Use of a short-term isotope-dilution method for determining the vitamin A status of children

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ABSTRACT

Background: After a dose of labeled vitamin A is given to humans for estimating body stores of vitamin A, blood is customarily drawn at pseudo-equilibration times ranging from 11 to 26 d. Objective: The objective was to determine whether a shorter sample collection interval (6 h or 3 d), which would be more realistic in field settings, can be used.

Design: Correlations of enrichment at 6 h or 3 d with enrichment at 21 d were made after an oral dose of deuterium-labeled vitamin A was given to Chinese schoolchildren (aged 10–11 y; n = 58) with marginal-to-normal vitamin A status. A predictive equation was then derived and applied to data obtained from a separate group of children to verify that the calculated enrichment at 21 d (determined by using data obtained at an earlier time point to predict 21-d enrichment) reflected directly measured enrichment at 21 d.

Results: Because 3-d isotope enrichment was found to correlate well with 21-d enrichment, a predictive equation was derived whereby 3-d data were used to predict isotope enrichment at pseudo-equilibration (ie, at 21 d). When the 3-d predictive equation was applied to a separate group of Chinese children, the calculated 21-d data (determined by using the 3-d data and the predictive equation) matched the directly measured 21-d data. Body stores of vitamin A determined from either the calculated or directly measured 21-d enrichment data also showed agreement.

Conclusion: Percentage enrichment at 3 d (but not at 6 h) can be used to evaluate vitamin A body stores in humans. Am J Clin Nutr 2002;76:413–8.

INTRODUCTION

Vitamin A deficiency remains a problem in developing countries worldwide, affecting 75–140 million children (1). In the past, vitamin A status has been evaluated by the use of dietary assessment tools, functional tests, biochemical methods, and isotope-dilution techniques (2). Of these methods, only isotope dilution using vitamin A labeled with a stable isotope (2H or 13C) can quantitatively estimate total body stores of vitamin A. When isotope-dilution techniques are used to determine body stores of vitamin A, blood samples are characteristically collected at pseudo-equilibration times ranging from 11 to 26 d after the test dose (3–5). During this long equilibration period, various factors that change retinol dynamics in the study population, such as infection, fever, or sickness (6, 7), can affect the degree of isotope enrichment and thereby interfere with the determination of vitamin A body stores. This fact prompted us to test whether a shorter sample-collection interval (6 h or 3 d), which would be more practical in field settings (8), could be used. An earlier oral isotope-dilution study in rats found that the best equation for quantitatively predicting liver vitamin A stores was derived from enrichment data obtained 3 d after the administration of a labeled vitamin A dose (9).

We report here the correlation of enrichment data at 6 h or 3 d with enrichment data at 21 d after an oral dose of labeled vitamin A was given to children. Because the 3-d isotope enrichment data correlated well with the 21-d enrichment data, a predictive equation was derived whereby 3-d data can be used to predict isotope enrichment at pseudo-equilibration (ie, at 21 d). The predictive equation was applied to data from a separate group of children to verify that the calculated 21-d enrichment (determined by using data obtained at an earlier time point to predict 21-d enrichment) reflected enrichment directly measured at 21 d. From 21-d enrichment data, body stores of vitamin A can then be derived.

SUBJECTS AND METHODS

Subjects

The study was carried out in an elementary school in the Shun-yi District at the outer edge of Beijing. Most inhabitants of the
district are middle-income farmers and their families. Informed consent was obtained from the parents before each child’s participation. The study was approved by the Committee on Human Research, Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, Beijing, and by the Human Investigation Committee at Tufts University and the New England Medical Center, Boston.

All children who regularly attended grade 4 of the Shun-yi Banqiao Central Elementary School, who were aged 10–11 y, who were generally healthy, and who could eat a test meal at school were eligible for enrollment in this study. A nutrition survey conducted in the Shun-yi District showed that 10–11-y-old children have marginal vitamin A status (8) but a low prevalence of parasitic infections (0.7% of the children in the district had parasitic infections in 1998). Children with fever (>38°C) at the time of enrollment were excluded from the study. Fifty-eight children, who had already been divided by teachers into 2 classroom groups with similar socioeconomic backgrounds, met the eligibility criteria and were enrolled in the study. One classroom group (group 1) comprised 24 subjects (14 boys and 10 girls) who provided blood samples 6 h and 21 d after receiving a 7.4-μmol (2.5-mg) dose of octadeuterated vitamin A: [10,19,19,14,20,20,20-[2H8]retinyl acetate [D8 vitamin A; 2116 retinol activity equivalent (RAE)]. Another classroom group (group 2) comprised 34 subjects (20 boys and 14 girls) who provided blood samples 3 d and 21 d after receiving a 7.4-μmol (2.5-mg) dose of D3 vitamin A (2116 RAE). From the correlation between the 6-h or 3-d data and the 21-d data, we attempted to derive predictive equations, whereby data from an early time point could be used to predict isotope dilution at pseudo-equilibration (ie, at 21 d). From the isotope-enrichment data at 21 d, body stores of vitamin A can then be calculated.

The demographic and anthropometric data for study participants in group 1 (height: 137.7 ± 5.4 cm; weight: 33.1 ± 6.4 kg) and group 2 (height: 142.5 ± 6.7 cm; weight: 35.0 ± 7.5 kg) did not differ significantly. The average height (140.7 ± 6.6 cm) and weight (34.4 ± 7.1 kg) of the entire group were 4 at the 75th percentile for height and the 60th percentile for weight of urban children who participated in the 1992 Chinese National Nutrition Survey (10). These anthropometric averages are at the 50th percentile for height and weight of US children of the same age (11).

The children’s nutrient intakes were estimated by 24-h dietary recall at each of the 2 blood sampling points. The results showed that the mean (±SEM) carotenoid intake was 1395 ± 429 mg/d, and the mean preformed vitamin A intake from animal foods was 137 ± 73 mg/d during the experimental period (spring 1998). These intakes are equivalent to 195 RAE/d, which is =50% of the recommended dietary allowance of vitamin A for Chinese in this age group.

On day 1 of the study, each child was given a physiologic dose of D3 vitamin A (2.5 mg; equivalent to ~0.26 μmol/kg body wt) dissolved in 80 mg corn oil and encapsulated in a no. 4 gelatin capsule. On the day when labeled retinyl acetate capsules were given, teachers and health workers administered the vitamin A capsule and water to each child. Children then ate a standard test breakfast of fried pancake (80 g) and soy milk (200 mL), which provided 40% of energy from fat, immediately after taking the oral dose of labeled vitamin A.

Either 6 h (group 1) or 3 d (group 2) and 21 d after the dose, a serum sample (<3 mL whole blood) was drawn from the forearm of each child by venipuncture. The serum samples were transferred to no-additive Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), which were covered with aluminum foil to prevent light damage and set at room temperature (25°C) for 0.5 h. The samples were centrifuged at 800 × g for 10 min at room temperature. Serum was separated, transferred to a Cryo vial (Nunc Inc, Rochester, NY), and kept at −20°C until it was shipped to Boston (≤1 mo) on dry ice. The serum samples were then kept at −70°C until they were analyzed. All samples were analyzed in Boston by gas chromatography–mass spectroscopy within 6 mo.

Serum enrichments of retinol at 6 h, 3 d, and 21 d were analyzed by linear regression to determine the significance of the enrichment correlation between either the 6-h and 21-d data for group 1 or the 3-d and 21-d data for group 2. A significant linear regression correlation of 3-d and 21-d enrichments was found (P < 0.001) and was used to develop a predictive equation for using 3-d measured enrichment data to predict 21-d enrichment (ie, at pseudo-equilibration). The calculated body stores of vitamin A for children in group 1 with a labeled-nonlabeled retinol ratio >6% were compared with those for children with a ratio <6% [analogous to the dehydroretinol-to-retinol ratio in the modified-relative-dose-response (MRDR) test].

To test the suitability of the predictive equation, we applied the equation to data obtained from a separate group of children, healthy preschoolers (age range: 5.3–6.4 y) residing in the city of Tai-an in Shandong province ≈600 km south of Beijing, who had previously participated in a vegetable intervention study (12). In that study, each child was given 3.0 mg D3 vitamin A (2545 RAE) dissolved in 170 mg corn oil 3 wk before the beginning of a 10-wk period of vegetable feeding. During this vegetable feeding period, children were given either green-yellow vegetables or light-colored vegetables at all 3 meals every school day. To detect possible changes in vitamin A stores that were due to the vegetable interventions, a 3.0-mg dose of 10,19,19,14,20,20,20-[2H8]retinyl acetate (D3 vitamin A; 2574 RAE) in corn oil was given at the conclusion of the intervention. The children’s blood was drawn either 3 d (3-d group; n = 23) or 21 d (21-d group; n = 18) after each child was given a labeled dose of vitamin A (ie, before and after the vegetable intervention). In our earlier report (12), the 21-d data (from the 21-d group) were used to calculate body vitamin A stores quantitatively, and 3-d enrichment data (from the 3-d group) were used to evaluate vitamin A stores qualitatively. In the present study, we used the predictive equation derived from the Shun-yi schoolchildren’s data to calculate (predict) a 21-d enrichment for the Tai-an preschool children by using their 3-d enrichment data (3-d group). The 21-d enrichment calculated from the 3-d data was then compared with the directly measured 21-d enrichment (before and after the vegetable interventions) in the Tai-an preschool children (21-d group). In this comparison, we assumed that the overall 21-d enrichment for the children in the 3-d group would be similar to that for the children in the 21-d group.

Sample analysis

An HPLC system equipped with a C18 column and using methyl tert-butylether:methanol:water as the mobile phase was used to analyze concentrations of carotenoids and retinoids in serum (13). An HPLC system equipped with a Pecosphere-3 C18 0.46 × 8.3-cm cartridge column with a 0.46 × 8.3-cm cartridge column as a guard column (both columns from Perkin-Elmer Inc, Norwalk, CT) was used to collect retinol in serum samples, as previously described (14). The gradient procedure at a flow rate of 1 mL/min was used in the following sequence: 100% solvent A
where \( F = \text{the labeled retinyl acetate (mmol)}, s = 0.65 \text{ [correction for H/D (CH}_3\text{CN:THF:water, 50:20:30, vol:vol:vol with 1% ammonium acetate in water], a -1-min gradient to 100% solvent B, a 9-min hold at 100% solvent B, and a 1-min linear gradient back to 100% solvent A. A gas chromatograph–electron capture negative chemical ionization mass spectrometer equipped with a 15-m DB-1 column and an on-column injector was used to determine the enrichment of labeled vitamin A in serum with a CV < 9% (15).}

**Body stores of vitamin A**

We used the modified Bausch-Rietz equation (4, 5, 12, 16) to determine body stores of vitamin A in the various groups of children by using their directly measured 21-d data and their calculated 21-d data (from the 3-d predictive equation). The calculation of body stores was done as follows:

Total liver reserve = \( F \times \text{dose} \times [s \times a \times (H/D - 1)] \)  (I)

where \( F = 0.5 \) (fraction of the dose to be stored in liver), dose is the labeled retinyl acetate (mmol), \( s = 0.65 \) [correction for H/D (hydrogen/deuterium) in serum], \( a = \exp (-kt) \) \((k = \ln 2t_{1/2}; t_{1/2} \) is the half-life of vitamin A turnover in the liver, and \( t \) is the time (d) since the dose was given—this factor is a correction to the \( H/D \) that is due to continued daily intake of dietary vitamin A), and \( H/D \) is the enrichment of retinol after a labeled dose of vitamin A.

**Statistics**

Unpaired Student’s \( t \) test was used to determine the difference in the anthropometric data for the 2 groups of Shun-yi schoolchildren. Analysis of variance (ANOVA) was used to determine differences in serum concentrations of the major carotenoids (\( \beta \)-carotene, cryptoxanthin, and lutein) between the 2 groups of children as analyzed by ANOVA. Serum concentrations of retinol, \( \beta \)-carotene, and lutein did not change significantly over time (6 h and 21 d for group 1; 3 d and 21 d for group 2). However, serum concentrations of cryptoxanthin measured at 21 d were significantly lower than those measured at 6 h (group 1) or 3 d (group 2), as analyzed by a univariate repeated-measures ANOVA. This was likely due to a decreased local availability of tangerines and dried persimmons, which are extremely high in cryptoxanthin but not in \( \beta \)-carotene or lutein. Tangerines and dried persimmons are commonly available as a snack food through the winter and early spring, but availability decreases as spring progresses, and our study was conducted in the spring. Analysis of the fasting blood samples 21 d after the 2.5-mg dose of the labeled vitamin A showed that 24% of the Shun-yi schoolchildren had serum concentrations of vitamin A \( \leq 0.87 \mu\text{mol/L} \) (25–30 g/dL), and 31% had serum concentrations of vitamin A > 0.87 \( \mu\text{mol/L} \) and \( \leq 1.05 \mu\text{mol/L} \) (25–30 \( \mu\text{g/dL} \)).

**Enrichment of \( D_8 \) retinol in serum measured at 6 h and 21 d in group 1**

In group 1, the mean enrichment of \( D_8 \) retinol in serum was 9.3 \( \pm 7.3\% \) 6 h after and 1.4 \( \pm 0.7\% \) 21 d after the \( D_8 \) vitamin A dose. Regression analysis showed no significant correlation between the percentage enrichments of retinol at 6 h and at 21 d (Figure 1).

Fifteen of 24 children in this group had a labeled-nonlabeled retinol ratio > 6% 6 h after the dose. In the MRDR test, a serum dehydroretinol-retinol ratio > 6% (which is analogous to a labeled-nonlabeled retinol ratio) is considered a positive response indicating marginal-to-low vitamin A status. When the modified Bausch-Rietz equation and the measured 21-d enrichment data were used to calculate body stores of vitamin A, the children with a labeled-nonlabeled retinol ratio > 6% (\( n = 15 \)) had mean vitamin A body stores of 0.175 \( \pm 108 \mu\text{mol} \), whereas the children with a labeled-nonlabeled retinol ratio < 6% (\( n = 9 \)) had vitamin A body stores of 0.292 \( \pm 227 \mu\text{mol} \) (\( P = 0.09 \)). Only 2 of 24 children had a serum retinol concentration < 0.87 \( \mu\text{mol/L} \) (25 \( \mu\text{g/dL} \)).

**RESULTS**

**Retinol and carotenoids in serum**

Mean serum concentrations of retinol and the major carotenoids measured 6 h, 3 d, and 21 d after administration of the test dose of labeled vitamin A are shown in Table 1. There were no significant differences in serum concentrations of retinol, \( \beta \)-carotene, cryptoxanthin, and lutein between the 2 groups of children as analyzed by ANOVA. Serum concentrations of retinol, \( \beta \)-carotene, and lutein did not change significantly over time (6 h and 21 d for group 1; 3 d and 21 d for group 2). However, serum concentrations of cryptoxanthin measured at 21 d were significantly lower than those measured at 6 h (group 1) or 3 d (group 2), as analyzed by a univariate repeated-measures ANOVA. This was likely due to a decreased local availability of tangerines and dried persimmons, which are extremely high in cryptoxanthin but not in \( \beta \)-carotene or lutein. Tangerines and dried persimmons are commonly available as a snack food through the winter and early spring, but availability decreases as spring progresses, and our study was conducted in the spring. Analysis of the fasting blood samples 21 d after the 2.5-mg dose of the labeled vitamin A showed that 24% of the Shun-yi schoolchildren had serum concentrations of vitamin A \( \leq 0.87 \mu\text{mol/L} \) (25–30 \( \mu\text{g/dL} \)).

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Retinol</th>
<th>( \beta )-Carotene</th>
<th>Cryptoxanthin</th>
<th>Lutein</th>
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<tbody>
<tr>
<td></td>
<td>( \mu\text{mol/L} )</td>
<td>( \mu\text{mol/L} )</td>
<td>( \mu\text{mol/L} )</td>
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<tr>
<td>Group 1</td>
<td></td>
<td></td>
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<tr>
<td>6 h (( n = 24 ))</td>
<td>1.028 ± 0.211</td>
<td>0.359 ± 0.174</td>
<td>0.189 ± 0.120²</td>
<td>0.510 ± 0.179</td>
</tr>
<tr>
<td>21 d (( n = 58 ))</td>
<td>1.023 ± 0.226</td>
<td>0.366 ± 0.150</td>
<td>0.134 ± 0.068²</td>
<td>0.471 ± 0.180</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3 d (( n = 34 ))</td>
<td>0.989 ± 0.244</td>
<td>0.426 ± 0.201</td>
<td>0.220 ± 0.158²</td>
<td>0.497 ± 0.160</td>
</tr>
<tr>
<td>21 d (( n = 58 ))</td>
<td>1.060 ± 0.146</td>
<td>0.410 ± 0.205</td>
<td>0.175 ± 0.122</td>
<td>0.503 ± 0.183</td>
</tr>
</tbody>
</table>

²\( s \) ± SD.

²Significantly different from data measured at 21 d, \( P < 0.05 \) (univariate repeated-measures ANOVA).

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Enrichment of D₈ retinol in serum measured at 3 d and 21 d in group 2

The mean enrichment of D₈ retinol in serum was 4.4 ± 2.1% 3 d after and 1.0 ± 0.6% 21 d after the D₈ vitamin A dose. As shown in Figure 2, regression analysis showed a linear correlation (r = 0.7, P < 0.001) of the percentage enrichments of retinol in serum samples collected 3 d and 21 d after the administration of the labeled vitamin A dose. The linear model is as follows:

\[ \text{Predicted } \% \text{ enrichment at } 21 \text{ d} = -0.061 + 0.243 \times \text{observed } \% \text{ enrichment at } 3 \text{ d}, \]

or,

\[ D/(D + H)_{21d} = -0.061 + 0.243 \times D/(D + H)_{3d} \text{ (3-d equation)} \]

By use of the 21-d enrichment data from all 58 grade 4 Shun-yi schoolchildren and of the modified Bausch-Rietz equation with a half-life set at 140 d to calculate the total body stores of vitamin A, both before and after the green-yellow vegetable and light-colored vegetable interventions. The body stores of vitamin A in the 2 vegetable-fed groups, both before and after the vegetable intervention as calculated with measured 3-d enrichment data, were not significantly different from the stores calculated from measured data obtained at 21 d (pseudo-equilibration), as shown in Table 3.

DISCUSSION

Vitamin A deficiency is 1 of the 3 most significant micronutrient problems in the world (17). Periodic supplementation of pre-formed vitamin A has been shown to effectively reduce childhood blindness (18) and mortality (19–21) in vitamin A–deficient populations. However, food-based interventions may not be as efficacious as hoped. For example, intervention studies using an increased intake of foods rich in carotenes showed no improvement in vitamin A status (22, 23). This may have been because vitamin A status in these studies was evaluated only by the measurement of serum retinol, which is not a sensitive indicator of vitamin A status over a wide range of vitamin A nutriture.

To estimate vitamin A status, the RDR test was developed, which is based on the response of serum retinol to an exogenous dose of vitamin A (24). The RDR is calculated as the ratio of the difference between serum retinol at baseline and 5 h after the administration of a small oral dose of vitamin A (450 μg retinyl acetate) and the baseline serum concentration of retinol (25). This enables a qualitative estimation of vitamin A stores, in that a ratio ≥20% suggests adequate liver stores of vitamin A (ie, <0.07 mmol/g liver) (25). The MRDR test uses 3,4 dihydroretinyl acetate and requires only one blood sample to be drawn 4–7 h after the test dose (26). The standard 3,4 dihydroretinyl acetate doses of 5.3 μmol for children aged <6 y, 7 μmol for children aged 7–12 y, and 8.8 μmol for children aged >12 y and adults are used in human studies as mentioned previously (27). A serum dehydroretinol-retinol ratio >6% is considered a positive response, indicating marginal-to-low vitamin A status. The
different (min A body stores in children with elevated labeled-nonlabeled vitamin A dose in children of different ages, so that body stores can be estimated more accurately. We then calculated a predicted enrichment at 21 d. Our results showed that the calculated 21-d data from the 3-d group matched the directly measured 21-d data from the 21-d group, as shown in Table 2. We further determined the body stores of vitamin A by using both calculated and measured 21-d enrichment data (Table 3). Again, the results showed agreement between the total body stores of vitamin A before and after the vegetable interventions when the 3-d enrichment data and the predictive equation or the directly measured 21-d enrichment data were used. Thus, the derived linear predictive equation can be used for children with marginal-to-normal vitamin A status. However, the difference in the 2 body store estimates (on the basis of calculated or directly measured 21-d data) varied from −2% to 25% in the 4 subgroups of children, as shown in Table 3. Moreover, the SD of the difference in the 2 body store estimates (before and after the intervention) were larger when the calculated 21-d data were used (0.042 and 0.076) than when the directly measured 21-d data were used (0.027 and 0.036) (Table 3). This implies that a different number of subjects will be needed for investigations when 3-d data are used to calculate the 21-d data to evaluate the body stores.

To account for the effect of continued, daily intake of unlabeled vitamin A on the subsequent isotopic enrichment of blood, a factor \( a = \exp (−kt) \) was introduced by Furr et al (4). This calculation is based on the assumptions that the depletion rate of liver vitamin A follows first-order reaction kinetics and that the mean half-life of vitamin A turnover in the liver is 140 d (3, 29). However, the half-life of vitamin A turnover in the liver can be in the range of 75–240 d with a CV of 35% for adults (29); there are no data on the half-life of vitamin A turnover in the livers of children. If the mean half-life of vitamin A in children were shorter than that in adults, estimated body stores as calculated with the equation of Furr et al would be lower. For example, with a half-life of 50 d instead of 140 d, estimated body stores would be 20% lower. Additional research is needed to determine the half-life of vitamin A in children of different ages, so that body stores can be estimated more accurately.

The liver concentrations of vitamin A in the Shun-yi and Tai-an children were 0.27 ± 0.19 \( \mu \text{mol/g} \) (range: 0.05–0.79 \( \mu \text{mol/g} \)) and 0.18 ± 0.08 \( \mu \text{mol/g} \) (range: 0.05–0.31 \( \mu \text{mol/g} \)), respectively, when 140 d was used as a half-life and assuming a liver weight of 0.03% of body weight (26). There were only 2 subjects among the Shun-yi children and 1 subject among the Tai-an children with liver concentrations of vitamin A ≤ 0.07 \( \mu \text{mol/g} \). In contrast, liver vitamin...
A concentrations at autopsy in children aged 4–8 and 8–15 y in the United States were reported to be $0.54 \pm 0.49 \mu mol/g$ (range: 0.08–1.70 $\mu mol/g; n = 12$) and $0.65 \pm 0.64 \mu mol/g$ (range: 0.16–1.59 $\mu mol/g; n = 4$), respectively (30).

Our observations provide evidence that it is possible to use percentage enrichment data at 3 d (but not at 6 h) to evaluate vitamin A body stores in children with marginal-to-normal vitamin A status. Further investigation will be needed to verify this 3-d equation in more subjects, in other populations of children of various ages and with different dietary vitamin A intakes, and in children with vitamin A deficiency from whom both 3-d and 21-d blood samples are available after the administration of isotope-labeled vitamin A.

REFERENCES


