Quantitative assessment of total body stores of vitamin A in adults with the use of a 3-d deuterated-retinol-dilution procedure[1–4]

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ABSTRACT

Background: The conventional deuterated-retinol-dilution (DRD) technique provides a quantitative estimate of total body stores of vitamin A in humans. The procedure requires equilibration of serum deuterated retinol with nondeuterated retinol after administration of an oral dose of deuterated vitamin A. Equilibration takes ≈3 wk to complete.

Objective: Our goal was to develop a predictive mathematical formula for quantitative assessment of total body stores of vitamin A in adults by using a procedure that takes less time to perform because serum isotope equilibration is not required, so that blood drawing can be done 3 d, instead of ≈3 wk, after isotope dosing.

Design: Ratios of serum deuterated to nondeuterated retinol (D:H retinol) were determined in Filipino adults (n = 68) 3 and 20 d after an oral dose of 0.015 mmol [$^2H_4$]retinyl acetate and in Guatemalan adults (n = 15) 3 and 21 d after a 0.030-mmol dose. D:H retinol values 20 or 21 d after the isotope dose were used in a mathematical formula to obtain quantitative estimates of total body stores of vitamin A that were then correlated with serum D:H retinol values 3 d after the isotope dose.

Results: The relation between these variables was nonlinear and was described by the following equation: total body stores of vitamin A (in mmol retinol) = $0.00468 \times 10^{37(\text{isotope dose in mmol})}$/D:H retinol in serum 3 d after the isotope dose.

Conclusion: A 3-d DRD technique could be used for quantitative assessment of total body stores of vitamin A; this technique takes less time than does the conventional DRD technique. *Am J Clin Nutr* 2003;77:694–9.

KEY WORDS Deuterated retinol dilution, DRD, 3-d DRD, stable isotope dilution, vitamin A body stores, vitamin A status assessment, Phillipines, Guatemala

INTRODUCTION

The deuterated-retinol-dilution (DRD) technique is useful for obtaining numerical estimates of the body's total vitamin A reserves. It is based on the principle that when a person's vitamin A status is poor, less dilution of a dose of deuterated vitamin A by endogenous vitamin A occurs, resulting in a higher ratio of deuterated to nondeuterated retinol (D:H retinol) in serum. The DRD procedure involves *1*) administration of an oral dose of deuterated retinyl acetate, *2*) determination of D:H retinol in serum after equilibration of deuterated retinol with endogenous nondeuterated retinol, and *3*) application of a mathematical formula (henceforth referred to as the DRD equation) described by Furr et al (1) to calculate total body reserves of vitamin A. This formula, which is a modification of the formula developed by Bausch and Rietz (2) in rats, has been used to assess vitamin A stores in US (1, 3), Bangladeshi (3–5), and Guatemalan (6) adults. The DRD technique has also been used to assess vitamin A stores in a US child (3) and in Chinese (7) and Nicaraguan (8) children, although work is needed to validate the DRD equation in this age group.

In our previous study among Guatemalan elders (6), we found an inverse relation between total body stores of vitamin A calculated by using the DRD equation (1) and serum D:H retinol values 3 d after the isotope dose (Spearman $r = -0.81$, $P = 0.004$); thus, we speculated that the retinol isotope ratio in serum 3 d after an oral dose of deuterated vitamin A might be used as an early indicator of vitamin A body stores. In that study (6), we also found that Guatemalan elders have adequate vitamin A status; their total body reserves are similar to values reported by Furr et al (1) for relatively healthy, adult American surgical patients. To confirm the relation between vitamin A body stores and serum D:H retinol values 3 d after the isotope dose and to determine any practical consequences thereof, in the present study, we sought a population of subjects with ages comparable to those of the subjects in the Guatemalan study (6) but with poorer overall vitamin A status. On the basis of our earlier finding of a high prevalence of hypovitaminosis A in rural Filipino schoolchildren (9), we anticipated that elders residing in similar rural Philippine communities would

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allow us to extend our observations into the lower range of total-body vitamin A reserves.

The aim of the present study was to formulate a predictive mathematical formula for the quantitative assessment of total body reserves of vitamin A in older adults with the use of serum D:H retinol values obtained 3 d after an oral dose of deuterated vitamin A. Our goal was to generate a predictive equation by using newly obtained data from Filipino elders and combining these data with previously obtained data from Guatemalan elders (6) to obtain a wide range of deficient-to-adequate values of vitamin A body reserves from which to derive the predictive equation. A 3-d DRD procedure, which does not require equilibration of deuterated retinol with endogenous retinol in serum, has the advantage of taking considerably less time than does the conventional DRD procedure.

SUBJECTS AND METHODS

Subjects

Elderly Filipino men and women (≥60 y old; $n = 172$) residing in the rural, low socioeconomic communities of Malabanan and Palsara in the municipality of Balete, Batangas province, ≈70 km south of Manila, underwent screening procedures that consisted of a medical history, complete physical examination, chest X-ray, electrocardiogram, and blood and urine tests. The inclusion criteria for study participation were being ambulatory and having no major chronic illnesses, prolonged diarrhea, or other conditions that might interfere with vitamin A absorption. Written informed consent was obtained from participants in the screening and study procedures. Sixty-eight subjects (33 women and 35 men) completed the procedures for both the conventional DRD and the 3-d DRD methods. Approval to conduct the study was obtained from the Tufts University-New England Medical Center Human Investigation Review Committee and the Ethical Review Board of the Philippine Council for Health Research and Development. The Guatemalan elderly subjects also resided in rural communities and were in generally good health, similar to that of the Filipino elders. Guatemalan subjects were treated with the antihelminthic drug albendazole before the study, whereas the Filipinos were found to have mild-to-moderate helminthic infections, which subsequent data analyses showed not to interfere with their vitamin A status measures. The procedures and ethical considerations for the Guatemalan study were described in our earlier publication (6).

Vitamin A isotopes, conventional DRD and 3-d DRD procedures, blood handling, and serum analyses

Tetradeuterated retinyl acetate (*all-trans*-retinyl-10,19,19,19-[$^2H_4$]acetate) was synthesized by Cambridge Isotope Laboratories (Andover, MA). Capsules containing a predetermined amount of the stable isotope in corn oil were prepared by first dissolving the isotope in absolute ethanol, adding corn oil, and evaporating off the ethanol under nitrogen, as previously described (6). In the Philippines, the capsules contained 0.015 mmol [$^2H_4$]retinyl acetate, whereas in Guatemala they contained 0.030 mmol of the same vitamin A isotope.

For conventional DRD and 3-d DRD procedures, study participants reported to the study center 3 times: on day 0, to ingest the [$^2H_4$]retinyl acetate capsule with a fat-containing meal consisting of fried rice and fried meat loaf; and 3 and 20 d later, for a fasting (12-h) venipuncture sample. To protect retinoids from photodegradation, blood was drawn into aluminum-wrapped evacuated tubes, allowed to clot, and centrifuged at 2800 × *g* for 10 min at room temperature in a dark room. Serum was pipetted into cryovials, which were stored at −20 °C until they were transported to Manila under ice and stored in a freezer at −70 °C; the frozen samples were carried by hand under dry ice to the Human Nutrition Research Center on Aging at Tufts University in Boston, where they were kept at −70 °C until they were analyzed. Similar blood drawing and handling procedures were used in the Guatemalan study (6).

All laboratory procedures in Boston were carried out under dim red light. Serum retinol in blood obtained during screening (before ingestion of the [$^2H_4$]retinyl acetate capsule) was extracted with chloroform:methanol (2:1, vol:vol) and hexane as previously described (10) and analyzed by gradient reversed-phase HPLC at 340 nm with a C_{30} column (11) for the Philippine samples and a C_{18} column (10) for the Guatemalan samples. Sera from blood drawn 3 and 20 or 21 d after the administration of [$^2H_4$]retinyl acetate were analyzed for deuterated and nondeuterated retinol isotopes by separating retinol from the other constituents of serum by using HPLC, collecting the retinol fraction, and derivatizing retinol into trimethylsilyl derivatives, which were then measured by gas chromatography–mass spectrometry (12).

Data analyses and formulation of a 3-d DRD predictive equation for total body stores of vitamin A

The DRD equation described by Furr et al (1) for obtaining an estimate of the total body stores of vitamin A is

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

where F is a factor that expresses the storage efficiency of an orally administered dose and is considered to be 0.5 on the basis of studies in rats by Bausch and Rietz (2), "dose" is the amount of labeled vitamin A (in mmol) administered orally, and 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 corrects for the irreversible loss of vitamin A and is based on the half-life of vitamin A turnover in the liver, which was estimated to be ≈140 d in 31–43-y-old adults (13). For simplicity, it is assumed that a is independent of the size of the vitamin A stores and is time invariant: $a = e^{-kt}$, where $k = 0.693/140$ or 0.5% per d, and t is time (in d) since the isotope dose was administered. The term "− 1" corrects for the contribution of the administered dose to the total-body vitamin A pool. The factors S and a correct for the facts that in humans, it is not possible to attain a truly equilibrated state because of continued ingestion of unlabeled dietary vitamin A and that with time unlabeled dietary retinol replaces labeled retinol lost in catabolism.

From gas chromatography–mass spectrometry data, calculations were done of D:H retinol in serum at 3 and 20 or 21 d after the oral dose of deuterated retinyl acetate. Regression analyses and Pearson's product-moment correlation analyses were used to study the relation between serum D:H retinol values at 3 d and those at 20 or 21 d. The D:H retinol values at 20 or 21 d were used in the DRD equation to obtain quantitative estimates of total body stores of vitamin A (1). These calculated values of vitamin A stores were then correlated with their corresponding serum D:H retinol values at 3 d, and because the relation between the 2 variables is nonlinear, logarithmic transformations were done. Data

TABLE 1
Characteristics and vitamin A status of the subjects in the Philippine and Guatemalan studies[1]

	Philippines (n = 35 M, 33 F)	Guatemala (n = 7 M, 8 F)
Age (y)	69 ± 1 (60–91)	70 ± 2 (60–81)
Body weight (kg)	47.8 ± 1.2 (30.0–75.1)	45.9 ± 1.7 (32.0–55.0)
Serum albumin (g/dL)	4.3 ± 0.03 (3.7–4.8)	4.3 ± 0.13 (2.7–5.1)
Serum retinol (μmol/L)	1.85 ± 0.07 (0.97–3.53)	1.68 ± 0.13 (0.58–2.63)
Serum D:H retinol at day 3[2]	0.117 ± 0.011 (0.028–0.559)	0.095 ± 0.017 (0.048-0.315)
Serum D:H retinol at day 20 or 21[3]	0.031 ± 0.003 (0.007–0.180)	0.014 ± 0.003 (0.006–0.047)
Estimated total-body vitamin A stores (mmol retinol)[4]	0.22 ± 0.02 (0.02–0.66)	0.83 ± 0.10 (0.18–1.43)[5]
Estimated liver vitamin A (μmol/g liver)[6]	0.17 ± 0.01 (0.02–0.56)	0.67 ± 0.07 (0.13–1.01)[5]

[1] $\bar{x}$ ± SEM; range in parentheses. D:H retinol, ratio of deuterated to nondeuterated retinol. Serum D:H retinol values at 3 and 20 or 21 d after the dose of [$^2H_4$]retinyl acetate may not be compared in the 2 studies because different doses of [$^2H_4$]retinyl acetate were administered.
[2] After an oral dose of 0.015 mmol [$^2H_4$]retinyl acetate (Philippines) or 0.030 mmol [$^2H_4$]retinyl acetate (Guatemala).
[3] At 20 d after an oral dose of 0.015 mmol [$^2H_4$]retinyl acetate (Philippines) or at 21 d after an oral dose of 0.030 mmol [$^2H_4$]retinyl acetate (Guatemala).
[4] Estimated by using the deuterated-retinol-dilution equation (1).
[5] Significantly different from Philippines, $P = 0.0001$ (Student's t test).
[6] Estimated by assuming that the weight of the liver is 2.4% of body weight and that 90% of total body vitamin A is in the liver.

from the Philippine and Guatemalan studies (in which the oral doses of deuterated retinyl acetate administered were 0.015 mmol and 0.030 mmol, respectively) were fitted in linear regression models, which were then combined to obtain a predictive mathematical formula for total body stores of vitamin A with the use of D:H retinol data at 3 d, at doses of orally administered [$^2H_4$]retinyl acetate between 0.015 and 0.030 mmol.

Student's t test was used to compare age, body weight, serum albumin, serum retinol, estimated total-body vitamin A stores, and estimated liver vitamin A concentrations between the Filipino and Guatemalan subjects. Liver vitamin A concentrations were estimated by making the assumption that the weight of the liver is 2.4% of body weight in nonobese adult subjects and that 90% of total body vitamin A is found in liver (13, 14). Serum D:H retinol values in the 2 studies cannot be compared because different doses of the vitamin A isotope were administered. The statistical software STATVIEW SE+GRAPHICS (Abacus Concepts, Inc, Berkeley, CA) and SYSTAT version 10 (SPSS Inc, Chicago) were used in data analyses.

RESULTS

The characteristics and vitamin A status of the subjects in the Philippine and Guatemalan studies are summarized in **Table 1**. The mean ages, body weights, and serum albumin concentrations of the subjects were not significantly different between the 2 studies. Mean (± SEM) serum retinol concentrations were also not significantly different between the 2 studies: the values were 1.85 ± 0.07 and 1.68 ± 0.13 μmol/L for the Filipinos and the Guatemalans, respectively ($P > 0.05$). However, the Filipino elders had very poor estimated total body stores of vitamin A compared with the Guatemalan elders (0.22 ± 0.02 compared with 0.83 ± 0.10 mmol retinol; $P = 0.0001$). Similarly, the estimated liver vitamin A concentrations in the Filipinos were low compared with those in the Guatemalans (0.17 ± 0.01 compared with 0.67 ± 0.07 μmol/g liver; $P = 0.0001$) when 90% of total body reserves of vitamin A was assumed to be in the liver. Because liver vitamin A stores may be ≥ 90% of total body stores in well-nourished persons and < 90% of total body stores in poorly nourished persons (13, 14), the values for liver stores may have been underestimated for some well-nourished subjects and overestimated for some malnourished subjects.

In the Philippine study, a strong linear relation existed between serum D:H retinol values 3 d after the [$^2H_4$]retinyl acetate dose and those 20 d after the dose ($r = 0.90$, $P = 0.0001$; **Figure 1**); a similar relation between serum D:H retinol values at 3 and 21 d occurred in the Guatemalan study ($r = 0.94$, $P = 0.0001$; Figure 1). When the retinol isotopic ratios at 20 or 21 d were used in the DRD equation to obtain estimates of total body stores of vitamin A (1), a nonlinear relation was found between these estimated values and serum D:H retinol values 3 d after the isotope dose (**Figure 2**). Logarithmically transformed data from each study were fitted by using linear regression models; the results from the 2 studies parallel one another (Figure 2). The models were combined, and data fitted into the combined model were described by the following equations.

$$\text{Log total body stores of vitamin A} = b_0 + b_1(\log \text{D:H retinol at 3 d}) + b_2(\text{isotope dose}) \quad (2)$$

where b_0 is the y intercept and b_1 and b_2 are coefficients.

$$\text{Log total body stores of vitamin A} = -2.297 - 0.965(\log \text{D:H retinol at 3 d}) + 37.148(\text{isotope dose}) \quad (3)$$

$$\text{Total body stores of vitamin A} = 10^{-2.297 + 37.148(\text{isotope dose})}/(\text{D:H retinol at 3 d})^{0.965} \quad (4)$$

$$\text{Total body stores of vitamin A} = 0.00505 \times 10^{37.148(\text{isotope dose})}/(\text{D:H retinol at 3 d})^{0.965} \quad (5)$$

Equation *5* can be further simplified to

$$\text{Total body stores of vitamin A (in mmol retinol)} = 0.00468 \times 10^{37[\text{isotope dose (in mmol)}]}/\text{D:H retinol in serum 3 d after the isotope dose} \quad (6)$$

The curves described by Equations *5* and *6* were virtually identical and superimposable. The orally administered isotope dose and the calculated value for total body stores of vitamin A are expressed in mmol.

DISCUSSION

The liver is the main storage organ for vitamin A in humans (15); thus, a good way to assess vitamin A status is to measure

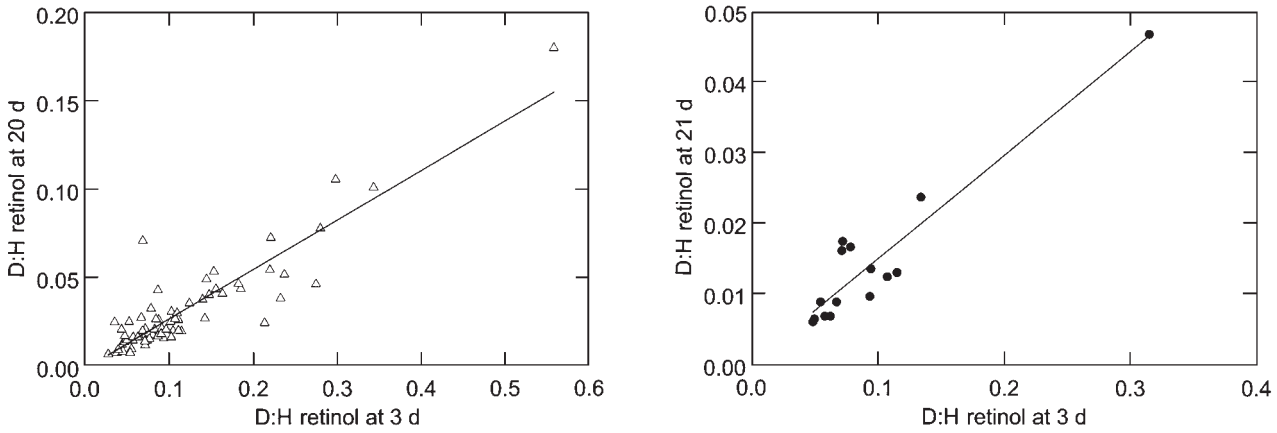

FIGURE 1. Serum ratios of deuterated to nondeuterated retinol (D:H retinol) 3 and 20 d after an oral dose of 0.015 mmol [$^2H_4$]retinyl acetate in Filipino elders ($r = 0.90$, $P = 0.0001$) and 3 and 21 d after an oral dose of 0.030 mmol [$^2H_4$]retinyl acetate in Guatemalan elders ($r = 0.94$, $P = 0.0001$).

hepatic stores. Because direct measurement of hepatic vitamin A is not feasible under normal circumstances, various indirect methods are used for biochemical assessment of vitamin A status, such as circulating retinol concentrations, the relative-dose-response test (16), and the modified-relative-dose-response test (17). Serum (or plasma) retinol is not a measure of vitamin A stores because it is subject to homeostatic control over a wide physiologic range of liver vitamin A concentrations (14). The relative-dose-response and modified-relative-dose-response tests are qualitative measures of the liver reserve of vitamin A because they do not provide a numerical estimate of liver stores. The DRD procedure (1) gives a quantitative estimate of total body reserves of vitamin A in humans, without the need for direct measurement of liver samples. The validity of the predictive mathematical formula used in the DRD technique for estimating total-body vitamin A stores has been verified in studies by 2 separate laboratories (1, 4). In generally healthy adult surgical patients in the United States, Furr et al (1) compared calculated values with values obtained by directly measuring vitamin A in liver biopsy specimens with the use of HPLC. A significant linear relation was found between these 2 measurements, with $r = 0.88$ and Spearman $r = 0.95$. Similarly, in adult Bangladeshi surgical patients with low-to-adequate vitamin A status, Haskell et al (4) found good agreement between values of vitamin A reserves calculated by using the DRD equation and values obtained directly by HPLC measurements of liver biopsy specimens ($r = 0.75$).

The DRD procedure, however, requires that deuterated retinol from the orally administered dose of stable isotope equilibrate with the endogenous retinol in serum before blood can be drawn for the determination of retinol isotopic ratios. Isotope equilibration in serum takes ≈3 wk to complete in adults (3, 6); thus, a stable-isotope-dilution method with a shorter procedure time would be preferable. In rats, Adams and Green (18) developed predictive equations for estimating total liver stores of vitamin A over a wide range of liver vitamin A values; unlike the formula developed by Bausch and Rietz in rats (2) and that described by Furr et al in humans (1), the predictive equations of Adams and Green do not require equilibration of the administered vitamin A isotope in

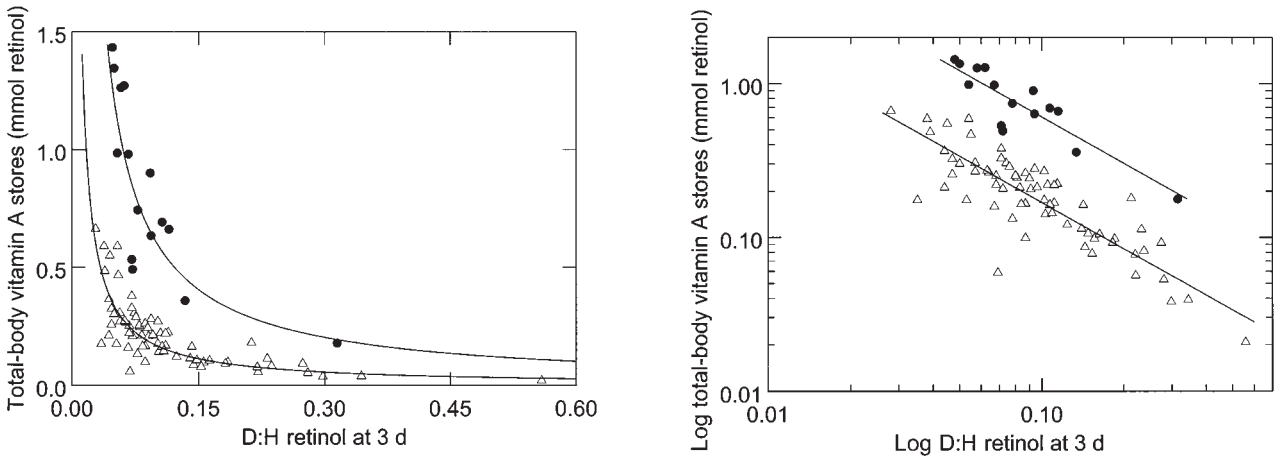

FIGURE 2. Left: nonlinear relation between serum ratios of deuterated to nondeuterated retinol (D:H retinol) 3 d after an oral dose of 0.015 (△) or 0.030 (●) mmol [$^2H_4$]retinyl acetate and total-body vitamin A stores. Right: linear relation between log D:H retinol in serum 3 d after an oral dose of 0.015 (△) or 0.030 (●) mmol [$^2H_4$]retinyl acetate and log total-body vitamin A stores. The DRD equation (1) was used for estimating total-body vitamin A stores.

serum. These equations also do not require an estimate of the efficiency of absorption and liver retention of the test dose of vitamin A, a correction for the difference between plasma and liver specific activities, or any assumption regarding metabolism of the test dose between the time of administration and that of blood sampling. All the aforementioned physiologic and metabolic processes are empirically reflected in their predictive equations (18).

In a study in Guatemalan elders (6), we reported a good correlation between serum D:H retinol 3 d after an isotope dose and total body stores of vitamin A as calculated by the DRD equation. In food-intervention studies among malnourished schoolchildren in the Philippines (9), we showed that serum D:H retinol values 3 d after a dose of deuterated vitamin A can be used to detect changes in vitamin A status in response to increased fruit and vegetable intakes; thus, we reported lower serum D:H retinol values after the dietary intervention than at baseline, indicating an improvement in vitamin A status. We also reported that the higher the baseline D:H retinol value at 3 d (ie, the poorer the vitamin A status), the greater was the reduction in D:H retinol values at 3 d (ie, improvement in vitamin A status) in response to the fruit and vegetable feeding intervention, thus indicating that bioconversion of plant carotenoids to vitamin A in humans varies inversely with their baseline vitamin A status. In that study, we reported an exceptionally high negative correlation between the preintervention isotopic ratio at 3 d and the reduction in isotopic ratio in response to the intervention ($r = -0.99$, $P = 0.0001$), denoting that the 3-d DRD procedure was a very promising tool for the assessment of vitamin A status. In contrast, the correlation between preintervention serum retinol concentrations and changes in serum retinol concentrations after the intervention was weak ($r = -0.38$, $P = 0.048$). At the time that study was conducted, only qualitative estimates of changes in vitamin A status were obtained, because a predictive equation for quantitative assessment of vitamin A body stores with the use of 3-d DRD data was unavailable. However, that study provided strong support to the notion that the 3-d DRD procedure could be an appropriate tool for assessment of vitamin A status (9).

In the present report, by combining new data from our studies among elderly Filipinos with poor-to-adequate vitamin A status with data from our earlier study among elderly Guatemalans with adequate vitamin A status (6), we were able to develop a predictive equation for estimating the amount of total body stores of vitamin A in adults by using serum D:H retinol data at 3 d. The better vitamin A status of the rural Guatemalan elders than of the rural Filipino elders is most likely due to the Guatemalan national program of fortifying domestic sugar with vitamin A rather than to socioeconomic differences between the rural populations. Because the liver may contain <90% of total body vitamin A when vitamin A status is poor (13), it is probable that in some of the Filipinos, the actual hepatic stores were even less than the calculated values reported here.

In both the Philippine and Guatemalan studies, we found a nonlinear relation between serum D:H retinol values and total body reserves of vitamin A as calculated by the DRD equation. The nonlinear relation between these variables indicates that when vitamin A status is poor, any vitamin A ingested will result in a decrease in serum D:H retinol values but not necessarily in an improvement in vitamin A stores, because vitamin A molecules are transported to target tissues, where they are needed and utilized for physiologic processes. In studies in rats, Adams and Green (18) also found a similar nonlinear relation between the fraction of an oral dose of [^{3}H]retinol recovered in plasma and total liver vitamin A as measured directly by using HPLC procedures.

The predictive 3-d DRD equation for quantitative assessment of total body stores of vitamin A in adults is expressed in Equation *6*. Because obtaining liver biopsy specimens is not normally feasible, Equation *6* was derived by using the DRD equation for obtaining estimates of total body stores of vitamin A (1) rather than by directly measuring the vitamin A content of liver samples. The main advantage of the 3-d DRD procedure over the conventional DRD procedure is the considerably shorter period from the time of isotope administration to the time of blood sampling (3 d compared with ≈3 wk). We conclude that a 3-d DRD procedure could be used for the quantitative assessment of total-body vitamin A stores, although further studies would have to be conducted to verify the validity of the proposed 3-d DRD formula. Such verification could be done by comparing results from both conventional DRD and 3-d DRD procedures in a new cohort of elders; the use of their respective predictive mathematical formulas should yield similar estimated values of total-body vitamin A stores. Because of different utilization rates of and requirements for retinol, the validity of both the conventional DRD and 3-d DRD equations for use in children, adolescents, and women of child-bearing age needs to be verified.

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JDR-M participated in the study design, HPLC and gas chromatography–mass spectrometry analyses of serum, data analysis, and the writing of the manuscript; FSS and LSF participated in the field work in the Philippines; GED participated in data handling and the generation of the predictive mathematical formula; NWS and MM participated in the field work in Guatemala; GGD participated in gas chromatography–mass spectrometry procedures; and RMR participated in the study design. All the authors critically reviewed the manuscript. They had no conflict of interest with the sponsoring organization.

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