Accelerator Mass Spectrometry Can Be Used to Assess Vitamin A Metabolism Quantitatively in Boys in a Community Setting

Emmanuel K. Aklamati, Modest Mulenga, Stephen R. Dueker, Bruce A. Buchholz, Janet M. Peerson, Emmanuel Kafwembe, Kenneth H. Brown, and Marjorie J. Haskell

Abstract
A survey indicated that high-dose vitamin A (HD-VA) supplements had no apparent effect on vitamin A (VA) status, assessed by serum retinol concentrations, of Zambian children < 5 y of age. To explore possible reasons for the lack of response, we quantified absorption, retention, and urinary elimination of either a single HD-VA supplement (209.8 μmol; 60 mg) or a smaller dose of stable isotope (SI)-labeled VA (17.5 μmol; 5 mg), which was used to estimate VA pool size, in 3- to 4-y-old Zambian boys (n = 4 for each VA dose). A tracer dose of \(^{14}C_2\)-labeled VA (0.925 kBq; 25 nCi) was coadministered with the HD-VA supplement or SI-labeled VA, and 24-h stool and urine samples were collected for 3 and 7 consecutive days, respectively, and 24-h urine samples at 4 later time points. Accelerator MS was used to quantify \(^14\)C in stool and urine. Estimates of absorption, retention, and the urinary elimination rate (UER) did not differ by size of the VA dose administered. Estimated absorption and retention were negatively associated with reported fever (r = -0.83; P = 0.011). The HD-VA supplement and SI-labeled VA were adequately absorbed, retained, and utilized in apparently healthy Zambian preschool-age boys; absorption and retention may be affected by recent fever.

Introduction
A national survey conducted in Zambia indicated that ~54% of children 6 mo to 5 y of age were marginally deficient in vitamin A (VA) (serum retinol concentration < 0.70 μmol/L) 1 mo after they received a high-dose VA (HD-VA) supplement (209.8 μmol; 1 μmol = 0.286 mg) through the national VA supplementation program (1). This apparent high prevalence of deficiency persisted even after controlling for the presence of systemic inflammation, based on serum concentrations of C-reactive protein (CRP) and α1-acid glycoprotein (AGP). These findings were unexpected, because the national VA supplementation program reaches ~80% of children < 5 y of age (1). Possible biological reasons for these observations are: 1) the HD-VA supplement was not adequately absorbed; 2) the HD-VA supplement was absorbed but rapidly excreted; 3) the HD-VA supplement was well absorbed and retained, but mobilization of the liver was impaired, possibly because of infection or inflammation; and 4) the HD-VA supplement was absorbed and retained, but daily utilization of VA in the children was greater than expected, such that by 1 mo after dosing, the HD-VA supplement no longer contributed to their VA status.

Limited information is available on absorption, retention, and utilization of VA from HD-VA supplements in children, primarily because of the lack of a safe and sensitive method for studying VA metabolism. A few studies were conducted >30 y ago using radiolabeled VA as a metabolic tracer in a small number of Indian children (2-4). However, radiolabeled tracers studies, particularly in children and women of childbearing age, have fallen out of favor because of concerns with administering the high levels of radioactivity that are necessary to be detectable by liquid scintillation spectrometry. Recently, accelerator MS (AMS) has been shown to be a powerful method for studying metabolism safely in humans. AMS was originally developed for

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3 Supplemental Figures 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

Abbreviations used: AGP, α1-acid glycoprotein; AMS, accelerator MS; CRP, C-reactive protein; HD-VA, high-dose vitamin A; MUAC, mid-upper arm circumference; SI, stable isotope; UCD, University of California, Davis; UER, urinary elimination rate; VA, vitamin A.

To whom correspondence should be addressed. E-mail: mjhaskell@ucdavis.edu.
carbon dating, which required a highly sensitive and quantitative method for measurement of carbon-14 (and subsequently other isotopes) with high precision (5,6). The method is ~4 to 5 orders of magnitude more sensitive than liquid scintillation counting and can measure isotopic ratios of $^{14}C/C$ to parts per quadrillion or attomole levels in milligram-sized samples (7). With AMS methodology, true tracer doses of radiolabeled compounds can be administered to humans that result in levels of radioactivity in biological tissues and fluids that do not differ substantially from the natural background. AMS has been used in human nutrition research to study the bioavailability of β-carotene and its bioconversion to VA, folate metabolism, and bone calcium balance (8–11).

To investigate possible reasons for the apparent lack of effect of HD-VA supplements on the VA status of Zambian children, we conducted a study to estimate the absorption, retention, and urinary elimination of an oral HD-VA supplement (209.8 μmol), in 3- to 4-yr-old Zambian boys using AMS methodology. This study was nested within a larger study to assess the impact of a single HD-VA supplement on total body VA pool size, using the paired-VA stable isotope (SI) dilution technique. Because there is no direct information in children on absorption, retention, and utilization of the oral dose of SI-labeled VA that is used to estimate total body VA pool size using the SI dilution technique, we also used AMS methodology to estimate absorption, retention, and utilization of the oral dose of SI-labeled VA (17.5 μmol). This information may be useful for refining the equation that is used for predicting VA pool size in this age group using the SI dilution technique (12).

Methods

Participants

The study was carried out in children from a low-income shanty compound on the outskirts of Ndola, Zambia. The study was restricted to boys to facilitate collection of 24-h urine samples. Boys 3–4 y of age were examined by a pediatrician to assess their general health. Twenty boys who were free from chronic disease, symptoms of malaria or diarrhea within the past 2 wk, and signs or symptoms of VA deficiency were eligible for the study. Those who had received a HD-VA supplement within the previous 2 mo were excluded. The study protocol was approved by the Institutional Review Boards of the Tropical Diseases Research Center, Ndola, Zambia, and the University of California, Davis (UCD) and the Radioactive Drug Research Committee of UCD. Informed, written consent was obtained from a parent or legal guardian before beginning the study procedures. The radiation dose the children were exposed to was 21 μSv (2.1 mrem; 1 μSv = 0.1 mrem). By comparison, the total effective dose of a 6-h airplane flight is 30 μSv and the dose of a dental X-ray examination is 200 μSv.

Study design

The boys were randomly assigned to 1 of 2 groups (10/group) to receive a tracer dose of [14C]2-retinyl acetate (0.925 kBq; 1 kBq = 27.0 nCi) with either an oral HD-VA supplement (209.8 μmol) or an oral dose of SI-labeled VA (17.5 μmol) (Fig. 1). On study d 0, the children were admitted to the research facility. On study d 1, 24-h collections of stool and urine were obtained for measurement of the baseline14C contents before the tracer dose was administered. On study d 2, children who were assigned to the HD-VA group (n = 10) received an oral dose of 209.8 μmol VA in corn oil (194 μL) followed immediately by an oral dose of [14C]2-retinyl acetate (0.925 kBq) in corn oil (98 μL). The children were not given a high-fat snack to facilitate absorption of the VA, because this is not done in the national VA supplementation program. Children who were assigned to the SI-labeled VA group (n = 10) received an oral dose of 17.5 μmol of SI-labeled VA in corn oil (194 μL) followed immediately by an oral dose of [14C]2-retinyl acetate (0.925 kBq) in corn oil (98 μL). All doses were administered directly into the children’s mouths using a positive displacement pipet. Thereafter, the children in the SI-labeled VA group were given a high-fat snack (bread and peanut butter) to enhance absorption of the dose of SI-labeled VA, as is recommended for the SI dilution technique (13). The 24-h stool samples were collected for 3 consecutive days (study d 2–4) for measurement of the cumulative amount of 14C excreted in stool to estimate VA absorption. The 24-h urine samples were collected for 7 consecutive days (study d 2–8) for measurement of the cumulative amount of 14C excreted in urine to estimate VA retention. Additionally, 24-h urine samples were collected on study d 14, 27, 45, and 59 for measurement of 14C in urine to estimate the daily urinary elimination rate (UER) of 14C.

In addition, as part of the larger study on the longer term impact of a HD-VA supplement on VA pool size, blood samples were collected before and 45 d after administration of the HD-VA supplement or placebo (corn oil) for measurement of the hemoglobin concentration and plasma concentrations of retinol, CRP, and AGP.

Preparation of the [14C]2-retinyl acetate dose

[11,12–14C2]-retinyl acetate was synthesized by Hoffman-La Roche. The [14C]2-retinyl acetate was purified by reverse-phase HPLC. Briefly, 255 μL of the stock solution of [14C]2-retinyl acetate in toluene was placed in a scintillation vial containing 100 μL BHT, dried under N2 gas, and reconstituted in 500 μL hexane. The [14C]2-retinyl acetate in hexane was loaded onto an NH2 solid phase extraction column (0.8×4.0 cm, 3 μm; GenPore) and eluted with 2 mL hexane containing 15% ethyl acetate. The eluent was dried under N2 gas and reconstituted in 200 μL methanol for purification by HPLC using a Class VP HPLC (Shimadzu) equipped with a photo-diode array detector. The [14C]2-retinyl acetate was injected onto a C18 HS Adsorbosphere column (150 × 4.6 mm, 5 μm) (Alltech) using a mobile phase of methanol/water (93:7). The retinyl acetate peak for each injection was collected into a vial containing BHT between 10.0 and 12.0 min. The HPLC fractions (n = 3) containing the [14C]2-retinyl acetate were combined, dried under N2 gas, reconstituted in methanol, reinfected onto the HPLC column as a second purification step, and eluted with methanol/water (93:7). The 14C activity in the purified fraction was measured by counting three 10-μL aliquots in a liquid scintillation counter (Wallac 1410). A radiochromatogram revealed that 96.9% of the 14C counts were associated with the retinyl acetate peak.

The remaining purified [14C]2-retinyl acetate was evaporated under N2 gas and dissolved thoroughly in corn oil. The radioactivity of 5 aliquots (10 μL) of [14C]2-retinyl acetate in corn oil was counted using liquid scintillation spectrometry to determine the volume required for a dose of 0.925 kBq (1.4 nmol VA).

SI-labeled VA and the HD-VA supplement

The SI-labeled VA[10,19,19,19–2H4]retinyl acetate was obtained from Cambridge Isotopes and transferred into corn oil, as previously described (13). The concentration of SI-labeled VA in corn oil was determined by UV-Vis spectrophotometry to determine the volume required for a dose of 17.5 μmol VA (13). Retinyl acetate in soybean oil (UNICEF, UN Essential Drugs Program, MEDICAP) was used as the source material.
for the HD-VA supplement; the VA concentration of the retinyl acetate in soybean oil was determined by UV-Vis spectrophotometry to determine the volume required for a dose of 209.8 μmol VA (13).

Collection of 24-h stool and urine samples. The 24-h stool samples were collected into plastic bags (0.4-mm thickness, Fisher Scientific) and were weighed using a triple beam balance (Ohaus). The samples were double-bagged, carefully sealed, and stored in a freezer at −20°C until shipped to UCD for analysis. The 24-h urine samples were collected into 2-L polypropylene jugs and stored at 4°C. Total urine volumes were measured and well-mixed samples were aliquotted into 2-mL cryovials and stored at −20°C until shipped to UCD for analysis.

Preparation of samples for AMS analyses. Urine and stool samples were processed for AMS analysis, as previously described (5). Briefly, stool samples were homogenized with 70% isopropyl alcohol (5:1, v:w) containing 0.5 mol/L potassium hydroxide; urine was untreated prior to combustion and graphitization (7). The 14C measurements were conducted at the Center for AMS at the Lawrence Livermore National Laboratory (Livermore, CA).

Total carbon analyses. Untreated urine (75 μL) and homogenized stool samples (75 μL) were lyophilized in aluminum capsules for 24 h and analyzed for total carbon content by using a Nitrogen/Carbon Analyzer (Thermo Finnegan FlashEA 1112 Series, Thermo Fisher Scientific) at the Division of Agriculture and Natural Resources Laboratory at UCD (14).

Calculation of 14C concentration. AMS data are expressed as isotopic ratios of 14C/12C and more conveniently expressed in units of fraction modern. One unit of fraction modern equals 0.226 mBq 14C/mg C (6.11 fCi 14C/mg C). The isotopic ratios were corrected for the baseline values by subtracting the ratio of the predose samples from postdose samples. The 14C concentration of samples was calculated using the following equation:

\[
\text{Modern} = \left( \frac{0.226 \text{ mBq} \text{ } 14C/\text{mg C}}{0.012 \text{ mg C}/\text{mol C}} \right) \times \left( \frac{\text{mol C/L suspension}}{\text{mBq} \text{ } 14C/\text{L suspension}} \right)
\]

The 14C content in the sample (mBq) divided by 1000 gives the 14C content in the sample in mBq/mL of suspension (15).

Estimation of 14C excreted in stool and urine as a percentage of dose administered. To determine the total 14C content in each 24-h collection, the 14C concentration (mBq/mL) was multiplied by the total volume of the suspension (for stool) and the total volume of urine collected in the 24-h period (for urine). The amount of 14C excreted per day is expressed as a percentage of the administered dose:

\[
\frac{\text{14C content of each 24-h sample (mBq)/Dose of 14C administered (mBq)}}{100} \times \text{Percent of dose excreted/d}
\]

Estimation of percent absorption. To estimate absorption, the cumulative amount of 14C excreted in stool during 72 h (3 d) after dosing was expressed as a percentage of dose administered and subtracted from 100%. Beyond 72 h, any 14C excreted in stool was assumed to be derived from biliary excretion of VA metabolites and the normal sloughing of intestinal cells (8).

Estimation of percent retention. To estimate retention, the cumulative amount of 14C in stool 72 h (3 d) after dosing and the cumulative amount of 14C excreted in urine 72 h (3 d) after dosing were expressed as percentages of dose administered and subtracted from 100%. 14C excreted in urine within 72 h after dosing was assumed to represent [3H]labeled VA that was absorbed but rapidly excreted. Beyond 72 h, 14C excreted in urine was assumed to be derived from end-products of VA metabolism and was assumed to have been retained initially.

Estimation of the UER. The UER of 14C in urine as a function of time and fitting an exponential equation derived from a log-linear plot to the observed data. The second coefficient (b2) of the final exponential term in the equation was used as an estimate of the UER, and t is time in days (16):

\[
-a_0 \exp(-b_0 t) + a_1 \exp(-b_1 t) + a_2 \exp(-b_2 t)
\]

Anthropometric, dietary, morbidity, and biochemical assessments

Anthropometry. Anthropometric assessments were done on study d 0. Weight was measured to the nearest 0.1 kg using a digital scale (Seca) and height was measured to the nearest 0.1 cm using a stadiometer (Salter). Mid-upper arm circumference (MUAC) of the children was measured to the nearest 0.1 cm using a nonstretchable tape.

Dietary. A FFQ was administered weekly to mothers to obtain information on the children’s consumption of selected VA-containing foods.

Morbidity. Questionnaires were administered to mothers throughout the study period to obtain information on the number of days on which children had selected signs or symptoms of illnesses during the previous week.

Malaria parasites. The presence of malaria parasites in blood was assessed by thick blood smears (slides) stained with Giemsa (17).

Biochemical assessments. Plasma retinol concentrations were measured by using HPLC (18). Marginal VA deficiency was defined as plasma retinol concentration < 0.70 μmol/L. Plasma concentrations of CRP and AGP were measured by using commercial radial immunodiffusion kits (Nanorid kit, The Binding Site). Cutoff values > 5.0 mg/L and > 1.0 g/L were used for plasma CRP and AGP, respectively, to identify children with infection and/or inflammation (19).

Hemoglobin concentrations were measured using the cyanmethemoglobin method with a Hemocue (Rankin Biomedical). Anemia was defined as hemoglobin concentration < 110.0 g/L.

Statistical analysis

Descriptive statistics were calculated for all variables. Values in the text are means ± SD. Mean estimates of absorption, retention, and UER of 14C were compared between the group of children who received the HD-VA supplement and those who received the smaller SI-labeled dose of VA by using the Student’s t test (α = 0.05). The Student’s t test was also used for comparing mean absorption, retention, and the UER between children with or without elevated plasma AGP (initial plasma AGP was used for the comparisons of estimated absorption and retention and initial and final plasma AGP were used for the comparison of UER). Mean amounts of VA excreted in urine at 3 d after dosing assessed by direct measurement or by the UER were compared within groups by using the signed-rank test (α = 0.05). Estimated absorption of 14C was examined in relation to reported symptoms and illnesses that occurred 1 wk before until 3 d after administration of the HD-VA supplement or the smaller dose of SI-labeled VA using Pearson correlation analysis. Estimated retention of 14C was examined in relation to reported symptoms and illnesses that occurred 1 wk before and 1 wk after administration of the HD-VA supplement or the smaller dose of SI-labeled VA. Similarly, the estimated urinary elimination of 14C was examined in relation to reported symptoms and illnesses 1 wk before administration of the test doses of VA and throughout the remaining weeks of the study period using Pearson correlation analysis.

Results

Participants. A total of 20 boys completed the study protocol. However, because of insufficient funding, AMS analyses have been completed for only 4 boys who received the HD-VA supplement and 4 boys who received the dose of SI-labeled VA. The preliminary results reported herein are, to our knowledge,
the first quantitative estimates of VA metabolism in preschool-age children based on the AMS tracer method, and they demonstrate the feasibility of applying this technique in this age group in a community setting. Children from the 2 groups did not differ in age, weight, height, MUAC, or anthropometric indices (Table 1).

VA absorption. The cumulative amount of $^{14}$C recovered in stools of children who received the HD-VA supplement was $16.2 \pm 7.1\%$ at 3 d after dosing (Fig. 2; individual data shown in Supplemental Fig. 1A,B). The estimated absorption, as percentage of the dose administered, was $83.8 \pm 7.1\%$ (range: $73.3$–$88.8\%$; $n = 4$). The cumulative amount of $^{14}$C recovered in stools of children who received the smaller dose of SI-labeled VA was $23.5 \pm 9.5\%$ at 3 d after dosing. The estimated absorption, as percentage of the dose administered, was $76.5 \pm 9.5\%$ (range: $62.4$–$82.9\%$; $n = 4$). Mean estimates of absorption did not differ between dose levels.

VA retention. The cumulative amount of $^{14}$C recovered in urine in children who received the HD-VA supplement was $7.4 \pm 2.4\%$ at 3 d after dosing (Fig. 2; individual data shown in Supplemental Fig. 2A,B). The estimated retention, as percentage of dose administered, was $76.3 \pm 6.7\%$ (range: $67.4$–$81.8\%$; $n = 4$). The cumulative amount of $^{14}$C recovered in urine in children who received the dose of SI-labeled VA was $5.4 \pm 1.6\%$ at 3 d after dosing. The estimated retention, as percentage of dose administered, was $71.1 \pm 9.4\%$ (range: $57.1$–$76.7\%$; $n = 4$). Estimates of mean retention did not differ by dose levels.

Urinary elimination of $^{14}$C. The UER declined rapidly during the first week after dosing. At 8.4 d after dosing, $>95\%$ of the fraction of $^{14}$C in urine was represented by the final exponential term of the equation and the fraction of $^{14}$C in urine continued to decline until 59 d after dosing for all children (Fig. 3). The UER of $^{14}$C in children who received the HD-VA supplement was $1.9 \pm 0.6\%/d$ (range: $1.5$–$2.7\%/d$; $n = 4$). The UER of $^{14}$C of the children who received the dose of SI-labeled VA was $1.8 \pm 1.2\%/d$ (range: $0.9$–$3.4\%/d$; $n = 4$). Mean estimates of the UER of VA did not differ between children who received the HD-VA supplement or the dose of SI-labeled VA.

Dietary assessment. The reported frequency of consumption of selected VA-containing foods was highest for VA-fortified sugar, $9.1 \pm 1.9$ times/wk, and dark green leafy vegetables, $8.0 \pm 1.0$ times/wk, and did not differ by group. The reported frequency of consumption of all other VA-containing foods was $<4$ times/wk and did not differ by group.

Biochemical and morbidity assessments

The initial hemoglobin concentration was $119.4 \pm 5.8$ g/L and none of the children had anemia. None of the children had elevated plasma CRP ($>5$ mg/L) at either time point. Seven of the 8 boys (87.5%) had elevated plasma AGP ($>1.0$ g/L) initially and all boys had elevated plasma AGP on study day 75. Initial plasma retinol concentrations in the HD-VA and SI-labeled VA groups were $1.10 \pm 0.28 \mu$mol/L and $1.09 \pm 0.28 \mu$mol/L, respectively and final plasma retinol concentrations were $1.03 \pm 0.20 \mu$mol/L and $1.09 \pm 0.26 \mu$mol/L, respectively; initial and final plasma retinol concentrations did not differ by group. No child had a concentration $<0.70 \mu$mol/L at either time point. Plasma retinol concentrations was not associated with absorption, retention, or urinary elimination of VA either before or after controlling for elevated initial AGP. None of the children had a positive malaria smear. The prevalence of reported morbidity was low around the time of administration of the $^{14}$C-labeled dose of VA (Table 2). Nevertheless, there was a negative association between estimated VA absorption and reported fever ($r = -0.83; P = 0.01$) and malaria ($r = -0.83; P = 0.01$) and between estimated VA retention and reported fever.

### TABLE 1 Initial characteristics of the boys who were administered HD-VA or SI-labeled VA

<table>
<thead>
<tr>
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<th>HD-VA</th>
<th>SI-labeled VA</th>
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<td>$3.6 \pm 0.3$</td>
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<td>Anthropometry</td>
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<td>$14.6 \pm 0.8$</td>
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<td>MUAC, cm</td>
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<td>$16.7 \pm 0.3$</td>
</tr>
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<td>$-0.9 \pm 0.8$</td>
</tr>
<tr>
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<td>$-0.9 \pm 0.7$</td>
</tr>
<tr>
<td>WHZ</td>
<td>$-1.1 \pm 0.2$</td>
<td>$-0.2 \pm 0.3$</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD. Mean values do not differ between groups.

2 Abbreviations: HAZ, height-for-age Z score; MUAC, mid-upper arm circumference; WAZ, weight-for-age Z score; WHZ, weight-for-height Z score.
Indian children received either 4–5 μCi[^3]H]-retinyl acetate or 14.4 μCi[^3]H]-retinyl acetate as a metabolic tracer (2–4). Absorption was estimated based on cumulative excretion of[^3]H in 24-h stool samples for 4 or 8 d after dosing and radioactivity was measured using liquid scintillation spectrometry (2–4). In the present study, a tracer dose of[^14]C]-retinyl acetate (0.925 kBq) was administered to children. Absorption was estimated based on cumulative excretion of[^14]C in 24-h stools for 3 d after dosing, and radioactivity in stool and urine samples was quantified using AMS, which is a far more precise and sensitive analytical method.

The number of 24-h stool samples that are required after dosing to obtain an accurate estimate of cumulative excretion of unabsorbed[^14]C is not certain. In a U.S. man, excretion of[^14]C-labeled β-carotene in stool was biphasic, with an initial spike from 0 to 48 h representing unabsorbed label and a slower output after 48 h, which was attributed to biliary excretion of metabolites or normal sloughing of intestinal cells that had accumulated β-carotene and/or its metabolites (8). In Indian children, the excretion of[^3]H in stool spiked initially and a low output was observed by 2–4 d after oral dosing with[^3]H]-retinyl acetate (2–4). In our study, we also observed an initial spike in fecal excretion of[^14]C but did not observe an obvious plateau by 3 d after dosing. In contrast, urinary excretion of[^14]C was very low by 7 d after dosing (0.162 ± 0.310% of the[^14]C-VA dose administered in the HD-VA group and 0.131 ± 0.950% of the[^14]C-VA dose administered in the SI-labeled VA group), suggesting that fecal excretion of[^14]C beyond 3 d after dosing represents metabolites of VA that are excreted into feces in bile. However, it is possible that some of the[^14]C in feces beyond 3 d was derived from unabsorbed VA that had accumulated in sloughed intestinal cells and that absorption was overestimated in children who did not reach an obvious plateau by 3 d after dosing. In the earlier study in Indian children, absorption was likely underestimated, because fecal excretion of label during the later days (−d4–8) probably represented metabolites of absorbed labeled VA (3). Additionally, some of the[^3]H may have exchanged with H⁺ in the gut and been excreted in feces.

It is also possible that VA status of the children is related to differences in estimates of absorption across studies. The Indian children in 2 of the earlier studies had marginal to low VA status based on reported serum retinol concentrations [ranging from 0.38 to 0.94 μmol/L; serum retinol concentrations were not reported for reference (4)], whereas children in the present study had adequate plasma retinol concentrations (2,3). However, in rats, absorption efficiency of VA does not differ among animals with low or marginal VA status, so it seems less likely that VA status might explain the observed differences in VA absorption (21).

**Discussion**

Using AMS methodology, we found that VA was adequately absorbed and retained from both a HD-VA supplement (209.8 μmol) and a lower dose VA supplement (17.5 μmol) among preschool-age Zambian boys. Their urinary elimination of VA was consistent with earlier estimates in children and somewhat greater than that reported for adults (20). This is the first time that VA metabolism has been quantified directly in preschool age children using a very low physiologic tracer dose of radiolabeled VA and AMS methodology. Importantly, this study demonstrates that AMS methodology can be carried out successfully in a pediatric population in a low-income community setting.

**Estimated absorption of VA.** Estimated absorption of the HD-VA supplement in Zambian boys tended to be higher than previously reported values for 4- to 5-y-old Indian children (68.2–79.4%) and 3- to 6-y-old Indian children (67.2%) (3,4). There are methodological differences across these studies that may account for the differences in estimates of absorption. The number of 24-h stool samples that are required after dosing to obtain an accurate estimate of cumulative excretion of unabsorbed[^14]C is not certain. In a U.S. man, excretion of[^14]C-labeled β-carotene in stool was biphasic, with an initial spike from 0 to 48 h representing unabsorbed label and a slower output after 48 h, which was attributed to biliary excretion of metabolites or normal sloughing of intestinal cells that had accumulated β-carotene and/or its metabolites (8). In Indian children, the excretion of[^3]H in stool spiked initially and a low output was observed by 2–4 d after oral dosing with[^3]H]-retinyl acetate (2–4). In our study, we also observed an initial spike in fecal excretion of[^14]C but did not observe an obvious plateau by 3 d after dosing. In contrast, urinary excretion of[^14]C was very low by 7 d after dosing (0.162 ± 0.310% of the[^14]C-VA dose administered in the HD-VA group and 0.131 ± 0.950% of the[^14]C-VA dose administered in the SI-labeled VA group), suggesting that fecal excretion of[^14]C beyond 3 d after dosing represents metabolites of VA that are excreted into feces in bile. However, it is possible that some of the[^14]C in feces beyond 3 d was derived from unabsorbed VA that had accumulated in sloughed intestinal cells and that absorption was overestimated in children who did not reach an obvious plateau by 3 d after dosing. In the earlier study in Indian children, absorption was likely underestimated, because fecal excretion of label during the later days (−d4–8) probably represented metabolites of absorbed labeled VA (3). Additionally, some of the[^3]H may have exchanged with H⁺ in the gut and been excreted in feces.

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**Estimated retention of VA.** The estimated retention of the HD-VA supplement in Zambian boys tended to be higher than previously reported values for 4- to 5-y-old Indian children (23–54%) and for 3- to 6-y-old Indian children (47.9%) (3,4). It is likely that the reasons for the discrepancies across studies are related to differences in analytical methodologies and in the number of days after dosing that radioactivity was measured in stool and urine to estimate retention. In the Indian studies, estimates of retention were based on cumulative excretion of radioactivity for 4 d in both stool and urine or 8 d in stool and 5 d in urine compared with 3d in stool and urine in the present study (3,4). Retention may have been underestimated in the earlier studies because of prolonged sampling times. It is also possible that VA status affects retention. In rats, VA utilization
varies with VA status (21,22). The daily VA disposal rate is higher in rats with adequate VA status than in rats with inadequate status, suggesting that degradative utilization occurs when VA status is adequate and VA intake increases, and that degradative utilization decreases when VA status and VA intake are inadequate. However, this is not a likely explanation of the differences in retention between boys in the present study and Indian children in the earlier studies, because boys in the present study had adequate VA status and higher VA retention than Indian children with inadequate VA status.

In the present study, the cumulative amounts of $^{14}$C lost in urine during the first 3 d after dosing did not differ from the amounts of $^{14}$C lost in urine in the first 3 d after dosing based on estimates of each individual’s estimated UER for the 59-d period (Table 3). This suggests that VA excreted in urine during the first 3 d after dosing is similar to the usual daily VA elimination rate and that retention is driven largely by absorption.

**UER.** The estimated UER of the HD-VA supplement and of the smaller dose of SI-labeled VA were 1.9 ± 0.6%/d and 1.8 ± 1.2%/d, respectively. These UERs are similar to the estimated system fractional catabolic rate (2.2%/d; 95% CI: 1.4, 3.0%/d) for Peruvian preschool-age children that was estimated according to population-based plasma retinol kinetic data using SI-labeled VA (16). An estimated daily VA elimination rate of ~2%/d in preschool-age children is higher than the reported value of 0.5%/d for adults (20). However, this is not unexpected, because children require VA for growth in addition to other physiologic functions.

**Effect of morbidity on estimates of absorption, retention, and urinary elimination of VA.** In the present study, the prevalence of symptoms of illnesses was low; however, VA absorption and retention were negatively associated with reported fever and malaria. These associations were driven by 1 child whose caretaker reported that the child was sick for 1 d, 3 d prior to receiving his doses of $^{14}$C-labeled retinyl acetate and SI-labeled VA. In areas where malaria and fever are endemic, fever is often assumed to be caused by malaria; however, this child tested negative for malaria. Estimates of absorption and retention of VA for that child were 62.4 and 57.1%, respectively. If the values for that child are omitted from the data set, estimates of absorption and retention of the smaller dose of SI-labeled VA are 81.2 ± 1.4% and 75.8 ± 0.8%, respectively. In the earlier studies in Indian children, severe infection at the time of dosing was associated with a reduction in absorption and retention of VA (2).

The results of the present study are useful for examining the modified Olson equation that is used for estimating total body VA stores in this age group using SI dilution methodology (13).

**TABLE 3** Comparison of VA eliminated in urine 3 d after dosing as estimated by direct measurement or by the UER in boys administered HD-VA or SI-labeled VA

<table>
<thead>
<tr>
<th>VA in urine</th>
<th>Direct measurement</th>
<th>Based on the UER</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-VA, n = 4</td>
<td>11.3 ± 3.4</td>
<td>9.1 ± 3.2</td>
</tr>
<tr>
<td>SI-labeled, n = 4</td>
<td>1.3 ± 0.4</td>
<td>0.8 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Mean values do not differ within groups.
2 Estimated amount of VA is based on the cumulative measured amount of $^{14}$C excreted in urine at 3 d after dosing.
3 Estimated amount of VA is based on the daily UER of $^{14}$C for 3 d after dosing.

In the Olson equation, a factor of 0.5 is used for the efficiency of retention (UER) of a dose of SI-labeled VA. In the present study, retention of an oral dose of SI-labeled VA (17.5 μmol) was estimated as ~75% at 3 d after administration of the dose. In preschool-age children, an oral dose of labeled VA takes between 8 and 12 d to fully mix with the endogenous VA pool. Using the estimated retention (75%) at 3 d after dosing and the UER of VA of 1.9%/d from the present study, it can be estimated that ~64.4% of the dose of SI-labeled VA would have been retained at 10 d after dosing, which is higher than the value of 50% that is used to estimate retention in the Olson equation. Improved estimates of VA pool size may be obtained for this age group if a value of ~64% is used in the Olson equation as the estimate for retention of the dose of SI-labeled VA. The data in the present study suggest that the SI dilution methodology should not be carried out in children whose caretakers report symptoms of fever within the previous week.

**Considerations regarding the study population.** The main purpose of this study was to assess whether HD-VA supplements are well absorbed and retained in Zambian preschool-age children in low-income communities who are likely to participate in the national HD-VA supplementation program. Our results indicate that the HD-VA supplements are adequately absorbed and retained. However, the children who participated in our study had better VA status and a lower prevalence of morbidity and subclinical infection than children who participated in the national VA status survey in Zambia in 2003 (1). We might have obtained different estimates of absorption, retention, and urinary elimination of VA from children with lower initial VA status and/or a higher prevalence of inflammation/infection, as suggested by the result for the child with reported fever. In the larger study, we estimated the impact of a HD-VA supplement on total body VA stores using the SI dilution method, and it is recommended that the test be carried out in children who are initially free from symptoms of illness to maximize the likelihood that they will adequately absorb and retain the oral dose of SI-labeled VA. Thus, we selected children for the larger study who were apparently healthy. Thus, our results for estimated absorption, retention, and urinary elimination of VA following administration of the HD-VA supplement reflect the responses of a small group of relatively healthy Zambian preschool-age boys from a low-income community; it is uncertain whether these findings would apply to the larger population of Zambian children who may experience more illnesses.

In summary, HD-VA supplements were adequately absorbed and retained in a small group of apparently healthy preschool-age Zambian boys from a low-income community. The estimated retention of the oral dose of SI-labeled VA was higher than the value of 50% that is currently used in the Olson equation for estimation of total body VA pool size using SI dilution methodology. This study demonstrates that AMS methodology can be carried out in a pediatric population in a low-income community setting and suggests that this methodology could be used to obtain direct, quantitative estimates of VA metabolism for other population subgroups for which data do not exist.

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responsibility for final content. All authors read and approved the final manuscript.

**Literature Cited**


