Assessment of total body stores of vitamin A in Guatemalan elderly by the deuterated-retinol-dilution method[1–4]

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ABSTRACT

Background: Deuterated retinol dilution (DRD) gives quantitative estimates of total body stores of vitamin A.

Objectives: In elderly people, we studied *1*) the time when an oral dose of deuterated vitamin A equilibrates with body stores, *2*) whether serum ratios of deuterated to nondeuterated retinol (D:H) at 3 or 6 d postdosing predicted body stores, and *3*) the ability of DRD to detect changes in the size of the body vitamin A pool.

Design: A 10-mg oral dose of [$^2H_4$]retinyl acetate was administered to 60–81-y-old Guatemalans (n = 47); percentage enrichment of serum retinol with deuterated retinol was determined at 1–3 time points per subject at 3, 6, 7, 14, 20, 21, and 54 d. In subjects from whom blood was obtained at 3 and 21 d (n = 15) and at 6 and 20 d (n = 9), total body stores were calculated by using the formula of Furr et al (Am J Clin Nutr 1989;49:713–6) with 21- or 20-d data and correlated with serum D:H at 3 or 6 d postdosing. Nine subjects received diets containing 982 ± 20 μg RE ($\bar{x}$ ± SEM) plus 800 μg RE as retinyl acetate supplements for 32 d. DRD, serum retinol, and relative dose response were used to assess vitamin A status before and after the intervention.

Results: Deuterated retinol equilibrated with the body pool by 20 d postdosing. Vitamin A supplementation for 32 d increased body stores, although unexplained exaggerated increases were seen in some subjects. An inverse linear relation was found between estimates of body stores and serum D:H at 3 d postdosing (r = −0.75, P = 0.002); at 6 d postdosing, the correlation was weaker.

Conclusions: DRD can detect changes in total body stores of vitamin A, although factors affecting serum D:H need to be elucidated. Serum D:H 3 d postdosing might be used as an early indicator of total body stores of vitamin A, although a predictive equation will need to be developed. *Am J Clin Nutr* 1999;69:278–84.

KEY WORDS Vitamin A, deuterated retinol dilution, stable isotope dilution, body stores, elderly, retinol, relative dose response, Guatemala

INTRODUCTION

The liver is the main storage organ for vitamin A in humans (1); thus, the best way to assess vitamin A status is to measure hepatic stores. Because direct measurement of hepatic vitamin A is not feasible under normal circumstances, various indirect methods are used to assess vitamin A status (2). Among these, the only method that gives a quantitative measure of total body stores is an isotope dilution procedure that involves *1*) administration of an oral dose of deuterated vitamin A, *2*) determination of isotopic ratios of retinol in serum after the isotope has equilibrated with the body's vitamin A pool, and *3*) application of the mathematical formula of Furr et al (3) to calculate total body stores of vitamin A. This formula, which is a modification of the formula developed by Bausch and Rietz (4), has been used successfully in adults (3, 5). The deuterated-retinol-dilution (DRD) technique for assessing body stores was validated by Furr et al (3) in generally healthy, adult American surgical patients and by Haskell et al (5) in adult Bangladeshi surgical patients with low to adequate vitamin A status. In these 2 studies, generally good agreement was found between the calculated values and values obtained directly through liver biopsies; the correlation coefficients were 0.88 (3) and 0.75 (5).

To use the prediction model described by Furr et al (3), blood should be drawn for determination of isotopic ratios of serum retinol after the administered isotope has mixed with the body's vitamin A pool. For young adults in the United States and Bangladesh, Haskell et al (6) determined the equilibration time to be 17.5 and 16.3 d, respectively; for a US child, the equilibration time is 14 d. The equilibration time in elderly subjects has not been studied previously.

See corresponding editorial on page 177.

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Our study examined the use of the DRD method for assessing the vitamin A status of elderly persons and the ability of the method to detect changes in the size of the body vitamin A pool after supplementation. Our goals were to *1*) determine the time when an oral dose of [$^2H_4$]retinyl acetate equilibrates with the body's vitamin A pool in elderly people, *2*) determine whether isotopic ratios of serum retinol at time points earlier than equilibration (eg, 3 or 6 d postdosing) could predict total body stores of vitamin A, *3*) study the response of measures of vitamin A status [eg, DRD, serum retinol concentrations, and percentage relative dose response (RDR)] to the provision of vitamin A supplements and controlled diets for ≈1 mo, and *4*) correlate the calculated values of total body stores of vitamin A with serum retinol concentrations and percentage RDR. The study was done in Guatemala, where 1989 surveys documented a 21% prevalence of submarginal vitamin A status among rural, elderly Guatemalans (7).

SUBJECTS AND METHODS

Subjects

Elderly Guatemalan men and women (≥60 y old) residing in rural communities along the periphery of Guatemala City were screened before admission into these studies. The subjects were in general good health with no febrile or infectious illness, no prolonged gastrointestinal disorders resulting in malabsorption and diarrhea, and no history of liver or kidney disease. They did not take vitamin supplements or antacids and were not chronic alcoholics or smokers. Of 85 subjects enrolled for measurement of vitamin A and carotenoid status (the results of which will be presented in a separate paper), a subgroup of 47 were recruited to participate in the isotope dilution procedures described here. Informed, written consent was obtained from subjects. Approval to conduct these studies was obtained from the Committee for Human Studies of CeSSIAM and from the Tufts University Human Investigation Review Committee.

Relative dose response

In the standard RDR procedure, serum retinol concentrations are measured 0 and 5 h after the administration of an oral dose of vitamin A (8). Studies in older Guatemalans, however, have shown that among those with abnormal responses, the maximum plasma retinol response to 480 μg retinol equivalents (RE) retinyl palmitate occurs 6 or 7 h after dosing (9). In our studies, baseline venous blood was obtained after a 12-h overnight fast. The subjects then ingested a capsule containing 480 μg RE (as retinyl acetate in corn oil) with a breakfast low in vitamin A and containing 12.5 g fat; the breakfast consisted of fried black beans, bread, and coffee sweetened with nonfortified brown sugar. A second blood sample was obtained 7 h after dosing. No food or drink other than water was ingested after breakfast until the test was completed. Percentage RDR is equal to the difference in serum retinol concentrations at 7 h and baseline, times 100, and divided by the value at 7 h. A response ≥14% was considered abnormal (8).

Blood handling and serum biochemistry

All blood was handled under minimal or red light to protect light-sensitive compounds from degradation. Venous blood from fasting subjects was drawn into a light-protected (ie, wrapped in aluminum foil) tube, allowed to clot in a dark place, cooled at 5°C so that the clot would shrink, and centrifuged for 15 min at 2800 × *g* at 5°C. Serum (portions of 0.5–1 mL) was pipetted into cryovials stored at −70°C at CeSSIAM in Guatemala until carried by hand under dry ice to Tufts University, Boston, where they were kept at −70°C until analyzed.

Serum retinol and carotenoids were analyzed under red light by gradient, reversed-phase HPLC (10) with retinyl acetate and echinenone as internal standards. C-reactive protein, ceruloplasmin, and α_1-antitrypsin were assayed by immunoprecipitation with the SPQ antibody reagent set II (Atlantic Antibodies, Stillwater, MN); albumin was assayed with Roche reagent for albumin (Roche Diagnostic Systems, Inc, Somerville, NJ).

Vitamin A isotopes

Tetradeuterated vitamin A, ie, *all-trans*-retinyl-10,19,19,19-[$^2H_4$]acetate, and octadeuterated vitamin A, ie, *all-trans*-retinyl-10,14,19,19,19,20,20,20-[$^2H_8$]acetate, were synthesized by Cambridge Isotope Laboratories (Andover, MA). We prepared capsules containing 5.0-mg amounts (15.04 μmol [$^2H_4$]retinyl acetate or 14.86 μmol [$^2H_8$]retinyl acetate) of these isotopes dissolved in corn oil. Because these compounds would not dissolve directly in corn oil, weighed amounts of isotope were first dissolved in absolute ethanol by sonication for ≈30 min; a predetermined amount of corn oil was then added to the flask of isotope and ethanol on a weighing scale. Ethanol was removed by evaporation under nitrogen for 3 h; residual ethanol was removed under a vacuum for 16 h. Amounts of corn oil containing 5.0 mg dissolved deuterated retinyl acetate (4.36 mg RE) were weighed precisely into empty gelatin capsules and stored in amber bottles at −20°C until used.

Determination of equilibration time

Time of equilibration of an orally administered dose of deuterated vitamin A with the body vitamin A pool was obtained by using data from 47 subjects who ingested 2 capsules of [$^2H_4$]retinyl acetate (10 mg) with a meal that provided 3473 kJ and 29 g fat (fried chicken, French fries, bread, and a soda). Fasting venous blood samples were obtained from each subject at 1–3 different time points, 3 d (n = 26), 6 d (n = 9), 7 d (n = 21), 14 d (n = 11), 20 d (n = 10), 21 d (n = 16), and 54 d (n = 9) after isotope administration. Serum retinol was separated from other serum components by HPLC (10); the retinol fraction was collected and derivatized to trimethylsilyl derivatives, and the deuterated and nondeuterated retinol in the derivatized preparations were analyzed by gas chromatography–mass spectrometry (GC-MS) with electron capture negative chemical ionization (11). We determined the accuracy of the GC-MS procedure to be 99%; serum sample analyses gave a CV of 6%. All HPLC and GC-MS procedures were done at Tufts University in Boston. Percentage enrichment of total serum retinol with deuterated retinol was calculated and plotted against time.

Calculation of total body stores of vitamin A

Estimates of total body stores of vitamin A were obtained by using the mathematical formula of Furr et al (3):

$$\text{Total body stores (in mmol retinol)} = F \times \text{dose} \times \{S \times a \times [(1/\text{D:H}) - 1]\} \quad (1)$$

where F is a factor that expresses the storage efficiency of an orally administered dose, which is considered to be 0.5 (4); dose is the amount of labeled retinol (in mmol) administered orally;

D:H is the ratio of deuterated to nondeuterated retinol in serum after the administered isotope has equilibrated with the body's vitamin A pool; and the factor −1 corrects for the contribution of the administered dose to the total body pool. In humans, it is not possible to attain a truly equilibrated state because of continued ingestion of unlabeled dietary vitamin A. Dietary vitamin A affects the isotopic ratio of serum retinol in that the contribution of the newly absorbed vitamin to the serum pool is greater than that of endogenous liver reserves (12). If no vitamin A is provided in the diet, experiments in rats have shown that the specific activities of labeled retinol in serum and liver are identical during the equilibration period (4). However, when dietary vitamin A is fed during the equilibration period, the mean ratio of specific activity in serum to liver falls to 0.65 over a wide range of liver vitamin A concentrations (12). Thus, the factor *S*, taken as 0.65, is a correction for the inequalities in specific activities in serum and liver. The factor *a* is the fraction of the absorbed deuterated retinol remaining in body stores at the time of blood sampling. It was introduced by Furr et al (3) as another correction to D:H to correct for the fact that, with time, unlabeled dietary retinol replaces labeled retinol lost in catabolism; *a* is based on the half-life of vitamin A in the liver, eg, 140 d [range: 75–240 d (13, 14)] and is assumed to be independent of the size of liver stores ($a = e^{-kt}$, where $k = 1/140$, and t is time in days since the isotope was administered).

Intervention phase: diets and procedures

A subgroup ($n = 9$) of elderly subjects from the rural community of Buena Vista participated in an intervention study for 32 d during which they were fed controlled diets daily containing 8661 ± 363 kJ ($\bar{x}$ ± SEM), with 14% protein, 64% carbohydrate, and 22% fat (equivalent to 50.6 g fat/d). Baseline energy intakes (assessed by three 24-h recalls during the month before the study) of this subgroup were 6276 ± 720 kJ, with 12% protein, 74% carbohydrate, and 14% fat (equivalent to 23.3 g fat/d). During the intervention period, 982 ± 7 μg RE was provided from usual food sources, an amount that was ≈16% higher than the subgroup's baseline vitamin A intake of 847 ± 152 μg RE/d. During the intervention, preformed vitamin A contributed 440 ± 32 μg RE and provitamin A carotenoids contributed 540 ± 5 μg RE (the subgroup's baseline intakes of these were 454 ± 70 and 394 ± 103 μg RE, respectively). Vitamin A from fortified sugar (10 mg retinol/kg sugar) constituted 78% of preformed vitamin A and 35% of total dietary vitamin A during the controlled feeding period; the corresponding values at baseline were 84% and 45%, respectively. The principal sources of provitamin A carotenoids were pumpkin, tomatoes, squash, and green leaves (*quiletes*). The subjects ate all meals at the feeding center and reported no consumption of dietary vitamin A away from the feeding center; if subjects chose to drink sweetened coffee in their homes, nonfortified sugar was provided. To ensure an increase in the size of the body vitamin A pool for measurement by the DRD technique, a capsule containing an arbitrarily chosen amount of 800 μg RE as retinyl acetate in corn oil was provided as a daily supplement.

The subjects were given a deworming dose of 400 mg albendazole (SmithKline Beecham Pharmaceuticals, Philadelphia) 1 to 2 wk before the start of the study because mild-to-moderate infections with *Ascaris lumbricoides* and *Trichuris trichiura* are prevalent among rural Guatemalan elderly (7). The sequence of procedures during the intervention phase were as follows. On day 1, after an overnight fast, subjects completed an RDR test; serum obtained at baseline was also used for measurements of baseline carotenoids and protein markers of infection. After 7 d (day 8), subjects started a DRD procedure by ingesting 10 mg [$^2H_4$]retinyl acetate. At 3 and 21 d postdosing (days 11 and 29), fasting blood was obtained for measurements of baseline isotopic ratios of serum retinol and for calculations of total body stores of vitamin A by using day 21 ratios. During this period (days 1–29), the subjects ate their usual diets in their homes. For the next 32 d (from days 29–60), subjects reported to the study center where they ate controlled diets 3 times daily and ingested vitamin A supplements. After the intervention (day 61), after an overnight fast, subjects completed an RDR test and serum measurements obtained at baseline were repeated. After 7 d (day 68), the subjects started a DRD procedure by ingesting 10 mg [$^2H_8$]retinyl acetate. [$^2H_8$]Retinyl acetate was used after the intervention to distinguish the newly introduced deuterated retinol in serum from residual [$^2H_4$]retinol. At 3 and 21 d postdosing (days 71 and 89), fasting blood was obtained for measurements of postintervention isotopic ratios of serum retinol and for calculations of total body stores of vitamin A by using day 21 ratios. From day 61 to the end of the study on day 89, the subjects ate their usual diets in their homes. Positive markers of infection in serum (C-reactive protein, ceruloplasmin, and α_1-antitrypsin) and a negative marker of infection (albumin) were assayed on days 1, 11, 29, 61, 71, and 89.

Relation of estimated total body stores of vitamin A to serum D:H at 3 or 6 d postdosing

We studied whether serum D:H at time points earlier than equilibration could be used to predict total body stores of vitamin A. Data from subjects who provided blood samples at days 3 and 21 ($n = 15$) and at days 6 and 20 ($n = 9$) after dosing with [$^2H_4$]retinyl acetate were used. Total body stores of vitamin A were calculated by using the formula of Furr et al (3) with isotopic ratios of serum retinol at equilibration time (day 20 or 21). The calculated values were correlated with D:H at the earlier time points (day 3 or 6).

Statistical analyses

Analysis of variance (ANOVA) was used to evaluate the means at various time points of percentage enrichment of total serum retinol with deuterated retinol; because F was significant ($P = 0.0001$), differences between pairs of means were evaluated by using Fisher's least-significant-difference method or Scheffe's F test. Unpaired Student's t tests were used to compare data between men and women; paired Student's t tests were used to compare values post- and preintervention. Pearson's product-moment correlation and Spearman's rank correlation analyses were used to correlate serum D:H at 3 or 6 d postdosing with the calculated values for total body stores of vitamin A by using the formula of Furr et al (3) with isotopic ratios of serum retinol at 21 or 20 d postdosing. All statistical analyses were performed with STATVIEW SE+ GRAPHICS software (Abacus Concepts, Inc, Berkeley, CA).

RESULTS

Equilibration time

The percentage enrichment of serum retinol with deuterated retinol at various time points after dosing with 10 mg [$^2H_4$]retinyl acetate is shown in **Figure 1**. The curve is a com-

TABLE 1
Estimated total body stores of vitamin A, estimated liver retinol concentrations, serum retinol concentrations, and percentage relative dose response (RDR) in elderly Guatemalan men and women[1]

	Men (n = 10)	Women (n = 16)	All subjects (n = 26)
Age (y)	72 ± 2 (65–81)	68 ± 2 (60–78)	70 ± 1 (60–81)
Weight (kg)	50.5 ± 1.9 (42.3–62.0)	48.3 ± 3.1 (31.8–83.0)	49.1 ± 2.0 (31.8–83.0)
Total body stores of vitamin A (mmol retinol)[2]	0.875 ± 0.148 (0.172–1.387)	0.714 ± 0.050 (0.346–1.016)	0.782 ± 0.059 (0.172–1.387)
Liver retinol concentration (μmol/g)[3]	0.752 ± 0.108 (0.148–1.127)	0.655 ± 0.057 (0.302–1.061)	0.692 ± 0.054 (0.148–1.127)
Serum retinol concentration (μmol/L)	1.78 ± 0.09 (1.35–2.22)	1.74 ± 0.14 (0.58–2.63)	1.75 ± 0.09 (0.58–2.63)
RDR (%)	−11.9 ± 7.9 (−68.3 to 11.7)	−1.3 ± 2.7 (−21.4 to 21.9)	−5.4 ± 3.5 (−68.3 to 21.9)

[1] $\bar{x}$ ± SEM; range in parentheses. There were no significant differences between men and women by unpaired Student's t test.
[2] Estimated by using the mathematical formula described by Furr et al (3).
[3] Estimated by assuming that liver weight is 2.4% of body weight (3, 14).

posite of responses from subjects with adequate vitamin A status (serum retinol: 1.85 ± 0.08 μmol/L, $\bar{x}$ ± SEM) who contributed data at 1–3 time points. At 3, 6, 7, 14, 20, 21, and 54 d postdosing, percentage enrichments of serum retinol with deuterated retinol ($\bar{x}$ ± SEM) were 9.4 ± 0.8%, 2.7 ± 0.2%, 3.2 ± 0.2%, 1.8 ± 0.2%, 1.1 ± 0.1%, 1.4 ± 0.2%, and 1.1 ± 0.2%, respectively. Values at 54 d were not significantly different from those at 20 or 21 d, but were significantly different from those at 14 d and at earlier time points. Values at 20 d were significantly different from those at 14 d and at earlier time points. Thus, equilibration of the oral dose of deuterated vitamin A with the body vitamin A pool occurred after 14 d, and by 20 d, postdosing.

Total body stores of vitamin A, serum retinol concentrations, and percentage RDR

As shown in **Table 1**, total body stores of vitamin A were calculated by using serum D:H at 20 or 21 d postdosing from 26 subjects (10 men and 16 women) who were 70 ± 1 y old ($\bar{x}$ ± SEM). Total body stores ranged from 0.172 to 1.387 mmol (49 to 397 mg) retinol, with a mean of 0.782 ± 0.059 mmol (224 ± 17 mg). To express the data per gram of liver in nonobese adults, liver weight was assumed to be 2.4% of body weight (3, 13). The subjects' mean body weight was 49.1 ± 2.0 kg. Expressed per gram of liver, retinol values ranged from 0.148 to 1.127 μmol/g (42.4 to 322.8 μg/g), with a mean of 0.692 ± 0.054 μmol/g (198.2 ± 15.5 μg/g). Thus, none of the subjects had liver concentrations below the cutoff value of 0.070 μmol/g (20 μg/g) for vitamin A adequacy (13, 15).

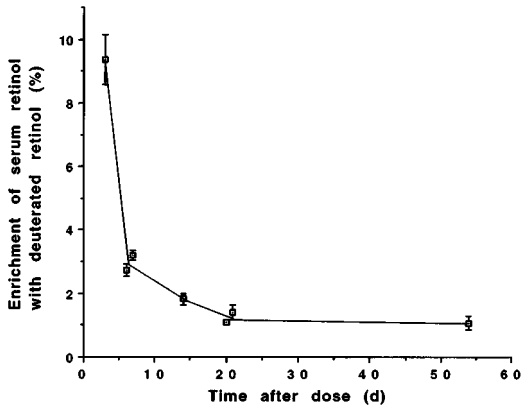

FIGURE 1. Time curve of percentage enrichment of serum retinol with deuterated retinol after an oral dose of 10 mg [$^2H_4$]retinyl acetate in Guatemalan elderly. Values are means ± SEMs at 3 d (n = 26), 6 d (n = 9), 7 d (n = 21), 14 d (n = 11), 20 d (n = 10), 21 d (n = 16), and 54 d (n = 9). ANOVA, Fisher's least-significant-difference method, and Scheffe's F test were used to analyze the data. Values at 54 d were not significantly different from those at 20 d (P = 0.88) or 21 d (P = 0.35), but were significantly different from values at 14 d and at earlier time points (P = 0.02). Values at 20 d were significantly different from those at 14 d and at earlier time points (P = 0.003).

Total body stores of vitamin A did not correlate with serum retinol concentrations or with percentage RDR. Serum retinol concentrations ranged from 0.58 to 2.63 μmol/L (16.6 to 75.4 μg/dL), with a mean of 1.75 ± 0.009 μmol/L (50.1 ± 2.5 μg/dL). One subject had a serum retinol value that was <0.70 μmol/L (ie, 0.58 μmol/L), whereas another had a value between 0.70 and 1.05 μmol/L (ie, 0.92 μmol/L); however, the calculated total body stores of vitamin A of these 2 subjects were adequate: 0.475 and 0.950 mmol retinol (136 and 272 mg), respectively. Their liver vitamin A concentrations were also adequate: 0.622 and 1.061 μmol/g (178 and 304 μg/g), respectively, and their percentage RDR values were normal: 12.4% and −21.4%, respectively. Only one subject had an abnormal RDR (21.9%), but her serum retinol concentration (1.53 μmol/L), liver retinol concentration (0.349 μmol/g), and total body stores of vitamin A (0.346 mmol retinol) were normal. There were no significant differences between men and women in age, body weight, total body stores of vitamin A, liver vitamin A concentrations, serum retinol concentrations, or percentage RDR.

Relation between estimated total body stores of vitamin A and serum D:H

Regression analysis showed a significant, inverse linear relation between serum D:H at 3 d postdosing and the values for total body stores of vitamin A calculated by using the formula of Furr et al (3) with isotopic ratios of serum retinol at 21 d postdosing (**Figure 2**). Pearson's product-moment coefficient of correlation (r) was −0.70 (n = 15; P = 0.004); the regression equation was $y = -3.858x + 1.169$. Spearman's rank correlation coefficient (r_S) was −0.85 (P = 0.002). When one outlier who had the highest serum D:H (or lowest total body stores of vitamin A) was excluded from data analyses, $r = -0.75$ (P = 0.002), the regression equation was $y = -9.597x + 1.605$, and $r_S = -0.81$ (P = 0.004). Serum D:H at 6 d postdosing versus total body stores of vitamin A calculated by using the formula of Furr et al (3) with isotopic ratios of serum retinol at 20 d postdosing gave $r = -0.59$ (P = 0.09) and $r_S = -0.76$ (P = 0.03) (**Figure 3**).

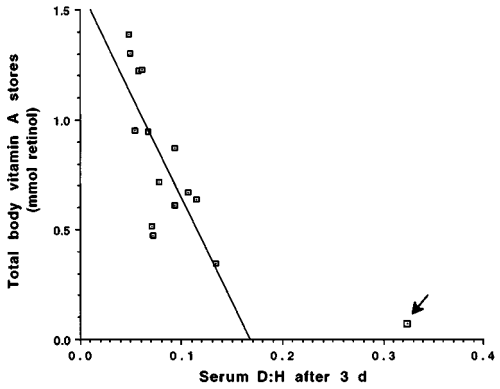

FIGURE 2. Correlation between serum ratios of deuterated to nondeuterated retinol (D:H) 3 d after a 10-mg oral dose of [$^2H_4$]retinyl acetate versus values for total body stores of vitamin A estimated by using serum D:H at 21 d postdosing in the mathematical formula described by Furr et al (3). For all values (n = 15), $r = -0.70$, $P = 0.004$, and $r_s = 0.85$, $P = 0.002$. When one outlier was excluded from the analysis (arrow), $r = -0.75$, $P = 0.002$, and $r_s = 0.81$, $P = 0.004$.

Intervention study

Provision of retinyl acetate supplements (800 μg RE) daily for 32 d in addition to controlled diets containing 982 ± 20 μg RE failed to alter serum retinol concentrations or percentage RDR, but increased total body stores of vitamin A in a subgroup of 4 men and 5 women who were 65–81 y of age (mean ± SEM: 71 ± 2 y; **Table 2**). The mean increase in body stores for the group was 0.264 ± 0.011 mmol retinol ($P = 0.03$). In 4 subjects, the mean increase was 0.117 ± 0.017 mmol retinol ($P = 0.02$), an increase of 23.5%. In 4 other subjects, the increase in total body stores of vitamin A was greater than could be accounted for by the sum of all vitamin A sources during the study period (diets, supplements, isotope dose, and RDR test doses). In these subjects, very low D:Hs were observed at 21 d postdosing (which translated into high calculated total body stores postintervention). Among these exaggerated responses, data from one subject with extremely high calculated body stores (3.77 mmol retinol postintervention) was excluded from the analyses; the remaining 3 subjects had a mean increase in body stores of 0.567 ± 0.076 mmol retinol ($P = 0.02$). One subject who had the highest body stores of vitamin A at baseline (1.301 mmol) showed a small decrease in body stores after supplementation (1.168 mmol).

Also shown in Table 2 is that concentrations of serum *trans*-β-carotene, 13-*cis*-β-carotene, α-carotene, α-cryptoxanthin, and lutein/zeaxanthin were not significantly different after the intervention. Serum lycopene increased significantly (tomato products were provided in the controlled diets) and β-cryptoxanthin was significantly lower postintervention, possibly because of the diets provided, although β-cryptoxanthin data for Guatemalan foods are not currently available.

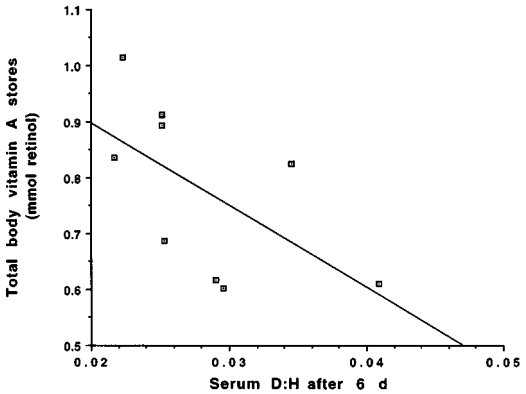

FIGURE 3. Correlation (n = 9) between serum ratios of deuterated to nondeuterated retinol (D:H) 6 d after a 10-mg oral dose of [$^2H_4$]retinyl acetate versus values for total body stores of vitamin A estimated by using serum D:H at 21 d postdosing in the mathematical formula described by Furr et al (3). $r = -0.59$, $P = 0.09$; $r_s = -0.76$, $P = 0.03$.

DISCUSSION

We applied the DRD procedure to assess the vitamin A status of elderly people. To use the mathematical formula of Furr et al (3) to calculate total body stores of vitamin A, blood for the determination of isotopic ratios of serum retinol should be drawn after the administered stable isotope has equilibrated with body stores. In this study, we found that the percentage enrichment of serum retinol with deuterated retinol at 20 d postdosing was not significantly different from values obtained 52 d postdosing, but was significantly different from those at day 14 and earlier. Thus, in this elderly group, equilibration occurred after 14 d and by 20 d postdosing, similar to in younger adults. Haskell et al (5) used isotopic ratios at 18–25 d and Furr et al (3) used ratios at 19–47 d postdosing to calculate total body stores of vitamin A in their study subjects, who were mostly younger adults. Recently, Haskell et al (6) studied the plasma kinetics of an oral dose of

TABLE 2
Effect of providing controlled diets plus supplements for 32 d on total body stores of vitamin A, serum ratios of deuterated to nondeuterated retinol (D:H) 3 d postdosing, serum retinol, percentage relative dose response (RDR), and serum carotenoids in Guatemalan elderly[1]

	Preintervention	Postintervention
Total body stores of vitamin A (mmol retinol)[2]		
All responses (n = 8)	0.635 ± 0.125	0.899 ± 0.136[3]
Good responses (n = 4)	0.498 ± 0.166	0.615 ± 0.183[4]
Exaggerated responses (n = 3)	0.595 ± 0.061	1.162 ± 0.137[4]
No response (n = 1)	1.301	1.168
Serum D:H 3 d postdosing[5]	0.107 ± 0.031	0.066 ± 0.016[6]
Serum retinol (μmol/L)	1.41 ± 0.17	1.39 ± 0.12
RDR (%)	−16.9 ± 10.1	−6.2 ± 2.6
Serum carotenoids (μmol/L)		
trans-β-Carotene	0.067 ± 0.019	0.080 ± 0.019
13-*cis*-β-Carotene	0.005 ± 0.002	0.007 ± 0.002
α-Carotene	0.019 ± 0.003	0.013 ± 0.003
β-Cryptoxanthin	0.088 ± 0.024	0.058 ± 0.016[6]
α-Cryptoxanthin	0.033 ± 0.009	0.027 ± 0.007
Lutein/zeaxanthin	0.411 ± 0.107	0.313 ± 0.069
Lycopene	0.088 ± 0.020	0.145 ± 0.020[6]

[1] $\bar{x}$ ± SEM. n = 9, unless stated otherwise.

[2] Calculated by using the mathematical formula described by Furr et al (3) with retinol isotopic ratios obtained 21 d after an oral dose of 10 mg [$^2H_4$]retinyl acetate preintervention and 10 mg [$^2H_8$]retinyl acetate postintervention. One exaggerated response was excluded from the analyses.

[3,4,6] Significantly different from preintervention (Student's paired t test): [3] $P = 0.03$, [4] $P = 0.02$. [6] $P = 0.04$.

[5] Obtained 3 d after an oral dose of 10 mg [$^2H_4$]retinyl acetate preintervention and 10 mg [$^2H_8$]retinyl acetate postintervention.

[$^2H_4$]retinyl acetate in subjects who were 24–44 y old and reported that the mean equilibration time for this age group was 16.6 d. They found no significant difference in equilibration time between US subjects (17.5 d) and Bangladeshi subjects (16.3 d) with estimated high or low total body stores of vitamin A.

We found a significant, inverse linear relation between the calculated total body stores of vitamin A and serum D:H 3 d after an oral dose of deuterated vitamin A; at 6 d postdosing the relation was weaker. This predictive ability of 3-d data is consistent with data obtained by Green et al (16–18) in kinetic studies in rats. These authors showed that the fraction of an injected or oral dose of [^{3}H]retinol remaining in plasma could be used to generate regression equations to predict liver vitamin A stores without the need to estimate the efficiency of absorption and liver retention of the test dose of vitamin A, the unity correlation between plasma and liver specific activity, or the metabolism of the test dose between the time of administration and blood sampling (18). If a predictive equation for use in humans can be developed by using isotopic ratios of serum retinol obtained 3 d after an oral administration of the stable isotope, and if this can be validated by liver biopsy measurements, total procedure time will be shortened considerably. Such a shortening of procedure time may be of practical importance in ensuring subject compliance, especially in field studies.

The elderly Guatemalans studied had adequate body stores of vitamin A. Their calculated stores (0.782 ± 0.059 mmol retinol) are similar to those reported by Furr et al (3) for healthy adult Americans (0.773 ± 0.191 mmol) and are much greater than those reported by Haskell et al (5) for adult Bangladeshis (0.110 ± 0.072 mmol). None of our study subjects had hepatic retinol concentrations <0.07 μmol/g, the cutoff for vitamin A adequacy (13, 15); their mean hepatic retinol concentration was 0.692 ± 0.054 μmol/g. For this age group (≥60 y), reported published values (in μmol/g) for mean retinol concentrations measured in livers obtained at autopsy include 0.435 in Canada (19); 0.632 in 5 US areas (Missouri, Iowa, Ohio, California, and Texas) (1); 1.219 in Washington, DC (20); and 0.338 in Illinois (21). By design, the present study reached only a small number of rural, elderly Guatemalans, who are not necessarily representative of rural, elderly Guatemalans throughout the country. However, our finding of adequate vitamin A status bodes well for elderly Guatemalans. The adequate status is most likely related to a national program mandating the fortification of sugar with vitamin A; the program had been in place for a decade at the time of this study.

It is well known that over the physiologic range of liver vitamin A concentrations (0.07–1.05 μmol/g), serum retinol concentrations are controlled homeostatically (15); thus, as expected in this population with adequate vitamin A status, total body stores of vitamin A did not correlate with serum retinol or with percentage RDR. In adult Americans, steady state serum retinol values <1.05 μmol/L are considered unusual, but not specific for poor vitamin A status (22). In this study, 2 elderly Guatemalans whose serum retinol concentrations were 0.58 and 0.92 μmol/L had adequate total body stores of vitamin A, liver vitamin A concentrations, and percentage RDR.

This study was an initial effort to apply the DRD technique to detect changes in the size of the body vitamin A pool in elderly subjects. We calculated that the subjects ingested a total of ≈97.2 mg RE from all sources, from the start of the study to just before the second DRD test was begun (days 1–67). These vitamin A sources included diet (61.92 mg RE), supplements (25.60 mg RE), first DRD isotope dose (8.72 mg RE), and the 2 RDR test doses (0.96 mg RE). The isotope dose administered during the second DRD test and all unlabeled vitamin A ingested during the subsequent 21-d equilibration period were excluded because, theoretically, they do not contribute to total body stores of vitamin A postintervention because they are accounted for by the terms -1, S, and a in the formula of Furr et al (3).

In the intervention study, we observed 3 responses: in 4 subjects, the mean increase was 33.51 mg (0.117 mmol); in 3 others, the response was exaggerated (mean increase: 162.39 mg, or 0.567 mmol) and could not be accounted for by the total amount of vitamin A provided; and in 1 subject, a 10% decrease in total body stores was observed. Interpreting these data in relation to the total amount of vitamin A consumed is difficult because of the many factors that affect the absorption and bioavailability of dietary vitamin A. We estimate that plant provitamin A carotenoids contributed ≈31.46 mg RE or 32.4% of the total vitamin A ingested during the 67-d period on the basis of the currently accepted, although uncertain, 6:1 equivalency for β-carotene bioconversion to retinol. Assuming that the storage efficiency of ingested vitamin A from all sources during the 67-d period was 50% and that the daily catabolic rate was 0.5% (13, 22), the theoretical expected increase in total body stores of vitamin A was 48.36 mg (0.169 mmol). Thus, the response of 33.51 mg observed in 4 subjects was reasonable.

The one subject who showed a decrease in total body stores of vitamin A had the highest stores at baseline. It is possible that the lack of response to ingested vitamin A may have been due to a protective mechanism that limits intestinal absorption or increases excretion when stores are already high. It has been shown that when liver reserves exceed 30 μg/g in rats, the biliary excretion of vitamin A metabolites is greatly increased (12).

In 4 subjects, an exaggerated increase in total body stores of vitamin A was observed in response to supplementation and feeding. One of the subjects had an elevated C-reactive protein concentration preintervention; the presence of infection may have resulted in a lower-than-normal baseline value for serum retinol and vitamin A stores, thus exaggerating the difference in stores before and after the intervention. In the other 3 subjects, for reasons that are unclear, the D:H in serum postsupplementation was very low, resulting in high calculated total body stores of vitamin A that could not be accounted for by the amount of vitamin A we provided. It is possible that these subjects also had falsely low baseline values or that they malabsorbed or poorly retained the [$^2H_8$]retinyl acetate dose given postintervention. Serum markers of infection in these 3 subjects, however, were normal. Haskell et al (5) showed that morbidity can affect isotopic ratios of serum retinol, resulting in an overestimation of the calculated total body stores of vitamin A. Although all subjects received albendazole before the study, they were not tested for helminths or for fat malabsorption postintervention.

In summary, the DRD procedure described by Furr et al (3) was used to determine the vitamin A status of elderly persons residing in rural Guatemala; estimates of total body stores indicative of adequate vitamin A status were found. The procedure can detect changes in the size of the body pool in response to supplementation; however, the factors that affect isotopic ratios of serum retinol need to be elucidated. Furthermore, the development of a quantitative estimate of total body vitamin A reserves in humans by using isotopic ratios of serum retinol at earlier time points, eg, 3 d after isotope dosing, would be useful.

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REFERENCES

1. Raica N Jr, Scott J, Lowry L, Sauberlich HE. Vitamin A concentration in human tissues collected from five areas in the United States. Am J Clin Nutr 1972;25:291–6.
2. Underwood BA, Olson JA, eds. A brief guide to current methods of assessing vitamin A status. Washington, DC: International Vitamin A Consultative Group, ILSI Press, 1993.
3. Furr HC, Amedee-Manesme O, Clifford AJ, et al. Vitamin A concentrations in liver determined by isotope dilution assay with tetradeuterated vitamin A and by biopsy in generally healthy adult humans. Am J Clin Nutr 1989;49:713–6.
4. Bausch J, Rietz P. Method for the assessment of vitamin A liver stores. Acta Vitaminol Enzymol 1977;31:99–112.
5. Haskell MJ, Handelman GJ, Peerson JM, et al. Assessment of vitamin A status by the deuterated-retinol-dilution technique and comparison with hepatic vitamin A concentration in Bangladeshi surgical patients. Am J Clin Nutr 1997;66:67–74.
6. Haskell MJ, Islam MA, Handelman GJ, et al. Plasma kinetics of an oral dose of [$^2H_4$]retinyl acetate in human subjects with estimated low or high total body stores of vitamin A. Am J Clin Nutr 1998;68:90–5.
7. King JE, Mazariegos M, Valdez C, Castaneda C, Solomons NW. Nutritional status indicators and their interactions in rural Guatemalan elderly: a study in San Pedro Ayampuc. Am J Clin Nutr 1997;66:795–802.
8. Flores H, Campos F, Araujo CRC, Underwood BA. Assessment of marginal vitamin A deficiency in Brazilian children using the relative dose response procedure. Am J Clin Nutr 1984;440:1281–9.
9. Bulux J, Carranza E, Castaneda C, et al. Studies on the application of the relative-dose-response test for assessing vitamin A status in older adults. Am J Clin Nutr 1992;56:543–7.
10. Ribaya-Mercado JD, Ordovas JM, Russell RM. Effect of β-carotene supplementation on the concentrations and distribution of carotenoids, vitamin E, vitamin A, and cholesterol in plasma lipoprotein and non-lipoprotein fractions in healthy older women. J Am Coll Nutr 1995;14:614–20.
11. Tang G, Qin J, Dolnikowski GG. Deuterium enrichment of retinol in humans determined by gas chromatography electron capture negative chemical ionization mass spectrometry. J Nutr Biochem 1998;9:408–14.
12. Hicks VA, Gunning DB, Olson JA. Metabolism, plasma transport and biliary excretion of radioactive vitamin A and its metabolites as a function of liver reserves of vitamin A in the rat. J Nutr 1984;114:1327–33.
13. Olson JA. Recommended dietary intakes (RDI) of vitamin A in humans. Am J Clin Nutr 1987;45:704–16.
14. Sauberlich HE, Hodges RE, Wallace DL, et al. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam Horm 1974;32:251–75.
15. Olson JA. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. J Natl Cancer Inst 1984;73:1439–44.
16. Green MH, Green JB, Lewis KC. Variation in retinol utilization rate with vitamin A status in the rat. J Nutr 1987;117:694–703.
17. Duncan TE, Green JB, Green MH. Liver vitamin A levels in rats are predicted by a modified isotope dilution technique. J Nutr 1993;123:933–9.
18. Adams WR, Green MH. Prediction of liver vitamin A in rats by an oral isotope dilution technique. J Nutr 1994;124:1265–70.
19. Hoppner K, Phillips WEJ, Erdody P, Murray TK, Perrin DE. Vitamin A reserves of Canadians. Can Med Assoc J 1969;101:84–6.
20. Mitchell GV, Young M, Seward CR. Vitamin A and carotene levels of a selected population in metropolitan Washington, D.C. Am J Clin Nutr 1973;26:992–7.
21. Schmitz HH, Poor CL, Wellman RB, Erdman JW Jr. Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. J Nutr 1991;121:1613–21.