

Assessment of vitamin A status by the deuterated-retinol-dilution technique and comparison with hepatic vitamin A concentration in Bangladeshi surgical patients¹⁻³

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ABSTRACT Hepatic stores of vitamin A were estimated in 31 Bangladeshi surgical patients (15 males and 16 females) by the deuterated-retinol-dilution (DRD) technique and by analysis of the vitamin A concentration of a liver biopsy specimen obtained during previously scheduled abdominal surgery. Patients ranged in age from 21 to 65 y and had an average body mass index (BMI; in kg/m²) of 17.7 ± 3.4 . They received $0.753 \mu\text{mol } [^2\text{H}_4]\text{retinyl acetate/kg body wt orally 9-11 d before surgery}$. Hepatic vitamin A reserves were estimated according to Furr et al (*Am J Clin Nutr* 1989;49:713-6) by using a single plasma isotopic-ratio measurement (18-25 d postdose). Estimated mean hepatic vitamin A stores were similar by both techniques, $0.110 \pm 0.072 \text{ mmol (by DRD)}$ compared with $0.100 \pm 0.067 \text{ mmol (by biopsy)}$. Regression analysis was used to compare results of the DRD and biopsy techniques. A significant linear relation was found between the two techniques ($r = 0.75$, $P < 0.0001$), and the least-squares regression line was not significantly different from $y = x$ ($P = 0.09$). The results indicate that the DRD technique provided a very good estimate of hepatic vitamin A reserves for this population. However, a wide prediction interval was observed for estimates of hepatic vitamin A reserves for individual subjects. Thus, further refinement of the prediction model is necessary to improve estimates of hepatic vitamin A reserves for individual subjects. *Am J Clin Nutr* 1997;66:67-74.

KEY WORDS Deuterated-retinol-dilution technique, vitamin A status assessment, stable isotope dilution, hepatic vitamin A reserves, vitamin A deficiency, humans, plasma-to-liver ratio

INTRODUCTION

Severe vitamin A deficiency continues to be an important cause of childhood blindness, affecting 250 000-500 000 children each year (1). Moreover, vitamin A deficiency is associated with increased morbidity and mortality from common childhood diseases in developing countries (2, 3), and supplementation with vitamin A can reduce mortality by $\approx 23\%$ among preschool children (4). Various strategies have been used for improving vitamin A status among vulnerable groups, such as the distribution of high-dose vitamin A supplements (5, 6), fortification of selected foods (7), and promotion of in-

creased consumption of foods rich in provitamin A carotenoids (8). However, the efficacy of these intervention strategies for improving vitamin A status has been difficult to determine because of the lack of a sensitive technique for estimating body stores of vitamin A.

The hepatic concentration of vitamin A is considered to be the best indicator of whole-body vitamin A status; however, direct biopsy of the liver for measurement of vitamin A concentration is not a feasible method for assessing vitamin A status in healthy individuals. The indirect biochemical assessment methods that are commonly used include serum retinol concentration (9) and the relative-dose-response tests (10, 11). However, these techniques do not provide quantitative estimates of vitamin A stores and cannot be used to classify individuals across the full range of vitamin A status.

Isotope-dilution techniques for quantitatively estimating total body stores of vitamin A have been validated in animal models by using radiolabeled vitamin A that was either injected intravenously (12-14) or administered orally (15). The deuterated-retinol-dilution (DRD) technique was introduced recently as an indirect method for estimating hepatic reserves of vitamin A quantitatively in humans (16). Briefly, the DRD technique involves administering an oral dose of stable isotope-labeled vitamin A ($[10,19,19,19\text{-}^2\text{H}]\text{retinyl acetate}$, or $[^2\text{H}_4]\text{retinyl acetate}$) to an individual, and after a period of equilibration, drawing a blood sample for the measurement of the isotopic ratio of retinol to $[^2\text{H}_4]\text{retinol}$ in plasma. Hepatic reserves of vitamin A can be estimated based on the principles of isotope dilution and a set of assumptions first described by Bausch and Rietz (13), and later expanded by Furr et al (16).

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The DRD technique was partially validated in a small group of generally healthy surgical patients in the United States (16). However, only one of the subjects had a low hepatic store of vitamin A. Thus, it was not certain whether the DRD technique would be useful for providing a quantitative estimate of hepatic reserves of vitamin A in a population with marginal to adequate vitamin A body stores. The objective of the present study was to examine the relation between the DRD technique and hepatic vitamin A reserves in a group of surgical patients with low to adequate body stores of vitamin A. The study was conducted in Dhaka, Bangladesh, where hepatic vitamin A concentrations at autopsy have been reported to be low (17) and previous studies suggest a low dietary intake of vitamin A (18).

SUBJECTS AND METHODS

Subjects

Thirty-one subjects 21–65 y of age (15 males and 16 females) who were scheduled for elective abdominal surgery were selected from two surgical wards at the Dhaka Medical College Hospital during a period of 10 mo. Patients were eligible for the study if they were free from infectious disease, nonfebrile, and had no recent history of malabsorption or liver or kidney disease. Subjects were screened for coagulation status (prothrombin/partial thromboplastin time), liver function (serum glutamine pyruvate aminotransferase), proteinuria (urinalysis), malabsorption (neutral fat in stools), intestinal parasites (microscopic examination of stool), protein status (serum albumin), and zinc status (plasma zinc). All of the patients had evidence of intestinal helminthic infections and were treated with levamisole ≥ 3 d before any of the vitamin A assessment techniques were performed. Written, informed consent was obtained from each of the subjects, and all of the study procedures were approved by the Human Subjects Review Committee of the University of California–Davis and the Ethical Committee of the Bangladesh Medical Research Council.

[$^2\text{H}_4$]Retinol-dilution study

An oral dose (0.753 $\mu\text{mol/kg}$ body wt) of [$^{10,19,19,19}\text{H}$]retinyl acetate (Cambridge Isotopes, Woburn, MA) in corn oil was administered to the subjects after an overnight fast. Immediately thereafter, they consumed a breakfast low in vitamin A and high in fat (fried unleavened bread with curried white potatoes) to enhance absorption of the dose. Blood was drawn (10 mL) on the day of surgery (9–11 d postdose) and on the day before discharge from the hospital (18–25 d postdose) for measurement of the isotopic ratio of [$^2\text{H}_4$]retinol to retinol in plasma. The subjects were hospitalized throughout the study period and were provided with a standard hospital diet that contained ≈ 450 μg retinol equivalents (REs)/d.

Estimation of hepatic retinol by isotope-dilution method

Hepatic reserves of vitamin A were estimated by using a single plasma isotopic-ratio measurement (18–25 d postdose) based on the equation reported by Furr et al (16). As described previously, total liver reserves (TLR) = $F \times \text{dose} \times (S \times a [(1/D:H) - 1])$, 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 based on the previous work of Bausch and Rietz (13),

the dose is the amount of isotope administered expressed as mg REs, S is the ratio of [$^2\text{H}_4$]retinol to retinol in plasma to that in liver, a is the half-life of vitamin A turnover in the liver, and $D:H$ is the isotopic ratio of [$^2\text{H}_4$]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 (16). For these reasons, the isotopic ratios in plasma and liver do not reach equality. Thus, a factor of 0.65 is used to estimate S to correct for the inequality of the ratios of [$^2\text{H}_4$]retinol to retinol in plasma and liver. The value of 0.65 is based on the observed mean ratio of specific activities of radiolabeled vitamin A in serum to liver in a group of rats with a wide range of hepatic vitamin A concentrations (0.02–0.12 $\mu\text{mol/g}$ liver) (19). The factor a , which is used to correct for the irreversible loss of vitamin A, is based on the half-life of vitamin A turnover in the liver, estimated as 140 d (20). This factor is assumed to be independent of the size of the hepatic store of vitamin A and time-invariant ($a = e^{-kt}$, where $k = 1/140$ d and t = time in days since the dose). Finally, the value -1 corrects for the contribution of the dose of labeled vitamin A to the hepatic vitamin A stores.

Hepatic vitamin A concentration

At the time of surgery, a wedge biopsy of liver tissue (≈ 200 mg) was obtained from the inferior central portion of the right lobe by the attending surgeon. The biopsy specimen was placed in an amber glass vial, protected from light, and immediately transported in a cold box to the laboratory for processing. A small portion of the liver tissue was placed in formalin and forwarded to a pathologist for examination. The remainder of the specimen was weighed accurately and placed in a 0.5-mL polyethylene vial, wrapped in film to prevent evaporation, and stored at -20°C until analyzed.

For measurement of the vitamin A concentration, a portion of the liver tissue (≈ 25 mg) was weighed accurately and ground in ≈ 100 mg anhydrous sodium sulfate until dry. The samples were saponified in 1.0 mL ethanol containing 100 μL of a 10% solution of pyrogallol in ethanol and 200 μL of a 30% aqueous KOH solution. This mixture was heated at 60°C for 1 h in the dark. After cooling, 1.0 mL deionized water was added, followed by 1.0 mL hexane containing 2 mg RE *O*-methyl-retinal-oxime/L as an internal standard. The samples were extracted into 3.0 mL hexane, the hexane layer was separated by centrifugation for 10 min at $2000 \times g$ and 4°C , transferred to a clean vial, and evaporated to dryness. The residue was redissolved in 0.070 mL methanol, vortexed vigorously, and analyzed by HPLC (model 5100; Varian, Sunnyvale, CA) using a mobile phase of 85:15:0.01 acetonitrile:methanol:ammonium acetate (vol:vol:wt), at a flow rate of 1.0 mL/min. Samples were injected onto a C-18, 5- μm column (250 mm $\times$ 4.6 cm internal diameter; Econosphere, Alltech Assoc, Deerfield, IL), and retinol was detected by ultraviolet absorption at 325 nm by using an ultraviolet/visible detector (model UV50; Varian). The liver samples were run in duplicate, and two samples from a reference plasma pool were extracted according to the same protocol and analyzed with each batch of liver samples. Mean ($\pm$ SD) within-run precision (CV) of the vitamin A measurements, based on the reproducibility of the vitamin A concentration of reference plasma, was

$2.7 \pm 1.7\%$. Within-run precision of the vitamin A concentration in liver, based on repeated measurements of chicken liver, was $9.3 \pm 3.0\%$. To assess whether any vitamin A was destroyed during the saponification process, samples of coconut oil were supplemented with a known quantity of retinyl acetate and analyzed according to the same protocol. Mean ($\pm$ SD) recovery of retinol ($\mu\text{g RE}$) from the added retinyl acetate was $99 \pm 5.5\%$.

Isotopic ratio of [$^2\text{H}_4$]retinol to retinol in plasma and liver

The isotopic ratio of [$^2\text{H}_4$]retinol to retinol in plasma and liver samples was determined by gas chromatography–mass spectrometry (GC–MS) as first described by Clifford et al (21) and modified by Handelman et al (22). Briefly, retinol was isolated from plasma and liver samples by HPLC and the *tert*-butyldimethylsilyl (tBDMS) derivative of retinol was formed. The tBDMS derivative is stable at 4°C for ≥ 1 y. Isotopic ratios were estimated by GC–MS on a Trio-2 quadrupole mass spectrometer (VG Masslab, Altrincham, United Kingdom) by using 70-eV electron ionization. Selected ion monitoring (SIM) was carried out for fragment ions of the tBDMS derivatives at m/z 255 (retinol) and m/z 259 ([$^2\text{H}_4$]retinol).

The chemical purity of the isotope was $> 99\%$, as assessed by HPLC, and the isotopic purity was $\approx 80\%$ [$^2\text{H}_4$]retinol and $\approx 20\%$ [$^2\text{H}_3$]retinol. The calibration of the mass spectrometer corrected for the fact that the isotope contained $\approx 80\%$ [$^2\text{H}_4$]retinol, as described below.

A set of calibration standards was prepared and analyzed with each batch of derivitized plasma and liver samples to adjust for the isotopic impurity. Stock solutions of deuterium-labeled retinol and unlabeled retinol were prepared individually in hexane and were standardized based on their extinction coefficients of $51\,000\text{ M}^{-1} \cdot \text{cm}^{-1}$ at 325 nm and molecular weights of 290 and 286, respectively. Calibration standards were prepared by adding 20 μg retinol to 10.00, 3.33, 1.00, 0.33, and 0.00 μg labeled retinol to provide [$^2\text{H}_4$]retinol-retinol weight ratios of 0.500, 0.167, 0.050, 0.0167, and 0.00.

To generate the calibration curve, the area ratios of m/z 259 ([$^2\text{H}_4$]retinol) and m/z 255 (retinol) were determined in SIM mode by GC–MS, as described above. The peak area response for m/z 259 was less than the expected response if the isotope had been 100% [$^2\text{H}_4$]retinol. When the observed isotopic ratios (m/z 259: m/z 255) were plotted against the weight ratios of the calibration standards (labeled retinol:retinol), the slope of the calibration curve was ≈ 0.75 rather than 1.0 because of the isotopic impurity. After subtracting the area ratio obtained for the standard containing no [$^2\text{H}_4$]retinol, a linear-regression equation was calculated between the weight ratios of the calibration standards and the corrected ratios of integrated areas for m/z 259 and m/z 255. The area ratio for the calibration standard that does not contain any [$^2\text{H}_4$]retinol is typically 0.003, whereas the isotopic ratios of the plasma and liver samples from the subjects ranged from ≈ 0.04 to 0.40. Thus, the area ratio of the background is relatively low and can be subtracted without influencing the accuracy of the measurement of the isotopic ratios in the plasma and liver samples. The background is probably caused by hydrogenation of unlabeled retinol due to collisional processes in the mass spectrometer's ionization

source. The hydrogen may come from traces of water in the ionization chamber.

To adjust for the isotopic impurity, a set of calibration standards was analyzed with each set of plasma and liver samples. The area ratio for the standard containing no [$^2\text{H}_4$]retinol was subtracted from the isotopic ratios obtained for the plasma and liver samples. The corrected area ratios were then substituted into the regression equation of the calibration curve to solve for the weight ratios. The within-run precision of the isotopic-ratio measurements was estimated by analyzing standards periodically with each set of plasma or liver samples. The CV for the mean isotopic-ratio measurements for the standards was typically $< 5\%$.

Statistical analysis

Descriptive statistics were calculated for all variables, and those that were not normally distributed were log transformed. The relation between hepatic vitamin A reserves as estimated by the DRD and biopsy techniques was examined by regression analysis and by comparison of means using Student's paired *t* test. All statistical analyses were performed by using the SAS software package (23).

RESULTS

Subjects' characteristics

The physical and clinical characteristics of the subjects at the time of entry into the study are shown in **Table 1**. Subjects ranged in age from 21 to 65 y with a mean ($\pm$ SD) age of 40 ± 10 y. Their clinical diagnoses were chronic duodenal ulcers ($n = 19$), cholelithiasis ($n = 11$), and chronic appendicitis ($n = 1$). The mean BMI was 17.7 ± 3.4 , and 23 (74%) of the subjects had a BMI < 19 . One subject with peptic ulcer disease had a very low BMI of 13.8. The serum albumin concentration was low (31 g/L) in one patient. Serum glutamine pyruvate aminotransferase activity was slightly elevated (40–66 U/L) in nine patients; however, liver tissue was normal by histologic examination in all cases. One subject developed an infection with fever shortly after the dose of labeled vitamin A was administered and was excluded from the analysis.

Plasma and hepatic retinol concentrations

Mean plasma and liver vitamin A concentrations were $1.32 \pm 0.41\text{ }\mu\text{mol/L}$ (0.61–2.33 $\mu\text{mol/L}$) and $0.100 \pm 0.067\text{ }\mu\text{mol/g}$ (0.021–0.252 $\mu\text{mol/g}$), respectively. Eight patients had plasma retinol concentrations $< 1.05\text{ }\mu\text{mol/L}$; however, two of those subjects had elevated concentrations of C-reactive protein, suggesting that their plasma retinol concentrations may have been reduced because of infection or inflammation. Thirteen patients had hepatic retinol concentrations $< 0.070\text{ }\mu\text{mol/g}$, which is the suggested cutoff for marginal vitamin A stores (24).

Plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol

The mean plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol at ≈ 10 d and 18–25 d postdose was compared in subjects with low or adequate hepatic vitamin A concentrations, as assessed by the biopsy technique. As shown in **Table 2**, at ≈ 10 d postdose the mean plasma isotopic ratio among subjects with adequate hepatic vitamin A concentrations ($\geq 0.07\text{ }\mu\text{mol/g}$)

TABLE 1

Physical and clinical characteristics of the surgical patients

Characteristic	Diagnosis					
	Duodenal ulcer or appendicitis		Cholelithiasis		All	
	Males (<i>n</i> = 14)	Females (<i>n</i> = 6)	Males (<i>n</i> = 1)	Females (<i>n</i> = 10)	Males (<i>n</i> = 15)	Females (<i>n</i> = 16)
Age (y)	44.1 ± 11.2 (29–65) ¹	34.3 ± 4.5 (26–40)	52	35.1 ± 8.9 (21–50)	44.7 ± 11.0 (29–65)	34.8 ± 7.2 (21–50)
Height (cm)	161.6 ± 8.7 (146–172)	148.3 ± 7.4 (138–159)	166	152.7 ± 5.5 (145–162)	161.9 ± 8.4 (146–172)	151.1 ± 6.4 (138–162)
Weight (kg)	42.1 ± 6.3 (31–55)	37.7 ± 4.4 (34–45)	39	48.4 ± 10.3 (36–66)	41.9 ± 6.2 (31–55)	44.4 ± 9.9 (34–66)
BMI (kg/m ²)	16.1 ± 1.7 (13.8–19)	17.2 ± 2.8 (13.8–21.5)	14.2	20.7 ± 3.9 (15.4–27.1)	16.0 ± 1.7 (13.8–19.0)	19.4 ± 3.8 (13.8–27.1)
Albumin (g/L)	41 ± 6 (31–50)	45 ± 3 (42–50)	4.5	45 ± 3 (40–50)	41 ± 6 (31–50)	45 ± 3 (40–50)
SGPT (u/L) ²	26.1 ± 16.1 (9–58)	19.4 ± 7.6 (10–31)	44.0	31.3 ± 16.2 (14–66)	27.3 ± 16.2 (9–58)	27.1 ± 14.4 (10–66)

¹ $\bar{x} \pm SD$; range in parentheses.² Serum glutamine pyruvate aminotransferase.

was 0.210 ± 0.103 compared with 0.107 ± 0.057 among subjects with low hepatic vitamin A concentrations ($< 0.07 \mu\text{mol/g}$) ($P < 0.001$). Similarly, at 18–25 d postdose, the mean plasma isotopic ratio among subjects with adequate vitamin A concentrations was 0.181 ± 0.119 compared with 0.095 ± 0.063 ($P < 0.05$) among subjects with low hepatic vitamin A concentrations, as would be expected based on the principle of isotope dilution.

Correlation between the DRD and biopsy techniques

Mean hepatic vitamin A reserves, as estimated by the DRD and biopsy techniques, were compared to examine the relation between the two assessment methods. Total hepatic vitamin A reserves based on the biopsy technique were estimated by multiplying the hepatic retinol concentration by an estimate of liver weight (2.4% of body weight). As shown in **Table 3**, estimates of mean hepatic vitamin A reserves were nearly identical by the two techniques, 0.110 ± 0.072 mmol (DRD) compared with 0.100 ± 0.067 mmol (biopsy) ($n = 30$, $P = 0.27$). Likewise, for those subjects with hepatic vitamin A concentrations $< 0.070 \mu\text{mol/g}$, estimated mean hepatic vitamin A reserves were similar, 0.077 ± 0.050 mmol (DRD) and 0.048 ± 0.017 mmol (biopsy) ($n = 13$, $P = 0.06$). The comparison of means among this subgroup of subjects im-

proved if two outliers, as described below (*see* Outliers), were excluded from the analysis, 0.061 ± 0.036 mmol (DRD) compared with 0.050 ± 0.016 mmol (biopsy) ($P = 0.24$). However, these subjects were not excluded from the analysis because they would be difficult to identify under field conditions.

Regression analysis was used to examine further the relation between the results of the DRD and biopsy techniques. When results of the two procedures were compared, a linear relation provided the best fit (**Figure 1**, $r = 0.75$, $P < 0.0001$, $n = 30$), and the equation for the least-squares line was not significantly different from $y = x$ ($P = 0.09$). The prediction intervals for estimating hepatic vitamin A reserves for a given individual and the 95% CI for the regression line are shown in **Figure 1**. The prediction interval for individual subjects is quite large, indicating that the DRD technique does not provide a precise estimate of hepatic vitamin A reserves for an individual subject. However, the DRD technique provided a very good quantitative estimate of hepatic vitamin A stores for this population of subjects, as indicated by the estimated mean hepatic vitamin A stores assessed by the two techniques.

Isotopic ratio of [²H₄]retinol to retinol in liver

The hepatic isotopic ratio of [²H₄]retinol to retinol was determined to 1) estimate the retention of the test dose of

TABLE 2Plasma isotopic ratio of [²H₄]retinol to retinol in subjects with low or adequate hepatic vitamin A stores¹

Subject groups	Plasma isotopic ratio of [² H ₄] retinol to retinol		Estimated hepatic vitamin A stores ²
	≈10 d postdose	18–25 d postdose	
Subjects with hepatic retinol concentrations $< 0.070 \mu\text{mol/g}$ ³ (<i>n</i> = 11)	0.210 ± 0.103^a (0.092–0.439)	0.181 ± 0.119^a (0.071–0.450)	0.050 ± 0.016 (0.022–0.073)
Subjects with hepatic retinol concentrations $\geq 0.070 \mu\text{mol/g}$ (<i>n</i> = 17)	0.107 ± 0.057^b (0.059–0.28)	0.095 ± 0.063^b (0.041–0.266)	0.139 ± 0.064 (0.062–0.252)

¹ $\bar{x} \pm SD$; range in parentheses. Means with different superscript letters within columns are significantly different, $P < 0.05$.² Based on biopsy technique.³ Excluding three outliers.

TABLE 3

Hepatic vitamin A stores as estimated by the deuterated-retinol-dilution (DRD) and biopsy techniques¹

Subject groups	Estimated hepatic vitamin A stores	
	DRD technique ²	Biopsy technique
	<i>mmol retinol</i>	
All subjects (<i>n</i> = 30)	0.110 ± 0.072 (0.011–0.272)	0.100 ± 0.067 (0.021–0.252)
Subjects with hepatic retinol concentrations < 0.070 μmol/g (<i>n</i> = 13)	0.077 ± 0.050 (0.011–0.178)	0.048 ± 0.017 ³ (0.021–0.073)

¹ $\bar{x} \pm \text{SD}$; range in parentheses.

² Based on a single plasma isotopic measurement (18–25 d postdose).

³ Nearly significantly different from DRD technique, *P* = 0.06.

labeled vitamin A in the liver ≈ 10 d postdose, and 2) examine the plasma-to-liver ratio of [²H₄]retinol to retinol. Retention of the test dose in the liver was estimated as $[D:H/(D:H + 1)] \times \text{THR}$, where D:H is the measured ratio of [²H₄]retinol to retinol in liver, and THR is total hepatic vitamin A reserves as estimated by the biopsy technique. The mean (± SD) hepatic isotopic ratio of [²H₄]retinol to retinol was 0.167 ± 0.094 (0.055–0.419). The estimated mean (± SD) retention of the dose of labeled vitamin A in the liver was 35.0 ± 14.5% (11.2–71.5%), and the measured mean plasma-to-liver ratio of [²H₄]retinol to retinol was 0.79 ± 0.24 (0.38–1.21).

Outliers

Agreement between the DRD and biopsy techniques was particularly poor in four subjects, all of whom had hepatic retinol concentrations < 0.070 μmol/g based on the biopsy technique (12.9%). In each case, the DRD technique overestimated hepatic vitamin A reserves. Three of these subjects had clinical histories consistent with either poor absorption or retention of the dose of labeled retinol, which would result in a lower plasma isotopic ratio and overestimation of hepatic vitamin A reserves. As mentioned above, one patient developed

a fever shortly after receiving the dose of labeled vitamin A. In hospitalized patients with fever, urinary losses of vitamin A can be substantial (25). It is possible that retention of the dose was poor in this patient for that reason. The second subject was diagnosed with a duodenal ulcer, but carcinoma of the stomach was discovered at the time of surgery. That patient had a low serum albumin concentration and slightly elevated serum glutamine pyruvate aminotransferase activity suggestive of impaired liver function. It is possible that this patient did not efficiently absorb or retain the dose of labeled vitamin A because of his illness. The third patient had a history of intestinal malabsorption, but had been asymptomatic for ≈ 3 mo. Although recent symptoms were not reported, the results of the DRD technique are consistent with poor absorption of the dose. This patient may have had a chronic condition resulting in impaired absorption. Furthermore, for these three patients the mean (± SD) estimated retention of the dose of labeled vitamin A in the liver was 12.2 ± 1.2%, which was significantly lower than the mean retention of the dose for the rest of the subjects with hepatic vitamin A concentrations < 0.070 μmol/g (29.8 ± 7.8%, *P* < 0.0003). The mean hepatic vitamin A concentration for these three subjects was 0.045 ± 0.022 μmol/g, which is not different from the mean concentration for the remaining subjects with low hepatic vitamin A stores (0.048 ± 0.017 μmol/g). This further suggests that the three subjects with outlier data did not efficiently absorb or retain the dose of labeled vitamin A. No explanation could be found for the lack of agreement between the two assessment techniques for the fourth outlier.

Only the subject who developed an infection with fever was excluded from the statistical analyses. Fever can be diagnosed easily in a field setting, and subjects who develop fever could be excluded. The individuals with malabsorption or cancer were included in the analyses because they would be difficult to identify in a field setting. However, for the purpose of comparing empirical values for the efficiency of storage of the test dose, and the plasma-to-liver ratio of [²H₄]retinol to retinol with those of the prediction equation, all three of the subjects were excluded from the analysis.

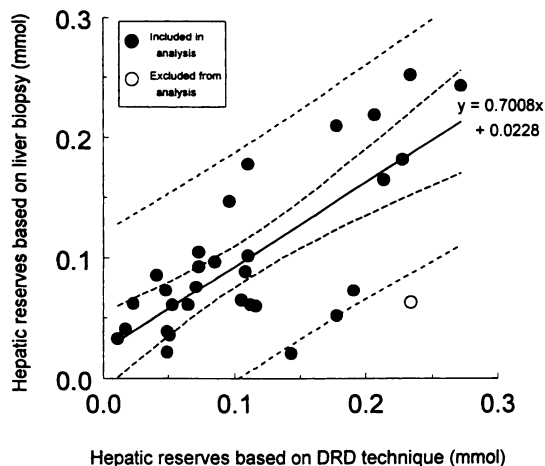


FIGURE 1. Comparison of hepatic vitamin A reserves as estimated by the deuterated-retinol-dilution (DRD) and biopsy techniques. *r* = 0.75, *P* < 0.0001, *n* = 30. Curved confidence bands are 95% CIs of the regression equation; straight confidence bands are 95% prediction intervals for individual subjects.

Evaluation of the equation for estimating hepatic vitamin A reserves

A comparison of empirical values for efficiency of storage of the test dose, and the plasma-to-liver ratio of [²H₄]retinol to retinol with the values used in the prediction equation is shown in Table 4. Retention of the test dose in the liver ranged from 18% to 72% (excluding three subjects), with a mean of 37.8 ± 12.7%, which is significantly different from the value of 50% used in the prediction equation (*P* < 0.0001). The mean plasma-to-liver ratio of [²H₄]retinol to retinol ranged from 0.38 to 1.21 (excluding three subjects), with a mean of 0.80 ± 0.23, which is significantly different from the value of 0.65 used in the prediction equation (*P* = 0.003). For subjects with hepatic retinol concentrations < 0.070 μmol/g, the mean retention of the dose was 29.8% compared with 42.3% for subjects with hepatic retinol concentrations ≥ 0.070 μmol/g (*P* < 0.006, Table 4). The estimated plasma-to-liver ratio of [²H₄]retinol to retinol for subjects with hepatic retinol concentrations < 0.070 μmol/g was 0.74 ± 0.31 compared with 0.83 ± 0.19 for subjects with hepatic retinol concentrations ≥ 0.07 μmol/g.

TABLE 4

Comparison of empirical values with values used in the prediction equation for efficiency of storage of the test dose, and plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol

	Prediction equation value	Observed mean values ¹		
		All subjects	Subjects with < 0.07 μmol retinol/g liver	Subjects with $\geq 0.07 \mu\text{mol}$ retinol/g liver
Efficiency of storage	0.50	$0.378 \pm 0.127^{2,3}$	0.298 ± 0.078	0.423 ± 0.129^4
Plasma-to-liver ratio	0.65	0.80 ± 0.23^3	0.74 ± 0.31	0.83 ± 0.19

¹ Excluding three outliers.

² $\bar{x} \pm \text{SD}$.

³ Significantly different from prediction equation value, $\alpha = 0.01$.

⁴ Significantly different from subjects with < 0.07 μmol retinol/g liver, $\alpha = 0.01$.

DISCUSSION

Hepatic reserves of vitamin A were estimated in 31 adult surgical patients in Bangladesh by the DRD technique and by direct measurement of the concentration of retinol in hepatic biopsy specimens that were obtained at that time of previously scheduled abdominal surgery. In contrast with the earlier study by Furr et al, nearly half of the patients in the present study had hepatic retinol concentrations below the cutoff of 0.070 $\mu\text{mol/g}$ for marginal vitamin A deficiency (24). Mean hepatic vitamin A reserves, as estimated by the DRD and biopsy techniques, were nearly identical for the full group of subjects, and were similar for the subgroup of subjects with low hepatic vitamin A stores. When three outliers were omitted from analysis, the mean hepatic vitamin A stores for the subgroup with low hepatic vitamin A stores were also nearly identical. A significant correlation ($r = 0.75$) between the results of the DRD and biopsy techniques was observed for the full group of subjects, whose estimated hepatic vitamin A reserves ranged from 0.021 to 0.252 mmol. The correlation improved ($r = 0.84$, data not shown) when three subjects who did not appear to absorb the dose of labeled vitamin A efficiently were excluded from the analysis. This is similar to the correlation ($r = 0.88$) observed between the results of the DRD and biopsy techniques in a group of surgical patients in the United States, whose hepatic vitamin A reserves ranged from 0.101 to 1.06 mmol after one of the subjects was omitted from the analysis (16).

To further examine the relation between the DRD and biopsy techniques, the possible sources of variation associated with the two techniques were examined. In the equation for estimating hepatic reserves, the factors that are used to estimate 1) the efficiency of storage of the test dose, 2) the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol, and 3) the irreversible loss of vitamin A over time will vary with vitamin A status. For this reason, the predictive ability of the equation may also vary with vitamin A status. On the other hand, the biopsy technique may not provide an accurate estimate of hepatic vitamin A reserves because the distribution of vitamin A in the liver is heterogeneous. However, it has been shown that in the human liver the retinol concentration of tissue obtained from the central portion of the right lobe is within 15% of the overall mean hepatic vitamin A concentration (26). The biopsy specimens from the subjects in the present study were obtained from that site.

In their early experiments, Bausch and Rietz (13) showed that after an injected dose of radiolabeled vitamin A had equilibrated with the vitamin A body pool in rats, ≈ 6 –40% of the dose was retained in the liver in rats with hepatic stores of

vitamin A < 0.07 $\mu\text{mol/g}$, whereas ≈ 50 –63% of the dose was retained in rats with adequate to high hepatic vitamin A stores. Presumably, this occurs because retinol is mobilized from the liver to meet the vitamin A needs of peripheral tissues, and stored to a lesser degree in rats with low hepatic stores of vitamin A. Similarly, in the present study, the retention of the dose in liver was significantly lower (29.8%) in subjects with low hepatic retinol concentrations (< 0.070 $\mu\text{mol/g}$) when compared with those with adequate hepatic retinol concentrations (42.3%), $P < 0.006$. Thus, efficiency of storage of the test dose appears to be different in subjects with low or adequate body stores of vitamin A. A value of 0.5 is used as an estimate of efficiency of storage in the prediction equation for well-nourished subjects. In the present study, the mean value for efficiency of storage (0.378) was significantly lower than the value of 0.50, which is used in the prediction equation. However, note that the hepatic isotopic-ratio measurement that was used to estimate retention was determined in biopsy specimens that were collected 9–11 d after the dose of labeled vitamin A was administered. Although there was a difference in retention of the test dose between subjects with low or adequate hepatic vitamin A reserves, the absolute mean value of 0.378 may not be a good estimate of the ultimate efficiency of storage because the test dose may not have fully mixed with the body pool at that time.

A factor of 0.65 is used as an estimate of the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol in the prediction equation to estimate hepatic reserves. That value was taken from a study in which the reported mean ratio of the specific activities of radiolabeled vitamin A in plasma-to-liver in rats with varying body stores of vitamin A was 0.65 (range: 0.46–0.81) (19). Although the ratio was variable, it did not vary systematically with dietary vitamin A intake or hepatic vitamin A stores in rats (19). In the present study, the mean ratio of [$^2\text{H}_4$]retinol to retinol in plasma to that in liver was 0.80 ± 0.23 , which was significantly different from the factor of 0.65 ($P < 0.005$). The ratio ranged from 0.38 to 1.21, but did not vary systematically with vitamin A stores, which agrees with previous observations in rats (19). The plasma and hepatic isotopic-ratio measurements that were used to calculate this ratio were determined in samples that were obtained 9–11 d after the dose of labeled vitamin A was administered. Some of the variation in the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol may be explained by interindividual differences in the extent to which the labeled vitamin A had mixed with the body pool of vitamin A at that time. While in the hospital the subjects received standard meals

that contained $\approx 450 \mu\text{g RE/d}$; thus, it is not likely that differences in dietary vitamin A intake among the subjects contributed to the variation in the estimate of the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol.

Although the factors for efficiency of storage of the test dose and the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol appear to be different in well-nourished and poorly nourished subjects, the factors used in the equation developed by Furr et al (16) provided a reasonably good estimate of hepatic vitamin A reserves for well-nourished subjects in the United States and for poorly nourished subjects in the present study. In the equation, the factor of 0.5 for efficiency of storage, and the factor of 0.65 for the plasma-to-liver isotopic ratio are multiplied together resulting in a coefficient of 0.325. If the mean values for the factors for efficiency of storage (0.378), and the plasma-to-liver ratio of [$^2\text{H}_4$]retinol to retinol (0.80) observed in the present study are multiplied together, the resulting coefficient is 0.302, which is not different from 0.325 ($P = 0.674$). Thus, it appears that the two factors compensate for one another to a certain extent as vitamin A stores change.

The factor that is used for the irreversible loss of vitamin A during the equilibration period is based on the half-life of vitamin A turnover in the liver, which is estimated as 140 d for well-nourished adults (16, 20). However, the half-life of vitamin A turnover in the liver has been determined in only a small number of adults and appears to vary substantially among individuals. The mean half-life for the depletion of body reserves of vitamin A has been reported as 154 d, with a range of 75 to 240 d and a CV of 35% (20). Furthermore, it has been shown that the fractional catabolic rate is similar in rats with low or marginal vitamin A stores, but significantly higher in rats with high stores (27). In a separate study (MJ Haskell, GJ Handelsman, MA Islam, AD Jones, JM Peerson, MA Wahed, D Mahalanabis, KH Brown, unpublished observations, 1996), we estimated the fractional catabolic rate for four adult Bangladeshi subjects with low to adequate vitamin A stores, based on the kinetics of the fraction of the dose of labeled vitamin A in plasma over a period of 90 d, after ingestion of an oral dose of [$^2\text{H}_4$]retinyl acetate. The mean fractional catabolic rate for the four subjects was $0.90 \pm 0.25\%$ of body stores/d (0.56–1.16%), which is not significantly different from the factor of 0.5%/d used in the prediction equation. This corresponds to an estimated mean half-life for vitamin A turnover in the liver of 111 ± 40 d (86–179 d) for these four subjects with a CV of 28%. This estimate is lower than the value of 140 d for well-nourished subjects, but also highly variable. Thus, use of a single estimate for the fractional catabolic rate in the prediction equation will contribute to error in the estimate of hepatic vitamin A reserves because of the large variation in the fractional catabolic rate among individuals.

Lastly, the proportion of total-body vitamin A that is stored in the liver will vary with vitamin A status. In well-nourished individuals, it is estimated that $> 90\%$ of total-body vitamin A is found in the liver, thus, an estimate of the total amount of vitamin A in the liver reflects total body stores of vitamin A. However, in marginal vitamin A deficiency, the proportion of total-body vitamin A that is stored in the liver is likely to be lower. The liver biopsy technique provides an estimate of total hepatic vitamin A, whereas the DRD technique provides an estimate of total body stores of vitamin A. Thus, the DRD technique may overestimate hepatic vitamin A reserves in

depleted subjects when compared with the biopsy technique. In the present study, the DRD technique provided a higher mean estimate of hepatic vitamin A reserves for depleted individuals than did the biopsy technique (Table 3). This supports the view that the DRD may overestimate hepatic reserves in depleted individuals. However, as shown in Figure 1, the DRD technique did not consistently overestimate hepatic vitamin A reserves for all depleted individuals.

In summary, the prediction equation originally developed by Furr et al (16) for well-nourished individuals in the United States provides a reasonably good quantitative estimate of hepatic vitamin A reserves for groups of individuals with low to adequate vitamin A body stores. The DRD technique is valuable in that it is the only indirect method that provides a quantitative estimate of vitamin A stores. The technique might be useful for estimating quantitative changes in hepatic stores of vitamin A in response to vitamin A supplementation, and therefore, as a method for evaluating vitamin A intervention strategies. It might also be useful as a primary reference method for validating other vitamin A assessment techniques. However, malabsorption, infection, and fever are likely to result in poor absorption or retention of the dose of labeled vitamin A. Likewise, dietary vitamin A intake will influence the plasma isotopic ratio of [$^2\text{H}_4$]retinol to retinol. Therefore, further work is needed to determine the sensitivity of the technique for detecting changes in vitamin A stores under conditions that are encountered in field situations. Whenever the DRD technique is used to estimate vitamin A stores, information on morbidity and dietary vitamin A intake should be collected throughout the study period to better interpret the results. 

REFERENCES

1. Sommer A, West K Jr. Vitamin A deficiency. Health, survival and vision. Oxford, United Kingdom: Oxford University Press, 1996.
2. Sommer A, Katz J, Tarwotjo I. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. *Am J Clin Nutr* 1984;40:1090–5.
3. Bloem MW, Wedel M, Egger RJ. Mild vitamin A deficiency and risk of respiratory tract disease and diarrhea in preschool and school children in northeastern Thailand. *Am J Epidemiol* 1990;131:332–9.
4. Beaton GH, Martorell R, L'Abbe KA, et al. Effectiveness of vitamin A supplementation in the control of young child morbidity and mortality in developing countries. Toronto: Department of Nutritional Sciences, University of Toronto, 1992.
5. Bloem MW, Wijnroks M, Ralte A, West KP Jr, Sommer A. The role of universal distribution of vitamin A capsules in combatting vitamin A deficiency in Bangladesh. *Am J Epidemiol* 1995;142:843–55.
6. West KP Jr, Pettiss ST. Control of vitamin A deficiency by the vitamin A periodic dosing approach. In: Bauernfeind JC, ed. Vitamin A deficiency and its control. Orlando, FL: Academic Press, 1986:325–57.
7. Bauernfeind JC, Arroyave G. Control of vitamin A deficiency by the nutrification of food approach. In: Bauernfeind JC, ed. Vitamin A deficiency and its control. Orlando, FL: Academic Press, 1986:359–88.
8. Teply LJ. Control of vitamin A deficiency by the agricultural crop and household food approach. In: Bauernfeind JC, ed. Vitamin A deficiency and its control. Orlando, FL: Academic Press, 1986:319–24.
9. Underwood BA. Biochemical and histological methodologies for assessing vitamin A status in human populations. *Methods Enzymol* 1990;190:242–51.
10. 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;40:1281-9.
11. Tanumihardjo SA, Muhilal, Yuniar Y, et al. Vitamin A status in preschool-age Indonesian children as assessed by the modified relative-dose-response assay. *Am J Clin Nutr* 1990;52:1068-72.
 12. Rietz P, Vuilleumier JP, Weber F, Wiss O. Determination of the vitamin A bodypool of rats by an isotopic dilution method. *Experientia* 1973;29:168-70.
 13. Bausch J, Rietz P. Method for the assessment of vitamin A liver stores. *Acta Vitaminol Enzymol* 1977;31:99-112.
 14. 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.
 15. Adams WR, Green MH. Prediction of liver vitamin A in rats by an oral isotope dilution technique. *J Nutr* 1994;124:1265-70.
 16. 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.
 17. Abedin Z, Hussain MA, Ahmad K. Liver reserve of vitamin A from medico-legal cases in Bangladesh. *Bangladesh Med Res Counc Bull* 1976;2:42-51.
 18. Institute of Nutrition and Food Science. Nutrition survey of rural Bangladesh 1981-82. Dhaka, Bangladesh: University of Dhaka, 1983.
 19. 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.
 20. Sauberlich HE, Hodges HE, Wallace DL, et al. Vitamin A metabolism and requirements in the human studied with the use of labelled retinol. *Vitam Horm* 1974;32:251-75.
 21. Clifford AJ, Jones AD, Tondeur Y, Furr HC, Bergen HR, Olson JA. Assessment of vitamin A status of humans by isotope dilution GC/MS. *Proc Mass Spectrom Allied Top* 1986;34:327-8.
 22. Handelman GJ, Haskell MJ, Jones AD, Clifford AJ. An improved protocol for determining ratios of retinol-d4 to retinol isolated from human plasma. *Anal Chem* 1993;65:2024-8.
 23. SAS Institute Inc. SAS/STAT user's guide, 6th ed. Cary, NC: SAS Institute, Inc, 1989.
 24. Olson JA. Recommended dietary intakes (RDI) of vitamin A in humans. *Am J Clin Nutr* 1987;45:704-16.
 25. Stephensen CB, Alvarez JO, Kohatsu J, Hardmeier R, Kennedy JI Jr, Gammon RB Jr. Vitamin A is excreted in the urine during acute infection. *Am J Clin Nutr* 1994;60:388-92.
 26. Olson JA, Gunning DB, Tilton RA. The distribution of vitamin A in human liver. *Am J Clin Nutr* 1979;32:2500-7.
 27. Green MH, Green JB, Lewis KC. Variation in retinol utilization rate with vitamin A status in the rat. *J Nutr* 1987;117:694-703.