

Use of the deuterated-retinol-dilution technique to assess total-body vitamin A stores of adult volunteers consuming different amounts of vitamin A¹⁻³

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ABSTRACT

Background: The deuterated-retinol-dilution (DRD) technique provides a quantitative estimate of total body stores of vitamin A. However, it is not known whether the technique can detect changes in vitamin A pool size in response to different intakes of vitamin A.

Objective: Our objective was to determine the responsiveness of the DRD technique to 3 different daily supplemental vitamin A intakes during a period of 2.5–4 mo.

Design: Two oral doses of [²H₄]retinyl acetate [52.4 μmol retinol equivalent (RE)] were administered on study days 1 and 91 to 26 men (18–32 y of age) who were consuming controlled, low-vitamin A diets, and receiving daily either 0, 5.2, or 10.5 μmol RE of unlabeled supplemental retinyl palmitate during a 75- or 129-d period. Plasma isotopic ratios of [²H₄]retinol to retinol on day 115 were used to estimate final vitamin A body stores per Furr et al (Am J Clin Nutr 1989;49:713–6).

Results: Final ($\bar{x} \pm SD$) estimated vitamin A pool sizes were 0.048 ± 0.031 , 0.252 ± 0.045 , and 0.489 ± 0.066 mmol in the treatment groups receiving 0, 5.2, and 10.5 μmol RE/d, respectively ($P < 0.001$). Estimated mean changes in vitamin A pool sizes were similar to those expected for the vitamin A-supplemented groups [estimated:expected (95% CI of change in pool size): 1.08 (0.8, 1.2) and 1.17 (1.0, 1.3)].

Conclusions: The DRD technique can detect changes in total body stores of vitamin A in response to different daily vitamin A supplements. However, abrupt changes in dietary vitamin A intake can affect estimates of total-body vitamin A stores. Am J Clin Nutr 1999;70:874–80.

KEY WORDS Vitamin A, deuterated-retinol-dilution technique, DRD technique, pool size, isotope dilution, stable-isotope study, supplementation, Bangladesh

INTRODUCTION

The deuterated-retinol-dilution (DRD) technique is an indirect, quantitative method for estimating total body stores of vitamin A (1, 2). The DRD technique consists of administering a known dose of deuterium-labeled vitamin A orally and measuring the plasma isotopic ratio of [²H₄]retinol to retinol after the dose has mixed fully with endogenous vitamin A body stores (≈ 20 d in adults; 3). Total body stores of vitamin A are estimated

according to the principles of isotope dilution, a set of assumptions regarding retention of the dose of labeled vitamin A, the ratio of specific activities of vitamin A in plasma to that in liver, and the irreversible loss of vitamin A over time (2). As described previously, total-body vitamin A stores = $F \times \text{dose} \times (S \times a \times [(1/D:H) - 1])$; where F is a factor for the efficiency of storage of an orally administered dose of vitamin A, which is estimated to be 0.5 on the basis of the previous work of Bausch and Rietz (1); the dose is the amount of isotope administered, expressed as μmol retinol equivalents (RE); S is the assumed ratio of [²H₄]retinol to retinol in plasma to that in liver; a is the half-life of vitamin A turnover in the body; and $D:H$ is the isotopic ratio of [²H₄]retinol to retinol measured in plasma.

The dose of labeled vitamin A does not truly equilibrate with vitamin A body stores because 1) unlabeled vitamin A is continually consumed in the diet, and 2) newly absorbed dietary vitamin A contributes preferentially to the plasma pool (2). For these reasons, the isotopic ratios in plasma and liver do not reach equality. On the basis of the observed mean ratio of specific activities of radiolabeled vitamin A in serum to those in liver in a group of rats with a wide range of dietary vitamin A intakes and hepatic vitamin A concentrations (0.02–0.12 μmol/g liver; 4), a factor of 0.65 is used to estimate S to correct for the inequality of the [²H₄]retinol-retinol ratios in plasma and liver. The factor a is used to correct for the irreversible loss of vitamin A and is based on the half-life of vitamin A turnover in the body, which is estimated as 140 d (5). This factor is assumed to be independent of the size of total-body vitamin A stores and time invariant ($a = e^{-kt}$, where k is 0.5%/d and t is time in days since the dose). Finally, the value -1 corrects for the contribution of the dose of labeled vitamin A to total-body vitamin A stores.

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The DRD technique has been validated in well-nourished surgical patients in the United States (2) and in surgical patients with low-to-adequate vitamin A status in Bangladesh (6). The results of these studies have shown that the technique provides an accurate quantitative estimate of total-body vitamin A stores for groups of subjects. The DRD technique may also be useful for assessing the effect of vitamin A supplementation programs by measuring the change in vitamin A pool size in response to an intervention. However, the sensitivity of the technique for detecting changes in total-body vitamin A stores is not known. Therefore, the present study was carried out to evaluate the usefulness of the DRD technique in assessing changes in total body stores of vitamin A in response to supplementation with 3 different amounts of vitamin A. We expected that final estimated total-body vitamin A pool sizes would vary in relation to the total amount of unlabeled vitamin A consumed over a 90-d supplementation period.

SUBJECTS AND METHODS

Subjects

Adult, male Bangladeshi volunteers between the ages of 18 and 35 y were recruited for the study and screened for plasma concentrations of retinol, serum albumin, and C-reactive protein. We selected from the group of volunteers those who had the lowest plasma retinol (0.51–1.22 $\mu\text{mol/L}$), adequate serum albumin ($>3.5 \text{ g/L}$), and normal C-reactive protein ($<12 \text{ mg/L}$) concentrations to participate in the study, provided that they had no clinical evidence of vitamin A deficiency or illness that would interfere with absorption of vitamin A. Subjects were treated with albendazole (800 mg; Smith Kline Beecham Pharmaceuticals, Philadelphia) 1 wk before the initial screening tests were conducted. Stool samples were collected from subjects who were selected for the study on enrollment (2 wk after treatment with albendazole) and midway through the study protocol. None of the subjects had evidence of intestinal helminths on either occasion. Informed consent was obtained from each subject and the study protocol was approved by the Ethical Review Committee of the International Centre for Diarrhoeal Disease Research in Dhaka and the Human Subjects Review Committee of the University of California, Davis.

Study site

The study was carried out at the International Centre for Diarrhoeal Disease Research in Dhaka, Bangladesh. The subjects reported to a research study ward daily from 0730 to 1930 for a period of 129 d. During that time, they consumed all of their meals (breakfast, lunch, and dinner) and a midafternoon snack in the study cafeteria under supervision. Because of space limitations in the research ward, the study was conducted in 2 separate rounds, each lasting a period of 129 d, with 15 subjects participating in the first round and 11 subjects in the second round.

Treatment groups

Subjects in round 1 ($n = 15$) were ranked in groups of 3 according to their initial plasma retinol concentration and those within each group were randomly assigned to 1 of 3 treatment groups to receive a basal low-vitamin A diet and a daily capsule containing one of the following: corn oil only (0 mg RE), 5.2 μmol RE retinyl palmitate/d (1.5 mg RE/d) in corn oil, or 10.5 μmol RE

retinyl palmitate/d (3.0 mg RE/d) in corn oil. Subjects in round 2 ($n = 11$) were ranked similarly according to their initial plasma retinol concentration and randomly assigned to treatment groups. The subject who fell in the middle of the retinol concentration ranking (no. 6) was selected for the placebo group ($n = 1$) and the remaining subjects were assigned to the vitamin A-treatment groups ($n = 10$). Thus, considering both rounds, there was a total of 6 subjects in the placebo group and 10 subjects in each of the vitamin A-supplemented groups (total $n = 26$).

Basal diet and administration of capsules

The basal diet consisted primarily of rice and lentils with small amounts of curried meats (mutton, chicken, and fish), vegetables (cabbage, cauliflower, white squash, and white potato), and fruit (banana), all with low vitamin A content. We estimated that the basal diet provided $\approx 0.94 \mu\text{mol}$ RE/d (270 μg RE/d) (7). Subjects were allowed to consume rice and lentils ad libitum at mealtimes so that they could self-adjust their food intake to satisfy their energy needs; however, portions of vegetables, meat, and fruit were controlled. Each subject received a single portion of these foods, which was weighed out onto a tared plate. All meals and snacks were prepared in the hospital kitchen under the supervision of a dietitian. Subjects were free to return home after the evening meal, but were instructed not to consume any food after leaving the study ward. If they did consume any food (which was reportedly rare), they were asked to inform the supervisor the next morning.

In both rounds 1 and 2, the placebo and unlabeled vitamin A capsules were administered in midafternoon with a high-fat snack (*shingara*, deep-fried white potato pastry). Subjects were weighed and measured at baseline and examined by a physician weekly for clinical evidence of vitamin A deficiency throughout the study periods. At the end of the full set of studies, subjects in the placebo group received a single high-dose vitamin A capsule (210 μmol RE as retinyl palmitate in corn oil).

Isotope-dilution studies

Subjects began consuming the basal diet and their assigned dose of unlabeled vitamin A 1 d before initiating the isotope-dilution studies (study day 0), then continued with their assigned treatment for a period of either 75 d (round 1; $n = 15$) or 129 d (round 2; $n = 11$). On study day 1, the subjects received a single oral dose of 52.4 μmol RE (15 mg RE) [$^2\text{H}_4$]retinyl acetate. Blood samples (7 mL) were drawn 12 h after the dose and on days 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 20, 30, 60, 75, and 90 for measurement of the plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol. The purpose of collecting multiple samples during the first 90 d of the study was to evaluate the effect of consumption of different amounts of vitamin A on the plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol. Plasma isotopic ratios on day 20 were also used to estimate vitamin A pool sizes at baseline (2).

On study day 91, a second dose of 52.4 μmol (15 mg RE) [$^2\text{H}_4$]retinyl acetate was given orally and blood samples were drawn 9, 15, 24, and 36 d after the dose for measurement of the plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol. We intended to use the plasma isotopic ratio on study day 115 (24 d after the second dose) to estimate total-body vitamin A stores on day 91 (2) for subjects in both rounds 1 and 2. This would also help us to evaluate the ability of the DRD technique to detect differences in vitamin A pool size in response to the different amounts of vitamin A provided during the 90-d supplementation period. However,

TABLE 1Characteristics of study subjects by treatment group¹

	Vitamin A supplement group		
	0 $\mu\text{mol RE/d}$ (<i>n</i> = 6)	5.2 $\mu\text{mol RE/d}$ (<i>n</i> = 10)	10.5 $\mu\text{mol RE/d}$ (<i>n</i> = 10)
Age (y)	23.6 $\pm$ 3.8	23.3 $\pm$ 5.1	20.7 $\pm$ 1.6
Body weight (kg)	48.1 $\pm$ 5.8	51.7 $\pm$ 5.1	45.8 $\pm$ 4.1
Height (m)	1.61 $\pm$ 0.05	1.63 $\pm$ 0.05	1.60 $\pm$ 0.04
BMI (kg/m ²)	18.5 $\pm$ 1.8	19.6 $\pm$ 1.3	17.9 $\pm$ 1.4

¹ $\bar{x} \pm \text{SD}$. There were no significant differences by supplement amount between rounds 1 and 2.

because of a problem with the original study design, which is described below, it was not possible to estimate reliably the final body stores of vitamin A for the subjects in the vitamin A-supplemented groups in round 1.

Before estimation of total-body vitamin A stores on day 91 for subjects in round 2, the plasma isotopic ratios on day 115 were corrected for the amount of isotope remaining in plasma from the first dose of [²H₄]retinyl acetate by using the terminal slope of each individual's initial plasma kinetic curve to estimate, by extrapolation, the contribution of the first dose to the observed isotopic ratio on day 115. Although supplementation with unlabeled vitamin A continued throughout the 129-d study period for those in round 2, the estimates of vitamin A pool sizes based on the plasma isotopic ratios on study day 115 theoretically reflect the pool sizes on the day that the second dose of labeled vitamin A was administered (study day 91). (The "e^{-kt}" term in the prediction equation for estimating total-body vitamin A stores corrects the isotopic ratios for irreversible loss of vitamin A during the period between administration of isotope and measurement of the plasma isotopic ratio.) Thus, the estimated pool sizes reflect the effect of 90 d of supplementation with 3 different amounts of vitamin A on final vitamin A stores.

Laboratory procedures

Plasma retinol concentrations were determined by HPLC (8). The within- and between-day CV of the mean plasma concentration of replicate, pooled plasma samples was <5%. The accuracy of the measurements was assessed by analyzing the retinol concentration of control serum (Fat Soluble Vitamins, 968A; National Institute of Standards and Technology, Gaithersburg, MD). The measured concentration of the control serum was within 2% of the certified retinol concentration. The isotopic ratio of [²H₄]retinol to retinol in plasma was determined by gas chromatography-mass spectrometry (9) as described previously (6). Briefly, retinol was isolated from plasma by HPLC and the *tert*-butyldimethylsilyl (tBDMS) derivative of retinol was formed. Isotopic ratios were estimated by gas chromatography-mass spectrometry on a Shimadzu QP-5000 quadrupole mass spectrometer (Shimadzu Scientific Inc, Columbia, MD) using 1.12 $\times$ 10⁻¹⁷ J (70 eV) electron ionization. Selected ion monitoring was carried out for fragment ions of the tBDMS derivatives at a mass-to-charge ratio of 255 (retinol) and 259 ([²H₄]retinol). The within-run precision of the isotopic ratio measurements was determined by analyzing standards with each set of plasma samples. The CV for the mean isotopic ratio measurements for the standards was <5%.

Statistical analysis

Mean plasma isotopic ratios of [²H₄]retinol to retinol were calculated for each of the 20 time points and compared by study group using repeated-measures analysis of variance. Total-body vitamin A pool sizes were estimated by using the method of Furr et al (2) and were compared by treatment group by using analysis of variance and Tukey's test for multiple comparisons. Mean plasma retinol concentrations were calculated on study days 1, 30, and 90 and compared by treatment group using two-factor repeated-measures analysis of covariance, with initial values controlled for, and by Tukey's test for multiple comparisons.

RESULTS

Subjects

A total of 26 men 18–32 y of age participated in the study. Because subjects in the placebo group in both rounds received the same treatment, the results for the subjects in the placebo group from round 1 and the single placebo subject from round 2 were combined (*n* = 6). The anthropometric characteristics of the subjects at baseline are shown in **Table 1**. Their body weights ranged from 39 to 58 kg and their body mass indexes (in kg/m²) ranged from 15.5 to 21.1. The mean values for each of these measurements did not differ by treatment group. None of the subjects developed clinical symptoms or signs of vitamin A deficiency during the study period.

Plasma retinol concentration

Initial plasma retinol concentrations ranged from 0.51 to 1.22 $\mu\text{mol/L}$ and mean concentrations were similar across treatment groups (**Table 2**). The mean plasma retinol concentrations of the 2 vitamin A-supplemented groups increased significantly by day 30 (*P* < 0.05) when compared with values on day 1, whereas there was no significant change in the placebo group. The mean plasma retinol concentration of the group that received 5.2 $\mu\text{mol RE/d}$ decreased slightly between days 30 and 90; however, the day 90 value was still significantly higher than the day 1 value (*P* < 0.01). The mean serum retinol concentration of the group that received 10.4 $\mu\text{mol RE/d}$ did not change significantly from day 30 to day 90.

TABLE 2Plasma retinol concentrations by day of study and vitamin A supplement amount¹

Study day	Vitamin A supplement group		
	0 $\mu\text{mol RE/d}$ (<i>n</i> = 6)	5.2 $\mu\text{mol RE/d}$ (<i>n</i> = 10)	10.5 $\mu\text{mol RE/d}$ (<i>n</i> = 10)
	$\mu\text{mol/L}$		
1 ²	0.93 $\pm$ 0.23 ^a	0.95 $\pm$ 0.22 ^a	0.85 $\pm$ 0.28 ^a
30	1.04 $\pm$ 0.19 ^a	1.63 $\pm$ 0.28 ^b	1.38 $\pm$ 0.44 ^b
90	0.95 $\pm$ 0.16 ^a	1.38 $\pm$ 0.27 ^b	1.32 $\pm$ 0.44 ^b

¹ $\bar{x} \pm \text{SD}$. Means with different superscript letters within rows and columns are significantly different, *P* < 0.05 (repeated-measures ANOVA and Tukey's test for multiple comparisons). The placebo group included 5 subjects from round 1 and 1 subject from round 2; the supplemented groups included 5 subjects from each round.

²Day 1 values were not significantly different between groups (Tukey's test).

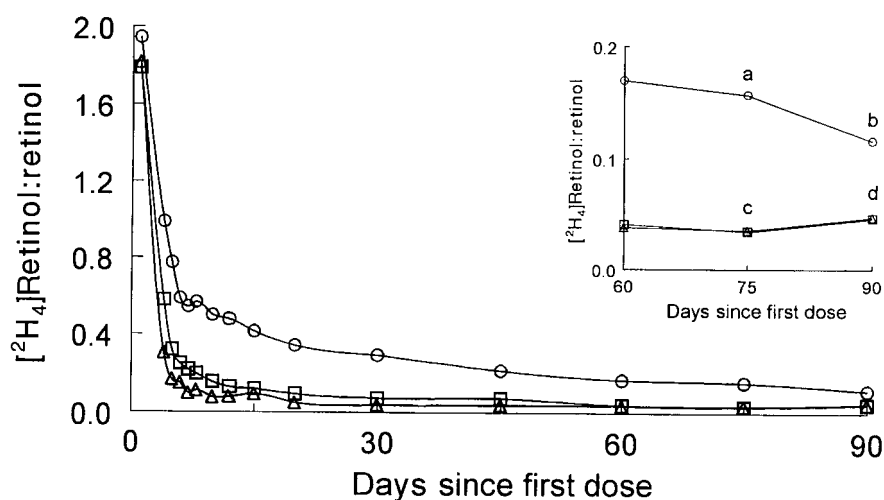


FIGURE 1. Mean ratio of [$^2\text{H}_4$]retinol to retinol in plasma by day since the first dose of deuterated vitamin A and by supplementation group in round 1 ($n = 15$): ○, 0 mg retinol equivalents (RE)/d; □, 1.5 mg RE/d; △, 3.0 mg RE/d. Isotopic ratios were significantly different by treatment group on day 4 and subsequently, $P < 0.001$. Inset: within-group isotopic ratios were significantly different for day 75 compared with day 90 (a and b, $P < 0.01$; c and d, $P < 0.02$).

Plasma isotopic ratios

The plasma isotopic ratios peaked within the first 24 h and declined sharply by day 2 (**Figure 1**). Plasma isotopic ratios were similar for all groups on day 1 but were significantly different by day 4 and thereafter ($P < 0.001$). In round 1, the mean plasma isotopic ratios of both vitamin A-supplemented groups increased significantly from day 75 to day 90 ($P \leq 0.011$), after daily consumption of supplemental unlabeled vitamin A had been discontinued (**Figure 1**). By contrast, the mean plasma isotopic ratio of the placebo group decreased significantly from day 75 to day 90 ($P < 0.01$), although the rate of decrease was not significantly different from that measured from day 30 to day 75. For subjects in round 2, supplementation with corn oil or unlabeled vitamin A was continued throughout the 129-d period. Thus, there was no perturbation of the plasma kinetic curves after day 75 for any of the 3 treatment groups, and the differences in patterns of isotopic ratios between days 75 and 90 that were observed in round 1 did not occur (**Figure 2**).

Estimated initial total-body vitamin A stores

We assumed that subjects in each of the 3 groups had similar total-body vitamin A reserves initially because of the way they were assigned to treatment groups and the fact that their mean plasma retinol concentrations did not differ. However, with use of the DRD technique, the estimated mean pool sizes of the vitamin A-supplemented groups at baseline were significantly greater than that of the placebo group ($P < 0.05$) (**Table 3**).

Estimated final total-body vitamin A stores in round 1

It was not possible to estimate final total body stores of vitamin A for the first group of subjects ($n = 10$, round 1) because of the observed “rebound” effect of the plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol when daily supplementation with unlabeled vitamin A was discontinued at 75 d. Specifically, because of the observed increase in the plasma isotopic ratios between days 75 and 90 for the vitamin A-supplemented groups, it was not possible to use the terminal slope of these subjects’ plasma kinetic curves

to estimate by extrapolation the contribution of the first dose to the observed isotopic ratio on day 115. Thus, their plasma isotopic ratios on day 115 could not be used to estimate their vitamin A stores. However, it was possible to estimate the final vitamin A stores of the placebo group because those subjects maintained a consistent dietary vitamin A intake throughout the 129-d study period. The mean ($\pm$ SD) total-body pool size of vitamin A on day 90 was 0.051 ± 0.034 mmol, in the placebo group, which did not differ significantly from the initial mean value.

Because of the perturbation of the plasma isotopic ratio from day 75 to day 90 described above in the 2 vitamin A-supplemented groups in round 1, the protocol was modified in round 2. The estimated final total-body vitamin A stores are presented for the second group of supplemented subjects ($n = 10$) and compared with the subjects who received placebo in both rounds ($n = 6$).

Estimated final total-body vitamin A stores in round 2

As shown in **Table 4**, the estimated final total-body vitamin A stores were significantly different across the three treatment groups following continuous supplementation with different levels of unlabeled vitamin A ($P < 0.001$). The estimated and expected theoretical differences in total-body vitamin A pool sizes for the vitamin A-supplemented groups are shown in **Table 4**. The expected theoretical differences in pool size were estimated as 50% of the total amount of supplemental vitamin A provided from day 1 to day 90, adjusted for the irreversible loss of vitamin A over time (0.5%/d) (5, 10). The difference in pool size was estimated as the observed mean final pool sizes of the vitamin A-supplemented groups in round 2 minus the observed mean final pool size of the placebo group (rounds 1 and 2 combined), assuming that the groups had similar pool sizes initially. A mean ($\pm$ SE) difference in pool size of 0.204 ± 0.024 mmol was estimated for the group that received $5.2 \mu\text{mol RE/d}$ (1.5 mg RE/d) and a difference of 0.441 ± 0.033 mmol was estimated for the group that received $10.5 \mu\text{mol RE/d}$ (3.0 mg RE/d). The ratio of estimated to theoretical pool sizes was 1.08 (95% CI: 0.82, 1.33) for the group that received $5.2 \mu\text{mol/d}$ (1.5 mg RE/d) and 1.17

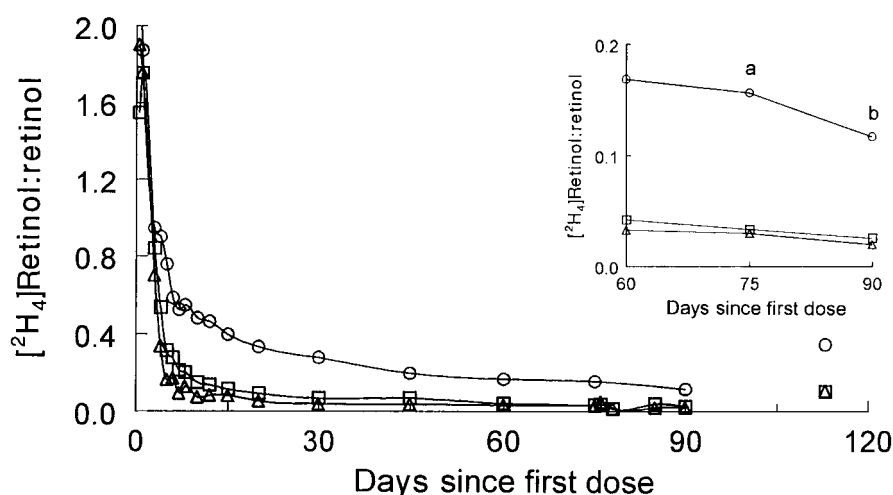


FIGURE 2. Mean ratio of [$^2\text{H}_4$]retinol to retinol in plasma by day since the first dose of deuterated vitamin A and by supplementation group in round 2 ($n = 16$): $\circ$, 0 mg retinol equivalent (RE)/d; $\square$, 1.5 mg RE/d; $\triangle$, 3.0 mg RE/d. Isotopic ratios were significantly different by treatment group on day 4 and subsequently, $P < 0.001$. Inset: there were no significant within-group differences in isotopic ratios from day 75 to day 90 in the vitamin A-supplemented groups; there was a significant within-group difference in isotopic ratios from day 75 to day 90 in the placebo group (a and b, $P < 0.01$).

(95% CI: 1.00, 1.34) for the group that received $10.5 \mu\text{mol/d}$ (3.0 mg RE/d). The estimated vitamin A pool size of the combined placebo group at baseline and after 90 d of supplementation was $0.040 \pm 0.023 \text{ mmol}$ and $0.048 \pm 0.031 \text{ mmol}$, respectively, and did not change significantly over time.

DISCUSSION

The difference in estimated total-body vitamin A pool sizes after 90 d of supplementation with 3 amounts of unlabeled vitamin A was evaluated using the DRD technique in a group of adult male Bangladeshi volunteers who had low-to-adequate plasma retinol concentrations at the onset of the study. Plasma isotopic ratios of [$^2\text{H}_4$]retinol to retinol were measured during the equilibration period after the first dose of isotope while the subjects were adjusting to different vitamin A intakes. The mean plasma isotopic ratios of [$^2\text{H}_4$]retinol to retinol varied in relation to dietary intake of unlabeled vitamin A during the equilibration period. These data suggest that the total vitamin A pool size of subjects who consume relatively high amounts of vitamin A during the equilibration period is likely to be overestimated.

Theoretically, the DRD technique estimates total body stores of vitamin A on the day that the test-dose is administered because the term e^{-kt} in the prediction equation corrects the observed plasma isotopic ratio for the irreversible loss of vitamin A over time. Thus, if our assumption is correct that the total vitamin A reserves of each of the supplementation groups were similar initially, the DRD test, with isotope being administered on day 1, should have provided similar estimates of total vitamin A pool size in each group. By contrast with this theoretical situation, the plasma isotopic ratios of [$^2\text{H}_4$]retinol to retinol were significantly lower in the vitamin A-supplemented groups than in the placebo group by day 4 and thereafter, and the estimates of total body stores of vitamin A based on plasma isotopic ratios on day 20 were correspondingly ≈ 4 –7-fold greater in the vitamin A-supplemented groups than in the placebo group. Plasma isotopic ratios may have been lower in the supplemented groups

on day 20 for 3 possible reasons: 1) a true difference in pool size on day 1, 2) a true increase in the vitamin A pool size between days 1 and 20 and incomplete correction for this with the assumptions of the DRD technique, and 3) a differential dilution of [$^2\text{H}_4$]retinol in plasma by the 3 supplementation levels of unlabeled vitamin A leading to a spurious overestimate of the pool size on day 20.

The first reason seems unlikely given that the subjects were randomly assigned to treatment groups and there were no differences in serum retinol concentrations at baseline. It is highly improbable that a true increase in the total body pool of vitamin A could have explained the differences in isotopic ratios on day 20 when it is assumed that the total vitamin A pool sizes were similar in each group initially. As described above, the DRD technique supposedly estimates total-body vitamin A stores on the day the test dose of isotope is administered rather than on the day that the isotopic ratio is measured. If we consider, for the moment, that the technique is estimating total-body vitamin A stores on the day that the isotope ratio is measured rather than on the day the isotope is administered, the estimates of vitamin A pool size are still higher than can be explained by the supplemental vitamin A that was provided during the 20-d period. If 50% of

TABLE 3

Initial total body stores of vitamin A as estimated by the deuterated-retinol-dilution technique on day 20 by supplement amount¹

Estimated total body stores of vitamin A	Vitamin A supplement group		
	0 $\mu\text{mol RE/d}$ ($n = 6$)	5.2 $\mu\text{mol RE/d}$ ($n = 5$)	10.5 $\mu\text{mol RE/d}$ ($n = 5$)
<i>mmol</i>			
Round 1	0.040 ± 0.023^a	0.153 ± 0.034^b	0.303 ± 0.064^c
Round 2	0.040 ± 0.023^a	0.166 ± 0.067^b	0.262 ± 0.061^c

¹ $\bar{x} \pm \text{SD}$. Means with different superscript letters are significantly different, $P < 0.001$ (ANOVA and Tukey's test for multiple comparisons). The placebo group included 5 subjects from round 1 and 1 subject from round 2.

TABLE 4

Estimated final total body stores of vitamin A (round 2) and estimated and expected theoretical differences in total vitamin A pool sizes on day 115

	Vitamin A supplement group		
	0 $\mu\text{mol RE/d}$ ($n = 6$) ¹	5.2 $\mu\text{mol RE/d}$ ($n = 5$)	10.5 $\mu\text{mol RE/d}$ ($n = 5$)
	<i>mmol</i>		
Estimated final total body stores of vitamin A ²	0.048 $\pm$ 0.031 ^a	0.252 $\pm$ 0.045 ^b	0.489 $\pm$ 0.066 ^c
Estimated difference ³	—	0.204 (0.180, 0.228)	0.440 (0.376, 0.505)
Expected difference ⁴	—	0.189	0.377
Estimated difference:expected difference	—	1.08 (0.82, 1.33)	1.17 (1.00, 1.34)

¹Included subjects from rounds 1 and 2.² $\bar{x} \pm \text{SD}$; means with different superscript letters are significantly different, $P < 0.05$ (ANOVA and Tukey's test for multiple comparisons).³Observed final pool size of vitamin A-supplemented groups minus observed final pool size of the placebo group; 95% CI in parentheses.⁴Based on 50% retention of supplemental vitamin A and a catabolic rate of 0.5%/d; 95% CI in parentheses.

the supplemental vitamin A that was consumed was retained (as is typically assumed; 1) and 0.5% of the total pool was irretrievably disposed of each day, the pool sizes could have increased by only 0.048 and 0.095 mmol during the first 20 d of supplementation in the groups that received 5.2 and 10.5 $\mu\text{mol RE vitamin A/d}$. However, the observed increments in vitamin A pool size, as estimated by the DRD technique, were 0.120 and 0.243 mmol for the vitamin A supplemented groups (Table 5). Thus, using these commonly applied assumptions regarding vitamin A retention and disposal, the DRD test provided a 2.5-fold overestimate of the maximum potential increment in pool sizes. Even if all of the vitamin A that was consumed was retained (an assumption that is extremely unlikely), the DRD test would have provided overestimates of the likely true pool size. Thus, the high estimates of total body stores of vitamin A in the vitamin A-supplemented groups cannot be explained by the amounts of vitamin A provided by the diet and supplements during the 20-d period. It is more likely that the vitamin A pool sizes of the vitamin A supplemented groups were overestimated because of the diluting effect of the supplemental unlabeled vitamin A on [²H₄]retinol in plasma. In particular, the newly absorbed unlabeled vitamin A of dietary origin probably had a greater diluting effect on the plasma pool of [²H₄]retinol than was anticipated by the prediction equation.

Although consumption of unlabeled vitamin A during the equilibration period can affect the plasma isotopic ratio of [²H₄]retinol to retinol and subsequent estimates of total body

stores of vitamin A, it is unlikely that free-living individuals in vitamin A-deficient populations would be consuming on a daily basis the amounts of vitamin A that were provided to the supplemented groups in this study. Nevertheless, occasional consumption of vitamin A-rich foods, such as liver, during the equilibration period might affect the plasma isotopic ratio and the subsequent estimate of total-body vitamin A stores.

In vitamin A-deficient populations, the usual intake of vitamin A is likely to be low and constant; thus, it would be preferable in future studies to conduct baseline and postintervention studies of pool size of such individuals while they consume their usual level of intake, before and after any supplemental vitamin A is provided. However, in populations with variable amounts of vitamin A in their usual diet, it might be preferable to conduct baseline and postintervention studies of vitamin A pool size while subjects are consuming controlled diets to ensure that daily intake of vitamin A is constant during the equilibration period.

When the DRD technique is used to evaluate the efficacy of vitamin A supplementation programs by comparing vitamin A pool sizes before and after an intervention, the evaluation should be designed to avoid problems in interpreting the data because of the observed rebound effect of the plasma isotopic ratio of labeled to nonlabeled retinol. After the supplementation period, subjects from populations with low dietary intakes of vitamin A should resume their usual (preintervention) vitamin A intakes before the second dilution study is initiated to estimate the postsupplementation vitamin A pool size. This will allow the subjects to adjust to

TABLE 5Comparison of increment in vitamin A body stores estimated by using the deuterated-retinol-dilution (DRD) test results on day 20 with the estimated maximum potential increment in vitamin A pool size after 20 d of supplementation in the vitamin A-supplemented groups¹

	Vitamin A supplement group	
	5.2 $\mu\text{mol RE/d}$ ($n = 10$)	10.5 $\mu\text{mol RE/d}$ ($n = 10$)
Maximum potential increment in vitamin A stores by day 20 (mmol) ²	0.048	0.095
Estimated increment in vitamin A stores as measured by the DRD technique on day 20 (mmol) ^{3,4}	0.120	0.243
Estimated increment in vitamin A stores as measured by the DRD technique on day 20 as a percentage of the maximum likely increment in vitamin A stores (%)	250.0	255.8

¹Initial vitamin A stores assumed to be 0.040 mmol for rounds 1 and 2 combined.²If 50% of the vitamin A provided by the supplements were retained over 20 d of supplementation and assuming a fractional catabolic rate of 0.5%/d.³The DRD technique theoretically estimates total body stores of vitamin A on the day that the test dose is administered; this comparison considers the possibility that the DRD technique estimated total body stores of vitamin A on day 20 rather than at baseline.⁴Calculated as the estimated vitamin A pool size of the supplemented groups on day 20 minus the estimated pool size of the placebo group (0.040 $\pm$ 0.023 mmol) at baseline.

their usual vitamin A intakes so that the plasma isotopic ratios that are measured to estimate the postsupplementation pool size will not be affected by an abrupt change in dietary vitamin A intake. The amount of time that subjects should consume their usual amounts of vitamin A before initiating the second dilution study has not yet been determined. In round 1 of the present study, supplementation with unlabeled vitamin A was discontinued on day 75. Thereafter, plasma isotopic ratios were measured on day 90 only, before the second isotope dilution study was initiated. Thus, the point at which the mean plasma isotopic ratios attained a new equilibrium, or plateau, after day 75 could not be determined. Nevertheless, it is clear that abrupt changes in dietary vitamin A intake can affect plasma isotopic ratios and estimates of total-body stores of vitamin A when the DRD technique is used.

It was possible to estimate total body stores of vitamin A in subjects participating in the second round of the study by using the DRD technique because these subjects continued to receive their assigned supplement throughout the 129-d study period. The estimated mean total-body vitamin A stores were significantly different across the 3 treatment groups and varied in relation to the amount of supplementary vitamin A that was provided. The ratio of observed to theoretical difference in pool size for the 2 vitamin A-supplemented groups was 1.08 and 1.17, and neither ratio was significantly different from 1.0. Thus, the DRD technique detected a differential increment in the total-body vitamin A pool size in the vitamin A-supplemented groups that was very similar to the expected difference, based on theoretical estimates.

Dietary vitamin A intake was strictly controlled during the study period, although it is conceivable that the subjects consumed additional food sources of vitamin A when they returned to their homes at night. However, this is unlikely because the typical Bangladeshi diet consists of rice and lentils and small amounts of curried meats and vegetables and is generally low in vitamin A content (11). Moreover, there was no significant change in the mean plasma retinol concentration or estimated pool size within the placebo group during the study, which suggests that vitamin A intake remained fairly constant throughout the observation period.

In summary, these data show that the DRD technique can detect changes in total-body vitamin A stores in response to supplementation in populations with low vitamin A status. The data therefore suggest that the DRD technique can be used to evaluate the impact of vitamin A intervention programs. However,

note that the subjects in this study consumed controlled diets and remained healthy throughout the study period. Dietary vitamin A intake during the equilibration period, morbidity, and greater variation in vitamin A status in free-living populations may affect the ability of the technique to detect changes in vitamin A pool sizes in response to an intervention. 

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