinol isotopes in serum, retinol was separated from the other constituents of serum by using HPLC; the retinol fraction was collected and derivatized to trimethylsilyl derivatives and analyzed by gas chromatography–mass spectrometry (GC-MS) (31). All HPLC and GC-MS procedures were done at Tufts University, Boston.

#### Statistical analyses

Student's paired *t* test was used to compare mean serum biochemistries before and after intervention. Regression analyses and Pearson's product-moment correlation analyses were performed to determine whether there was an association between change in serum retinol and baseline retinol and between change in 3-d D:H retinol and baseline 3-d D:H retinol. Pooled data from both schools pre- and postintervention were used to correlate 3-d D:H retinol with serum retinol values. All statistical analyses were done by using STATVIEW SE+GRAPHICS (Abacus Concepts, Inc, Berkeley, CA).

## RESULTS

#### Characteristics of study participants

The characteristics of the study participants at baseline are shown in **Table 1**. The mean ( $\pm$ SEM) age of the subjects in school 1 ( $n = 27$ ) was  $9.5 \pm 0.4$  y (range: 6.8–13.2 y) and that in school 2 ( $n = 25$ ) was  $9.3 \pm 0.2$  y (range: 7.2–10.1 y). On the basis of Philippine standards of weight-for-age, height-for-age, and weight-for-height (32), many of the children, especially those in school 2, were found to be undernourished. Stool examination for helminth eggs showed that the intensity of the parasitic infestations ranged from very light to very heavy.

#### Dietary intakes

The estimated daily intakes of selected nutrients at baseline and in the schools during the 12-wk intervention period are shown in **Table 2**.

##### School 1

The mean energy intake of 5.6 MJ (1328 kcal) by participating subjects in school 1 at baseline represented 76% and 64% of

**TABLE 1**  
Characteristics of the study participants at baseline<sup>1</sup>

	School 1 (n = 13 M, 14 F)	School 2 (n = 13 M, 12 F)
Age (y)	9.5 ± 0.4 (6.8–13.2)	9.3 ± 0.2 (7.2–10.1)
Weight (kg)	22.6 ± 1.1 (13.5–35.4)	21.3 ± 0.7 (15.7–30.8)
Height (cm)	122.7 ± 2.4 (99.5–142.8)	121.2 ± 1.6 (106.5–139.8)
Percentage underweight (%) <sup>2</sup>	41.6	56.5
Percentage stunted (%) <sup>2</sup>	29.1	39.0
Percentage with wasting (%) <sup>2</sup>	25.0	40.7
Midupper arm circumference (cm)	18.1 ± 0.3 (15.0–22.3)	17.5 ± 0.3 (15.1–20.7)
Serum retinol (µmol/L)	0.68 ± 0.03 (0.33–0.93)	0.66 ± 0.03 (0.32–0.93)
Children with abnormal CIC results (%) <sup>3</sup>	20.8	12.0
<i>Ascaris lumbricoides</i>		
Percentage with infestation (%)	44.4	82.6
Intensity of infestation (eggs/g feces)	57 164 ± 34 122 (984–291 592)	41 729 ± 19 670 (738–369 656)
<i>Trichuria</i>		
Percentage with infestation (%)	55.6	21.7
Intensity of infestation (eggs/g feces)	10920 ± 5144 (205–46084)	2214 ± 1672 (328–8897)
Hookworm		
Percentage with infestation (%)	38.9	34.8
Intensity of infestation (eggs/g feces)	3749 ± 1606 (328–12 177)	456 ± 197 (123–1804)

<sup>1</sup> $\bar{x} \pm \text{SEM}$ ; range in parentheses.

<sup>2</sup>Based on Philippine standards of weight-for-age, height-for-age, or weight-for-height (32).

<sup>3</sup>CIC, conjunctival impression cytology.

the Philippine RDA for children aged 7–9 and 10–12 y, respectively, the RDA being 1740 and 2090 kcal, respectively, for these age groups. The baseline mean protein intake of 37 g represented 106% and 82% of the RDA for these age groups, which are 35 and 45 g, respectively. The mean vitamin A intake of 374 RE preintervention represented 94% and 88% of the Philippine RDA of 400 and 425 RE for these age groups (26).

The lunch and 2 snacks eaten in school 1 daily for 5 d/wk for 12 wk provided 2258 ± 53 RE, of which 2192 ± 52 RE was derived from plant sources (based on a 6:1 conversion for

β-carotene:retinol) and 5.3 ± 0.04 MJ (1277 ± 11 kcal) from 33 ± 0.3 g fat, 37 ± 0.2 g protein, and 209 ± 2 g carbohydrates.

#### School 2

At baseline, the mean energy intake by participating subjects in school 2 represented 57% and 48%, respectively, of the Philippine energy RDA for children aged 7–9 and 10–12 y; the mean protein intake represented 86% and 67% of the protein RDA, and the mean vitamin A intake (90% of which was derived from plant sources) represented 110% and 104% of the Philippine RDA for these age

**TABLE 2**  
Daily intakes of selected nutrients in schoolchildren at baseline and amounts provided at school during the intervention<sup>1</sup>

	School 1 (n = 27)		School 2 (n = 25)	
	Baseline <sup>2</sup>	During intervention <sup>3</sup>	Baseline <sup>2</sup>	During intervention <sup>4</sup>
Energy				
(MJ)	5.6 ± 0.5	5.3 ± 0.04	4.2 ± 0.3	2.5 ± 0.05
(kcal)	1328 ± 109	1277 ± 11	996 ± 80	601 ± 11
Protein (g)	37 ± 3	37 ± 0.2	30 ± 2	9.6 ± 0.2
Fat (g)	25 ± 3	33 ± 0.3	17 ± 2	9.4 ± 0.2
Carbohydrates (g)	239 ± 24	209 ± 2	181 ± 17	119 ± 2
Vitamin A (RE)				
From plant sources	246 ± 119	2192 ± 52	395 ± 138	0
From animal sources	128 ± 26	67 ± 1	45 ± 9	0
Total	374 ± 117	2258 ± 53	440 ± 137	0
Iron (mg)	9 ± 1	16 ± 0.1	10 ± 2	4.2 ± 0.1
Ascorbic acid (mg)	44 ± 13	276 ± 1	54 ± 14	11.1 ± 0.2

<sup>1</sup> $\bar{x} \pm \text{SEM}$ . Philippine food tables (26) were used to assess dietary intakes. The vitamin A content of plant sources was calculated by using a 6:1 conversion factor for β-carotene:retinol. RE, retinol equivalents.

<sup>2</sup>Based on 24-h dietary recalls of food intakes at home.

<sup>3</sup>Based on intakes from lunch and 2 snacks (midmorning and midafternoon) 5 d/wk for 12 wk.

<sup>4</sup>Based on intakes from 2 snacks (midmorning and midafternoon) 5 d/wk for 12 wk.

**TABLE 3**Vitamin A status of schoolchildren at baseline and after 12 wk of intervention<sup>1</sup>

	School 1 (n = 27)		School 2 (n = 25)	
	Baseline	Postintervention	Baseline	Postintervention
Serum retinol ( $\mu\text{mol/L}$ )				
$\bar{x} \pm \text{SEM}$	0.68 $\pm$ 0.03	1.06 $\pm$ 0.04 <sup>2</sup>	0.66 $\pm$ 0.03	0.86 $\pm$ 0.05 <sup>2</sup>
Median	0.71	1.08	0.68	0.78
Range	0.33–0.93	0.59–1.79	0.32–0.93	0.56–1.41
Serum D:H <sup>3</sup>				
$\bar{x} \pm \text{SEM}$	0.663 $\pm$ 0.130	0.299 $\pm$ 0.026 <sup>4</sup>	1.051 $\pm$ 0.143	0.546 $\pm$ 0.066 <sup>5</sup>
Median	0.434	0.269	0.788	0.477
Range	0.115–2.896	0.089–0.578	0.145–3.337	0.150–1.642

<sup>1</sup>The children in school 1 were treated with an anthelmintic drug 1–2 wk before being fed a carotene-rich lunch and 2 snacks with  $\approx$ 2258 retinol equivalents and  $\approx$ 5.3 MJ (33 g fat, 37 g protein, and 209 g carbohydrates) for 5 d/wk for 12 wk. The children in school 2 were treated with an anthelmintic drug and fed 2 snacks with  $\approx$ 2.5 MJ (9.4 g fat, 9.6 g protein, and 119 g carbohydrates) but no vitamin A or carotenes for 5 d/wk for 12 wk.

<sup>2,4,5</sup>Significantly different from baseline (Student's paired *t* test): <sup>2</sup>*P* = 0.0001, <sup>4</sup>*P* = 0.004, <sup>5</sup>*P* = 0.001.

<sup>3</sup>Ratio of deuterated to nondeuterated retinol in serum 3 d after an oral dose of 5 mg [<sup>2</sup>H<sub>4</sub>]retinyl acetate at baseline or 5 mg [<sup>2</sup>H<sub>8</sub>]retinyl acetate postintervention.

groups. The 2 daily snacks provided in the school at midmorning and midafternoon for 5 d/wk for 12 wk contained no sources of vitamin A or carotenes; they provided 2.5  $\pm$  0.05 MJ (601  $\pm$  11 kcal) from 9.4  $\pm$  0.2 g fat, 9.6  $\pm$  0.2 g protein, and 119  $\pm$  2 g carbohydrates.

### Analyses before and after intervention

#### Serum retinol

At baseline, the mean serum retinol concentration in school 1 was low: 0.68  $\pm$  0.03  $\mu\text{mol/L}$ ; after 12 wk, the value increased by 55.9% to 1.06  $\pm$  0.04  $\mu\text{mol/L}$  and the median value rose from 0.71 to 1.08  $\mu\text{mol/L}$  (by 52.1%) (Table 3). In school 2, baseline serum retinol was 0.66  $\pm$  0.03  $\mu\text{mol/L}$ ; after 12 wk, the mean value increased by 30.3% to 0.86  $\pm$  0.05  $\mu\text{mol/L}$ ; the median value rose from 0.68 to 0.78  $\mu\text{mol/L}$  (by 14.7%).

At baseline, 48% of participating children in school 1 had serum retinol concentrations that were  $<0.70 \mu\text{mol/L}$  ( $<20 \mu\text{g/dL}$ ); postintervention, there were 4% (Figure 1). At baseline, 89% of the children had serum retinol values that were  $<0.87 \mu\text{mol/L}$  ( $<25 \mu\text{g/dL}$ ); postintervention, this percentage was 19%. At baseline, 100% of the children had serum retinol values that were  $<1.05 \mu\text{mol/L}$  ( $<30 \mu\text{g/dL}$ ); postintervention, the corresponding percentage was 44%.

At baseline, 52% of participating children in school 2 had serum retinol values that were  $<0.70 \mu\text{mol/L}$ ; postintervention, this percentage was 24% (Figure 1). At baseline, 92% of the children had serum retinol values that were  $<0.87 \mu\text{mol/L}$ ; postintervention, the corresponding percentage was 64%. At baseline, 100% of the children had serum retinol values that were  $<1.05 \mu\text{mol/L}$ ; postintervention, the corresponding percentage was 84%.

#### Three-day deuterated-retinol-dilution procedure

In school 1 at baseline, D:H retinol was 0.663  $\pm$  0.130 after isotope administration; postintervention, this value was 0.299  $\pm$  0.026 (Table 3). The 55% decrease in D:H retinol was significant and represented an improvement in total-body vitamin A stores. In school 2 at baseline, D:H retinol was 1.051  $\pm$  0.143 three days after isotope administration; postintervention, this value was 0.546  $\pm$  0.066; the 48% decrease in D:H retinol was significant.

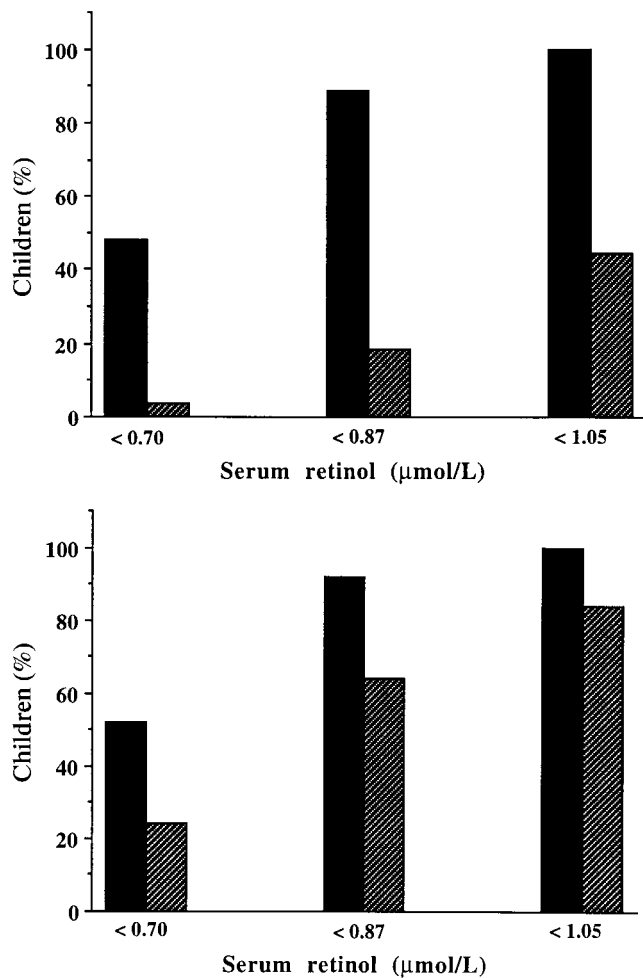
#### Serum carotenoids

In school 1, except for lutein and zeaxanthin, there were significant increases from baseline in serum carotenoids after 12 wk

of intervention (Table 4). Increases in *all-trans*- $\beta$ -,  $\alpha$ -, and 13-*cis*- $\beta$ -carotene were 5.2-, 7.5-, and 4.0-fold, respectively; increases in  $\beta$ - and  $\alpha$ -cryptoxanthin were 3.2- and 3.0-fold, respectively; lycopene was increased by 5-fold. In school 2, there were small but significant increases in all serum carotenoids after 12 wk. Increases in  $\beta$ -,  $\alpha$ -, and 13-*cis*- $\beta$ -carotene were 1.8-fold, 2.0-fold, and 1.2-fold, respectively; the increase in  $\beta$ -cryptoxanthin was 2.5-fold and in  $\alpha$ -cryptoxanthin, 1.5-fold; the increase in lutein and zeaxanthin was 1.4-fold and in lycopene was 3.3-fold.

#### Other blood measurements

In school 1 there was an improvement in blood hemoglobin concentration postintervention, a decrease in serum ferritin, no change in serum transferrin receptor and albumin, and increases in serum transthyretin and  $\alpha_1$ -antitrypsin (Table 5). The decrease in serum ferritin may have been a reflection of decreased liver stores as a result of improved vitamin A status. It has been postulated that vitamin A is needed for the mobilization of iron from the liver to the hematopoietic sites (33) via mechanisms that are yet to be elucidated. Many studies showed a positive association between changes in vitamin A status and iron status after food fortification (34) or supplementation with vitamin A (35). In the present study, because dietary iron and vitamin C also improved with food intervention, the rise in hemoglobin could not be ascribed to an improvement in vitamin A status alone. Serum albumin concentrations were within the normal range of 38–54 g/L for children in this age range (36) and did not change with food intervention. An improvement in mean serum transthyretin, however, was observed, from 154 to 180 mg/L. Transthyretin is considered to be a sensitive measure of nutritional assessment; it is more rapidly responsive to changes in nutritional status than is albumin (36). Although an increase in  $\alpha_1$ -antitrypsin, a positive acute-phase protein, was observed, the values were within the normal range; the observed increase may have been a response to increased protein intakes. The concentrations of C-reactive protein, another acute-phase protein measured, were normal both pre- and postintervention (data not shown because the method used did not differentiate values  $<60 \text{ mg/L}$ ). Thus, infections and inflammations were not confounding factors during the study.



**FIGURE 1.** Percentage of children with serum retinol <0.70 (poor), <0.87, and <1.05 (poor to marginal)  $\mu\text{mol/L}$  at baseline (black bars) and postintervention (shaded bars) in school 1 (top) and school 2 (bottom). Baseline versus postintervention comparisons within schools were significant (school 1,  $P = 0.02$ ; school 2,  $P = 0.03$ ). In school 1, the children were treated with an anthelmintic drug 1–2 wk before being fed a carotene-rich lunch and 2 snacks with 2258 retinol equivalents and 5.3 MJ (33 g fat, 37 g protein, and 209 g carbohydrates) daily for 5 d/wk for 12 wk. In school 2, the children were treated with an anthelmintic drug and fed 2 snacks with 2.5 MJ (9.4 g fat, 9.6 g protein, and 119 g carbohydrates) but no vitamin A or carotenes for 5 d/wk for 12 wk.

In school 2, there was a slight decrease in blood hemoglobin postintervention, possibly as a result of decreased intake of vitamin C; no change in serum ferritin and transferrin receptor; and a slight decrease in serum albumin. As in school 1, increases in serum transthyretin and  $\alpha_1$ -antitrypsin were noted, possibly in response to improved dietary protein; serum C-reactive protein was normal in all participating children both pre- and postintervention.

#### Residual [ $^2\text{H}_4$ ]-retinol

GC-MS analyses of serum samples obtained immediately after the 12-wk study period (before administration of [ $^2\text{H}_8$ ]retinyl acetate) showed that the percentage enrichment of serum retinol with [ $^2\text{H}_4$ ]retinol was  $2.7 \pm 0.3\%$  in school 1 and  $5.5 \pm 0.5\%$  in school 2.

This represents residual [ $^2\text{H}_4$ ]retinol in serum at 88 d after ingestion of 5 mg [ $^2\text{H}_4$ ]retinyl acetate; the lower amounts in school 1 subjects indicate a better vitamin A status than in school 2 subjects.

#### Relation between serum retinol at baseline and change in serum retinol

In school 1 an inverse correlation was found between serum retinol values at baseline and improvement in serum retinol after 12 wk of food intervention (**Figure 2**). In school 2, no correlation between these variables was found (**Figure 3**).

#### Relation between 3-d D:H retinol at baseline and change in 3-d D:H retinol

In school 1 (**Figure 2**) and school 2 (**Figure 3**), a strong inverse correlation was found between D:H retinol at baseline and changes in this measure after 12 wk of intervention; the higher D:H retinol (ie, poorer total-body vitamin A stores) at baseline, the greater the decrease in D:H retinol (ie, improvement in total-body vitamin A stores) postintervention.

#### Other regression analyses

No correlation was found between change in serum retinol and change in 3-d D:H retinol in response to the interventions in the 2 schools. Improvement in serum  $\beta$ -carotene was unrelated to baseline  $\beta$ -carotene, baseline vitamin A status (serum retinol or total body stores of vitamin A), or improvement in vitamin A status, a finding that supports the notion that serum  $\beta$ -carotene is not indicative of vitamin A status (18).

#### Relation of serum retinol and 3-d D:H retinol

D:H retinol 3 d after an oral dose of [ $^2\text{H}_4$ ]- or [ $^2\text{H}_8$ ]retinyl acetate was inversely correlated with serum retinol (**Figure 4**). The relation was nonlinear and was stronger with poorer vitamin A status, ie, stronger when serum retinol was <0.70  $\mu\text{mol/L}$  than when serum retinol was >0.70  $\mu\text{mol/L}$ . This finding agrees with the fact that serum retinol is under homeostatic control and tends to fall only when liver stores are low (18); it also supports the notion that D:H retinol 3 d after administration of deuterated vitamin A reflects total-body vitamin A stores.

## DISCUSSION

We reported earlier (22) that in healthy elderly subjects, D:H retinol 3 d after administration of deuterated retinyl acetate correlated well with estimates of total-body vitamin A stores derived by using the longer DRD procedure of Furr et al (19). In the present study, we evaluated the response of the 3-d procedure to changes in the body pool size of vitamin A after food intervention. Not only was a decrease in D:H retinol (ie, improvement in vitamin A status) observed postintervention, but also the magnitude of the decrease was correlated with the baseline measure; the higher the D:H retinol at baseline (ie, the poorer the vitamin A status), the greater the reduction in serum D:H retinol after intervention ( $r = -0.99$ ,  $P = 0.0001$ ). Total-body vitamin A stores (as measured by 3-d DRD) was an excellent predictor, whereas serum retinol was a poor predictor, of the subject's responses to interventions aimed at improving vitamin A status. The results of animal studies indicate that a feedback mechanism increases intestinal  $\beta$ -carotene 15,15'-dioxygenase activity when tissue vitamin A concentrations are low (11–13).

TABLE 4

Serum carotenoid concentrations of schoolchildren at baseline and after 12 wk of intervention<sup>1</sup>

	School 1 (n = 27)			School 2 (n = 25)		
	Baseline	Postintervention	Magnitude of increase	Baseline	Postintervention	Magnitude of increase
	$\mu\text{mol/L}$			$\mu\text{mol/L}$		
<i>all-trans</i> - $\beta$ -carotene						
$\bar{x} \pm \text{SEM}$	0.12 $\pm$ 0.02	0.62 $\pm$ 0.04 <sup>2</sup>	5.2	0.06 $\pm$ 0.01	0.11 $\pm$ 0.01 <sup>2</sup>	1.8
Median	0.11	0.54		0.05	0.10	
Range	0.01–0.56	0.38–0.98		0.01–0.17	0.03–0.24	
$\alpha$ -Carotene						
$\bar{x} \pm \text{SEM}$	0.02 $\pm$ 0.004	0.15 $\pm$ 0.01 <sup>2</sup>	7.5	0.01 $\pm$ 0.001	0.02 $\pm$ 0.002 <sup>2</sup>	2.0
Median	0.01	0.14		0.01	0.02	
Range	0.001–0.06	0.001–0.27		0.001–0.04	0.004–0.04	
13- <i>cis</i> - $\beta$ -carotene						
$\bar{x} \pm \text{SEM}$	0.007 $\pm$ 0.001	0.035 $\pm$ 0.002 <sup>2</sup>	5.0	0.006 $\pm$ 0.001	0.007 $\pm$ 0.001 <sup>3</sup>	1.2
Median	0.01	0.03		0.004	0.007	
Range	0–0.03	0.01–0.07		0.001–0.01	0.002–0.08	
$\beta$ -Cryptoxanthin						
$\bar{x} \pm \text{SEM}$	0.06 $\pm$ 0.01	0.19 $\pm$ 0.01 <sup>2</sup>	3.2	0.04 $\pm$ 0.01	0.10 $\pm$ 0.01 <sup>4</sup>	2.5
Median	0.04	0.17		0.03	0.09	
Range	0.002–0.25	0.10–0.30		0.004–0.18	0.006–0.29	
$\alpha$ -Cryptoxanthin						
$\bar{x} \pm \text{SEM}$	0.02 $\pm$ 0.004	0.06 $\pm$ 0.004 <sup>2</sup>	3.0	0.02 $\pm$ 0.002	0.03 $\pm$ 0.004 <sup>5</sup>	1.5
Median	0.02	0.05		0.01	0.03	
Range	0.002–0.07	0.03–0.11		0.002–0.04	0.009–0.08	
Lutein and zeaxanthin						
$\bar{x} \pm \text{SEM}$	0.50 $\pm$ 0.11	0.47 $\pm$ 0.05	0.9	0.21 $\pm$ 0.02	0.29 $\pm$ 0.03 <sup>6</sup>	1.4
Median	0.37	0.42		0.19	0.25	
Range	0.02–2.69	0.19–0.98		0.01–0.50	0.03–0.72	
Lycopene						
$\bar{x} \pm \text{SEM}$	0.05 $\pm$ 0.01	0.25 $\pm$ 0.02 <sup>2</sup>	5.0	0.03 $\pm$ 0.01	0.10 $\pm$ 0.01 <sup>2</sup>	3.3
Median	0.05	0.23		0.02	0.09	
Range	0.003–0.25	0.08–0.45		0.01–0.21	0.02–0.28	

<sup>1</sup>The children in school 1 were treated with an anthelmintic drug 1–2 wk before being fed a carotene-rich lunch and 2 snacks with  $\approx$ 2258 retinol equivalents and  $\approx$ 5.3 MJ (33 g fat, 37 g protein, and 209 g carbohydrates) for 5 d/wk for 12 wk. The children in school 2 were treated with an anthelmintic drug and fed 2 snacks with  $\approx$ 2.5 MJ (9.4 g fat, 9.6 g protein, and 119 g carbohydrates) but no vitamin A or carotenes for 5 d/wk for 12 wk.

<sup>2–6</sup>Significantly different from baseline (Student's paired *t* test): <sup>2</sup>*P* = 0.0001, <sup>3</sup>*P* = 0.05, <sup>4</sup>*P* = 0.0004, <sup>5</sup>*P* = 0.0002, <sup>6</sup>*P* = 0.01.

In this study, we observed an inverse relation between serum retinol concentrations and D:H retinol 3 d after administration of deuterated retinyl acetate. The correlation was not linear and was stronger when serum retinol was  $<0.70 \mu\text{mol/L}$ . This observation agrees with the fact that serum retinol is under homeostatic control and tends to fall only when liver stores are low (18). The notion that D:H retinol 3 d after isotope administration is a useful indicator of total-body vitamin A stores is consistent with data obtained by Green et al (37–39) in kinetic studies in rats. These authors showed that, in contrast with the isotope-dilution methods described by Bausch and Rietz (20) and Furr et al (19), it is possible to predict liver vitamin A stores over a wide range of liver vitamin A values without requiring an estimate of the efficiency of absorption and liver retention of the test dose of labeled vitamin A, the unity correlation between plasma and liver specific activities, equilibration of the test dose with body stores, or metabolism of the test dose between the time of administration and blood sampling, because all these physiologic and metabolic processes are empirically reflected in their prediction equations (37).

The results in school 1 show that it is possible to obtain a substantial improvement in the vitamin A status of malnourished children by treatment with an anthelmintic drug, followed by increased intakes of locally available carotene-rich plant foods in

a meal and snacks that provide increased energy from fat, protein, and carbohydrates. Recently, there has been renewed discussion about the effectiveness of plant foods for improving vitamin A status. Many studies showed that consumption of carotene-rich plant foods can improve serum retinol concentrations (3–6, 16, 40, 41); other studies, however, showed either no effect of plant foods on serum retinol (42–47) or only small improvements that were much less than predicted on the basis of conventional carotenoid conversion factors (1). The lack of effect of plant carotene ingestion on serum retinol found by some investigators may have been because the subjects had a satisfactory vitamin A status. In general, in studies that showed no improvement in serum retinol with increased fruit and vegetable intakes, the baseline serum retinol of subjects was higher [baseline mean values in  $\mu\text{mol/L}$ : 2.31 (43), 2.28 (42), 1.79 (47), 1.45 (44), 1.26 (45), and 0.89 (46)] than at baseline in studies in which improvements in serum retinol were found [baseline mean values in  $\mu\text{mol/L}$ : 0.50 (4), 0.54 (16), 0.58 (6), 0.59 (5), 0.65 (40), 0.71 (1), 0.73 (41), and 1.08 (3)].

In the present study in school 1, mean serum retinol at baseline was low (0.68  $\mu\text{mol/L}$ ); the intervention raised serum retinol to an adequate value of 1.06  $\mu\text{mol/L}$ , an increase of 0.38  $\mu\text{mol/L}$  or 56%; this was accompanied by 5- and 8-fold increases

TABLE 5

Concentrations of blood hemoglobin, serum ferritin, transferrin receptor, albumin, transthyretin, and  $\alpha_1$ -antitrypsin in schoolchildren at baseline and after 12 wk of intervention<sup>1</sup>

	School 1 (n = 27)		School 2 (n = 25)	
	Baseline	Postintervention	Baseline	Postintervention
Hemoglobin (g/L)	129 ± 2	133 ± 2 <sup>2</sup>	123 ± 2	117 ± 1 <sup>3</sup>
Ferritin (μg/L)	27.2 ± 5.3	18.5 ± 3.6 <sup>4</sup>	15.6 ± 4.8	18.7 ± 4.3
Transferrin receptor (mg/L)	6.5 ± 0.3	6.5 ± 0.3	6.4 ± 0.4	6.2 ± 0.4
Albumin (g/L)	46.3 ± 0.5	46.6 ± 0.5	47.3 ± 0.6	46.2 ± 0.4 <sup>5</sup>
Transthyretin (mg/L)	154 ± 5	180 ± 5 <sup>3</sup>	147 ± 6	173 ± 7 <sup>2</sup>
$\alpha_1$ -Antitrypsin (g/L)	1.13 ± 0.04	1.49 ± 0.03 <sup>6</sup>	1.18 ± 0.02	1.52 ± 0.03 <sup>6</sup>

<sup>1</sup>The children in school 1 were treated with an anthelmintic drug 1–2 wk before being fed a carotene-rich lunch and 2 snacks with ≈2258 retinol equivalents and ≈5.3 MJ (33 g fat, 37 g protein, and 209 g carbohydrates) for 5 d/wk for 12 wk. The children in school 2 were treated with an anthelmintic drug and fed 2 snacks with ≈2.5 MJ (9.4 g fat, 9.6 g protein, and 119 g carbohydrates) but no vitamin A or carotenes for 5 d/wk for 12 wk.

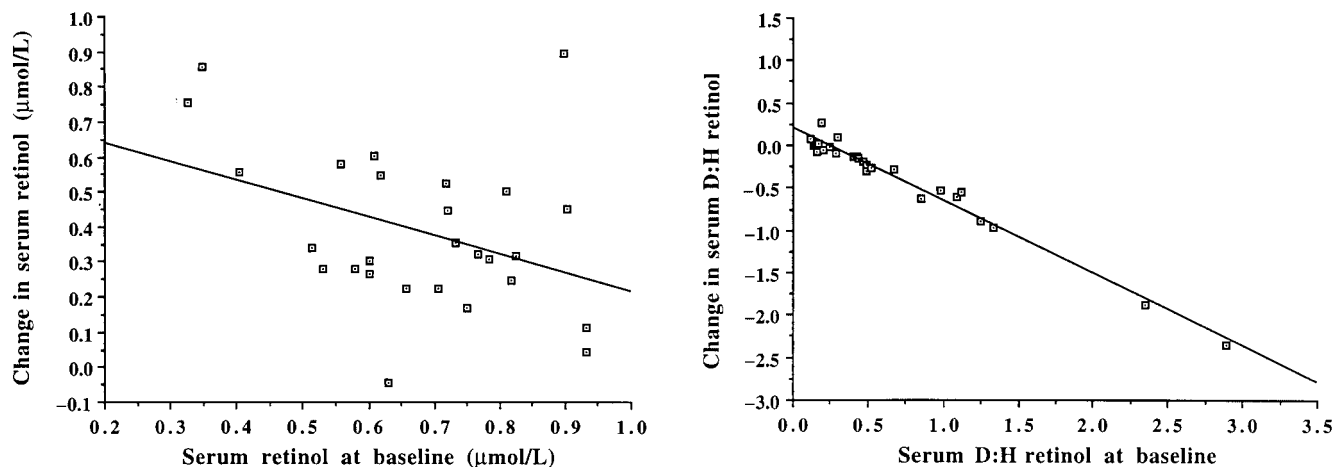
<sup>2–6</sup>Significantly different from baseline (Student's paired *t* test): <sup>2</sup>*P* = 0.002, <sup>3</sup>*P* = 0.001, <sup>4</sup>*P* = 0.01, <sup>5</sup>*P* = 0.05, <sup>6</sup>*P* = 0.0001.

in serum  $\beta$ - and  $\alpha$ -carotene and a 3-fold increase in  $\beta$ -cryptoxanthin. These favorable effects on vitamin A status agree with those reported by Jalal et al (5), who supplemented basic diets (containing ≈197 RE and ≈12.2 g fat) of 3–6 y-old vitamin A-malnourished Indonesian children with carotene-rich foods (750 RE/d, mainly from red sweet potatoes) plus 15 g fat/d for 6 d/wk for 3 wk. They found that in subjects with a mild *Ascaris lumbricoides* infection, mean serum retinol increased by 0.54 μmol/L, whereas in subjects with high fecal egg counts, the increase in mean serum retinol was less (0.23 μmol/L).

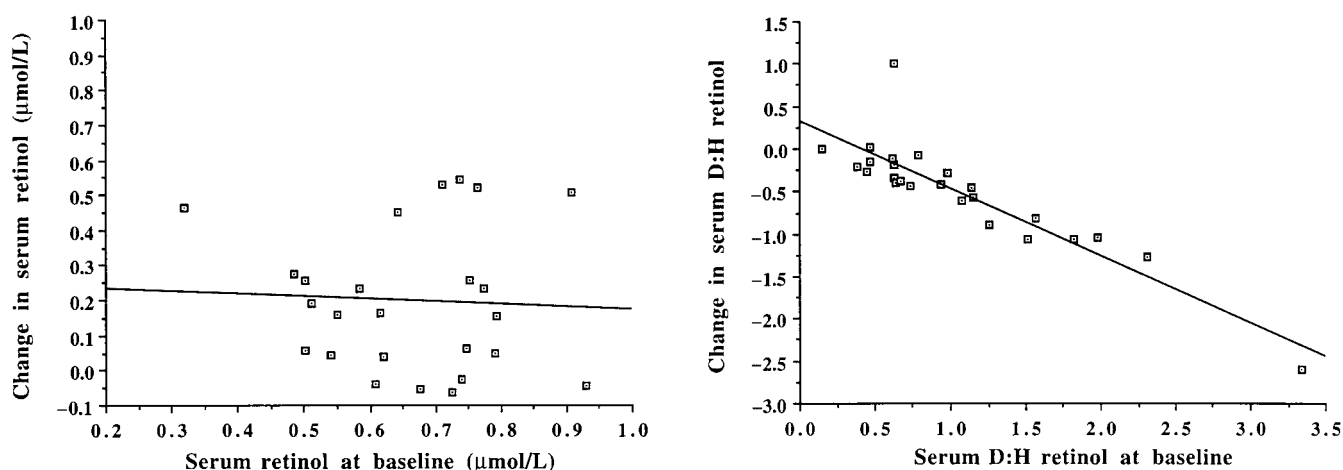
The improvement in serum retinol (by 0.20 μmol/L) in school 2 subjects may have been due to improved absorption and utilization of plant carotenes in the children's usual home diets. The improvement may have been brought about by decreased intestinal parasitic load (although this was not verified after anthelmintic drug treatment) and increased dietary fat provided in the mid-morning and midafternoon snacks at school. Some fat may have remained in the gastrointestinal tract long enough to aid in the absorption of carotenes provided by usual home diets 2–3 h later

at lunch and supper. Jalal et al (5) similarly reported an increase in serum retinol (by 0.17 μmol/L) in Indonesian children who were treated with an anthelmintic drug and provided with supplemental fat (but no carotenes) in a basic meal for 3 wk. An improvement in the protein intakes of school 2 subjects also may have contributed to the improvement in their vitamin A status. In rats fed a low-protein diet, carotene absorption and conversion to vitamin A were reputed to be impaired, resulting in lower newly formed vitamin A in intestine, liver, and serum than in rats fed adequate protein (7, 8). Furthermore, in 2-y-old Egyptian children with protein-energy malnutrition, treatment with energy and proteins without supplemental vitamin A for 4 wk improved not only serum retinol binding protein and transthyretin but also serum vitamin A (9). It is also possible that, in our study, with the knowledge that their children were participating in a vitamin A study, the parents of children in school 2 may have provided more dietary retinol or carotenes in their homes.

The decrease in D:H retinol in school 1 subjects after the intervention was 55%; in school 2 subjects, the corresponding percent-

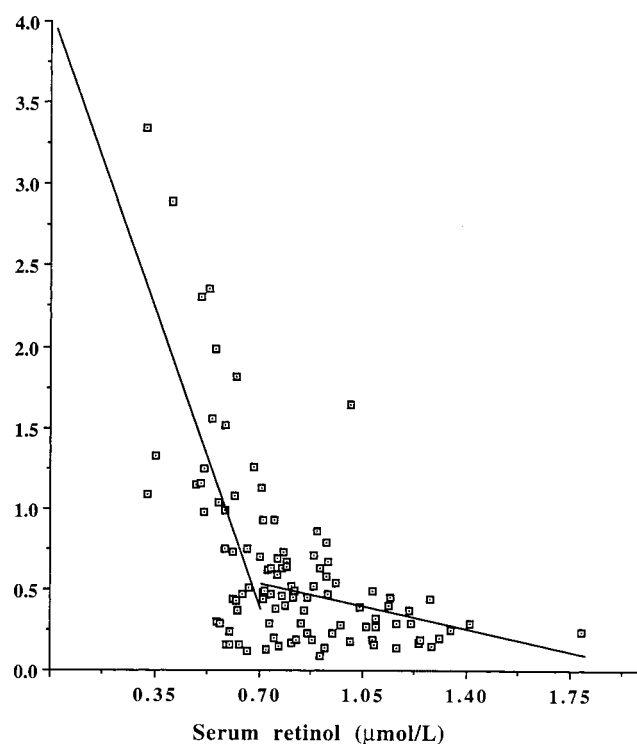


**FIGURE 2.** Correlation between serum retinol at baseline and change in serum retinol postintervention ( $r = -0.38$ ,  $P = 0.048$ ) and between the ratio of deuterated to nondeuterated retinol in serum (D:H retinol) at baseline and change in D:H retinol postintervention ( $r = -0.99$ ,  $P = 0.0001$ ) in the children in school 1. D:H retinol was obtained 3 d after an oral dose of 5 mg [<sup>2</sup>H<sub>4</sub>]retinyl acetate at baseline or 5 mg [<sup>2</sup>H<sub>3</sub>]retinyl acetate postintervention. The change in D:H retinol is the value postintervention minus the value at baseline. A high D:H retinol denotes poor total-body vitamin A stores; a decrease in D:H retinol denotes an improvement in vitamin A body stores. The children in school 1 were treated with an anthelmintic drug 1–2 wk before being fed a carotene-rich lunch and 2 snacks with 2258 retinol equivalents and 5.3 MJ (33 g fat, 37 g protein, and 209 g carbohydrates) daily for 5 d/wk for 12 wk.




**FIGURE 3.** Correlation between serum retinol at baseline and change in serum retinol postintervention ( $r = -0.05$ ) and between the ratio of deuterated to nondeuterated retinol in serum (D:H retinol) at baseline and change in D:H retinol postintervention ( $r = -0.89$ ,  $P = 0.0001$ ) in the children in school 2. D:H retinol was obtained 3 d after an oral dose of 5 mg [ $^2\text{H}_4$ ]retinyl acetate at baseline or 5 mg [ $^2\text{H}_8$ ]retinyl acetate postintervention. The change in D:H retinol is the value postintervention minus the value at baseline. A high D:H retinol denotes poor total-body vitamin A stores; a decrease in D:H retinol denotes an improvement in vitamin A body stores. The children in school 2 were treated with an anthelmintic drug 1–2 wk before being fed 2 snacks with 2.5 MJ (9.4 g fat, 9.6 g protein, and 119 g carbohydrates) but no vitamin A or carotenes for 5 d/wk for 12 wk.

age was 48%; the corresponding improvements in mean serum retinol were 56% and 30%, respectively. Although the reduction in D:H retinol was smaller in school 2 than in school 1, it was substantial and significant nevertheless. However, interpretation of these results should take into consideration that the vitamin A statuses of the children in the 2 communities were not similar at baseline. Although there were no significant differences in their mean serum retinol concentrations at baseline, the study participants in the community of Hukay (school 2) had higher mean D:H retinol at baseline (denoting lower total-body vitamin A stores) than did the children in the community of Santa Elena (school 1). In studies in rats, Adams and Green (37) showed that 3–6 d after oral administration of [ $^3\text{H}$ ]retinol the relation between liver total retinol and the fraction of the oral dose of [ $^3\text{H}$ ]retinol in plasma was nonlinear, so that when liver stores of vitamin A were low, appreciable decreases in plasma [ $^3\text{H}$ ]retinol did not correspond to appreciable increases in liver total retinol. In humans, a similar possible nonlinear relation between total body stores of vitamin A and D:H retinol 3 d after isotope administration was suggested in our previous study in older adults whereby one outlier who had a high D:H retinol did not fit in the linear regression analysis (22). Thus, it may be that in children in school 2, the observed reduction in D:H retinol postintervention reflected improved circulating retinol concentrations, although not necessarily improved liver stores, because when there is a vitamin A deficiency, newly ingested retinol molecules (from dietary carotenes or from preformed vitamin A) may not be stored in hepatic tissues but circulate to target tissues that require vitamin A. In school 2, improvement in vitamin A status was likewise strongly influenced by baseline D:H retinol; thus, the finding was consistent in both schools. The weak relation between baseline serum retinol and improvement in serum retinol postintervention that was seen in school 1 was not observed in school 2. We conclude that the response in humans to interventions for improving vitamin A status is strongly influenced by total body stores of vitamin A and is influenced little or not at all by serum retinol.



**FIGURE 4.** Correlation between serum retinol and the ratio of deuterated to nondeuterated retinol in serum (D:H retinol) 3 d after an oral dose of 5 mg [ $^2\text{H}_4$ ]retinyl acetate or [ $^2\text{H}_8$ ]retinyl acetate. Pooled data at baseline and postintervention from the children in schools 1 and 2 were used. For all values ( $n = 104$ ),  $r = -0.54$ ,  $P = 0.0001$ ; for serum retinol  $< 0.70$   $\mu\text{mol/L}$  ( $n = 35$ ),  $r = -0.59$ ,  $P = 0.0002$ ; for serum retinol  $\geq 0.70$   $\mu\text{mol/L}$  ( $n = 69$ ),  $r = -0.33$ ,  $P = 0.005$ .

Controlled studies using isotope-dilution techniques are needed to determine the specific contributions of different sources of dietary carotenoids (eg, green compared with yellow fruit and vegetables), fat and protein intakes, and deworming to improvements in total body stores of vitamin A. Furthermore, although D:H retinol 3 d after an oral dose of deuterated retinyl acetate is a promising measure of total body stores of vitamin A, this ratio gives only relative measures of total body stores of vitamin A; work is now needed to develop an equation to predict the amounts of total-body vitamin A stores in children and adults with the use of 3-d DRD data. 

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