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Vitamin A assessment by the isotope-dilution technique: good news from Guatemala[1,2]

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Unlike many vitamins, vitamin A is absorbed fairly well, is stored efficiently in the liver as well as in other organs, is recycled extensively within the body, and is catabolized irreversibly in healthy adults at a relatively slow, first-order rate (half-life: 126–140 d). Thus, the physiology of the vitamin lends itself well to analysis by isotope-dilution methods.

The concept involved in isotope dilution is simple: a dose of a labeled tracer, in the case of the study by Ribaya-Mercado et al (1) either tetradeuterated or octadeuterated retinyl acetate, is given orally to a subject, and then the investigator waits for the equilibration, or a state approaching it, of the labeled tracer with endogenous body reserves of the vitamin. Several blood samples are taken at various times, retinol is purified from its complex with retinol binding protein in the plasma, and the ratio of the deuterated to the nondeuterated compound is measured by mass spectrometry. The measure of endogenous reserves is related to the extent of dilution of the labeled tracer.

Rietz et al (2) pioneered the use of the technique in rats, Furr et al (3) validated the method in 11 human adults by comparing calculated total body reserves with measurements obtained by liver biopsy, and Haskell et al (4, 5) extended the technique first to a group of 31 Bangladeshi surgical patients and then to others with low or high vitamin A reserves. We now turn our attention to a similar study in elderly subjects in Guatemala (1).

The current study was initiated, at least in part, because 21% of a rural group of Guatemalan elderly (aged ≥60 y), primarily of Mayan descent, were reported to have serum retinol concentrations <1.05 μmol/L (30 μg/dL) as well as other nutritional inadequacies in 1989 (6). Because serum retinol concentrations in the range of 0.35–1.05 μmol/L can result from infections or other nutritional inadequacies, a more specific technique, namely isotope dilution, was used in the current study.

In this study, the 47 selected elderly Guatemalans (aged 60–81 y) resided in rural communities peripheral to Guatemala City. Each subject ingested 10 mg (30.5 μmol) [$^2H_4$]retinyl acetate in corn oil with a fat-containing meal. Blood samples were taken at various times up to 54 d afterward, and the extracted retinol was derivatized and analyzed by a valuable new procedure, gas chromatography–electron capture negative chemical ionization mass spectrometry (7). Total body reserves were then determined by using the formula of Furr et al (3).

The method worked well, with a relatively small variance at various times. The near-equilibration time of 20 d, or more properly >14 d, agrees with earlier findings (3–5). Thus, the near-equilibration time does not seem to be affected much by age or by the extent of vitamin A storage. Second, all elderly subjects had adequate vitamin A status, with a range of total body reserves from 0.17 to 1.39 mmol retinol and reported liver retinol concentrations of 0.15–1.13 μmol/g. The liver retinol concentrations, however, do not seem to have been corrected for the ≈10% of the total body reserves that are stored in other tissues. Nonetheless, the lowest value is still well above the generally accepted cutoff for adequacy of 0.07 μmol/g liver. As expected for vitamin A–sufficient subjects, the total body reserves did not correlate with either serum retinol concentrations or percentage relative dose response (RDR). Two subjects, one with a moderately low serum retinol value (0.58 μmol/L) and one with a slightly enhanced RDR (21.9%), showed adequate total body reserves.

Reported RDR values were variable (eg, many negative values were found, including one of −68.3%). RDR values, in theory, should vary from 0% (good vitamin A status) to 100% (very deficient state), but should not be negative, which occurs when the initial plasma concentration of retinol exceeds that obtained 5 h after dosing. Two factors probably contributed to these aberrant results: *1*) a small dose of vitamin A [480 μg retinol equivalents (RE)] was used (most investigators now use 1000 μg RE) and *2*) technical problems may have arisen from making comparisons between 2 different blood samples. The modified-relative-dose-response test, in which the probing dose is 3,4-didehydroretinyl acetate and a single blood sample is taken, minimizes these problems (8). Most investigators also currently use a cutoff of 20% for the RDR instead of 14%, as used in the current study, to minimize such technical discrepancies.

A new, interesting facet of the current study was the measurement of the extent of isotope dilution at 3 d. The values obtained at 3 d correlated well with those obtained at 20 d ($P < 0.004$). Clearly, a method with a 3-d waiting period would be much easier to handle in surveys than a method requiring 16–20 d.

Adams and Green (9) suggested using such an approach as a result of their studies in rats. They devised an empirical formula for estimating total body reserves over a wide range of liver vit-

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amin A concentrations (0.73–1097 nmol/g) solely by using the fraction of the administered dose of labeled vitamin A present in the plasma (FD_p) at 3 d and appropriate empirical constants. In their studies, however, the difference between the FD_p at 3 d and that at 6 d, which is the near-equilibrium time for rats, was ≤2-fold, compared with a 9-fold difference between 3 and 20 d in the current study. Furthermore, the correlation between 6-d and 20-d values in the current study was much worse. As Ribaya-Mercado et al suggest, studies that require shorter periods, despite the problem of dealing with rapidly changing isotope ratios, merit further attention.

The current study also examined the effect in 8 subjects of a 32-d supplementation period (800 μg RE plus an additional 155 μg RE in food) on total body reserves. In this second phase, the investigators used octadeuterated retinyl acetate to distinguish the isotope from that used earlier. Seven of 8 subjects showed expected increases in total body reserves. The increases were larger (and in 3 cases much larger) than those calculated on the basis of estimated dietary intake. In this calculation, the increase in dietary intake during the 32-d controlled supplementation period only rather than the total amount ingested during the first 67 d of the study might have better estimated the expected increase in total body reserves. Thus, the results from this part of the study, although well conceived, must have caused the authors more anguish than satisfaction. Possible explanations of these observations are that the given dose of octadeuterated retinyl acetate was less than calculated, an epidemic of diarrhea swept through the village on the day of dosing, or the citizens of Buena Vista held an impromptu festa, embellished with ample portions of chicken and lamb liver, during the last 29 d of the study to celebrate its termination.

To recapitulate, the isotope-dilution technique for estimating total body reserves of vitamin A has provided important information about vitamin A status in 3 different parts of the world as conducted by 3 independent groups of investigators. Concurrence among the major methodologic facets of these studies is excellent. Second, the elderly Guatemalans in the study by Ribaya-Mercado et al had a fully satisfactory vitamin A status. This finding must have been due, at least in part, to the fortification of sugar with vitamin A in Guatemala, a program initiated by Guillermo Arroyave (10). Third, shorter times for partial equilibration of labeled vitamin A with endogenous reserves, despite the time-dependent nature of the measurements, may provide adequate estimates of total body reserves in humans. The isotope-dilution technique for vitamin A assessment is clearly here to stay.

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