Eur J Nutr 41 : 141–147 (2002)
DOI 10.1007/s00394-002-0368-0

ORIGINAL CONTRIBUTION

Dual isotope test for assessing β-carotene cleavage to vitamin A in humans

Sabrina J. Hickenbottom
Shawna L. Lemke
Stephen R. Dueker
Yumei Lin
Jennifer R. Follett
Colleen Carkeet
Bruce A. Buchholz
John S. Vogel
Andrew J. Clifford

Summary *Background* The ability of β-carotene to deliver bioactive retinoids to tissues is highly variable. A clearer understanding of the environmental and genetic factors that modulate the vitamin A potential of β-carotene is needed. *Aim of study* Assess the vitamin A value of orally administered β-carotene relative to a co-administered reference dose of preformed vitamin A. *Methods* Equimolar doses (30 μmol) of hexadeuterated D_6 β-carotene and D_6 retinyl acetate were orally co-administered in an emulsified formulation to a male subject. The plasma concentration time courses of D_6 retinol (derived from D_6 retinyl acetate) and bioderived D_3 retinol (from D_6 β-carotene) were determined for 554 h postdosing using gas chromatography/mass spectrometry. Intact D_6 β-carotene plasma concentrations were determined by high-pressure liquid chromatography. The ratio of the two forms of vitamin A, D_6 retinol/D_3 retinol, at any single time point is postulated to reflect the quantity of vitamin A derived from β-carotene relative to preformed vitamin A. Additionally, a minute amount of ^{14}C β-carotene (50 nCi; 0.27 μg) was included in the oral dose and cumulative 24-h stool and urine samples were collected for two weeks to follow absorption and excretion of the β-carotene. The ^{14}C nuclide was detected using accelerator mass spectrometry (AMS). *Results* During the absorption/distribution phase (3–11 h) the D_6/D_3 ratio of the two retinols was not stable and ranged between a value of 3 and 16. Between 11 and 98 h postdosing the ratio was relatively stable with a mean value of 8.5 (95 % CI: 7.5, 8.7). These data suggest that in this subject and under these conditions, 8.5 moles of β-carotene would provide a vitamin A quantity equivalent to 1 mole of preformed vitamin A. On a mass basis, 15.9 μg of β-carotene was equivalent to 1 μg of retinol. The total administered β-carotene was found to be 55 % absorbed by AMS analysis of cumulative stool. *Conclusion* The co-administration of D_6 β-carotene and D_6 retinyl acetate provides a technique for assessing individual ability to process β-carotene to vitamin A. The results indicate that a single time point taken between 11–98 h after dose administration may provide a reliable value for the relative ratio of the two forms of vitamin A. However, results from more subjects are needed to assess the general utility of this method.

Key words vitamin A – β-carotene – AMS – stable isotope – carbon-14 – human

Received: 29 November 2001
Accepted: 3 June 2002

S. J. Hickenbottom · S. L. Lemke · S. R. Dueker (✉) · Y. Lin · J. R. Follett · C. Carkeet · A. J. Clifford
3217A Meyer Hall
Department of Nutrition
University of California, Davis
One Shields Ave
Davis, CA 95616, USA
E-Mail: srdueker@ucdavis.edu

B. A. Buchholz · J. S. Vogel
Center for Accelerator Mass Spectrometry
Lawrence Livermore National Laboratory
Livermore, CA 94551, USA

Work was performed under the auspices of the US Department of Energy by University of California Lawrence Livermore National Laboratory under Contract No. W-7405-Eng-48, the NIH/National Center for Research Resources (RR13461), NIDDK DK48307, and USDA CSREES # 99-35200-7584.

EJN 368

Introduction

Provitamin A β-carotene is absorbed intact or as intestinally cleaved vitamin A. The amount of vitamin A derived from β-carotene is highly variable and the factors that modulate vitamin A formation from β-carotene are under active investigation. Commonly referenced factors include food matrix [1, 2], oil content (emulsification) [3, 4], vitamin A status [5, 6], and competitive interaction with related non-provitamin A carotenoids [7–9]. Increasing interest is being directed towards genetic elements [10, 11]. Defining the underlying sources of inter-individual variance is of critical interest in developing countries where, despite abundant plant sources of β-carotene, vitamin A deficiencies persist [12, 13].

Tracer studies using isotopically labeled compounds coupled with mass spectrometric detection are arguably the best means of addressing in vivo metabolism of β-carotene and other nutrients. Early studies in the 1960's used radioisotopes in clinical patients [14–16], but these studies were gradually replaced by stable isotope approaches using carbon-13 and deuterium labeled substrates [17–25]. Recently, a reintroduction of carbon-14 is occurring as accelerator mass spectrometry (AMS) technology becomes available. AMS is reinvigorating the use of carbon-14 labeled substrates because of its high sensitivity (attomole). At attomolar levels of detection sensitivity, low nanoCurie amounts of ^{14}C labeled substrates provide ample signal for long-term in vivo testing and the detection of rare metabolites [19, 26, 27].

We previously reported estimates of β-carotene bioconversion to vitamin A in dual isotope studies using D_6 retinyl acetate (vitamin A) and D_6 β-carotene [21, 22]. In this design, deuterated D_6 retinyl acetate functioned as a reference dose for calculating the relative amount of retinol (D_3 retinol) derived from D_6 β-carotene. The test doses were administered one week apart with a low fat meal. Relative vitamin A value was calculated from area-under-the-curve (AUC) concentration-time plots for D_6 and D_3 retinol using multiple time points taken over several weeks. Tang [20] reported a similar approach, using different isotopomeric forms of the test compounds given up to 2 y apart. Potentially large inter-day variation in the absorption of the two compounds is a drawback of temporally separating the two doses.

These studies' designs do not lend themselves to the high throughput required for population surveys. Samples over many time points must be collected to adequately define the plasma response curves and generate AUC estimates. Significant modifications to the aforementioned protocols that would improve their value as tools for estimating the vitamin A equivalence of β-carotene are 1) co-administration of the two vitamin A sources so that inter-day variation is eliminated and so that the two forms are sampled on the same time axes. In this way a ratio at a single point will provide a ratio (D_6/D_3 integrated areas) of bioequivalence. 2) Deliver the dose in the emulsified state so that absorbability is maximized and variance in absorption due to incomplete solubilization is minimized. 3) Reduce the dose sizes to minimize competitive or inhibitory effects in absorption and bioconversion of the β-carotene and the two stable isotope forms of vitamin A.

To evaluate the dual isotope concept for a method amenable to population surveys, we performed an investigation in a single male subject utilizing three co-administered, isotopically labeled compounds. Equimolar doses of preformed vitamin A (D_6 retinyl acetate; reference dose) and D_6 β-carotene (experimental dose) were administered in a fatty drink. In addition, a minute amount of ^{14}C β-carotene (50 nCi, 0.27 μg, 0.51 nmol) was also given to determine bioavailability. Although a useful tool for confirming the results of our deuterated compounds, ^{14}C β-carotene would not be included in the field method other than to assess absolute bioavailability in certain sub-populations.

Subject and methods

Chemicals

All solvents and chemicals were purchased from Fisher Scientific (Santa Clara, CA) unless otherwise noted. β-apo-12'-carotenal-O-t-butyloxime and retinal-O-ethyloxime as internal standards for total plasma retinol and β-carotene determinations were prepared as described previously [21]. N-Methyl-N-(t-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) was purchased from Regis (Morton Grove, IL) in 1-mL glass ampoules.

The *all-trans*-[19,19,19,20,20,20-$^2H_6$]retinyl acetate (D_6 retinyl acetate) and [19,19,19,19',19',19'-$^2H_6$]β-carotene (D_6 β-carotene) were obtained from Cambridge Isotope Laboratories (Andover, MA). The isotopic purity of the D_6 retinyl acetate was determined by gas chromatography/mass spectrometry (GC/MS) to be 91% D_6 retinyl acetate, 6% D_5 retinyl acetate, 2% D_4 retinyl acetate, and 1% other forms. The isotopic purity of the D_6 β-carotene was determined by fast atom bombardment mass spectrometry to be 59% D_6 β-carotene, 34% D_5 β-carotene, 6% D_4 β-carotene, and 1% other forms [28]. *All-trans*-[6,6',7,7'–$^{14}C_4$] β-carotene was a gift from Roche Vitamins, Ltd. (Basel, Switzerland). Radiochemical purity was confirmed by analytical reversed phase high performance liquid chromatography (RP-HPLC). ^{14}C β-carotene was dissolved in ethanol, and the concentration determined spectroscopically at 450 nm ($\varepsilon = 141 \times 10^3$ [29]). The specific activity of the β-carotene was 98.8 mCi/mmol and was verified on two scintillation counters calibrated with National Institute

for Standards and Technology certified carbon–14 quench standards.

Radiation safety/human subjects

Informed written consent was obtained from the volunteer under the guidelines established by the Human Subject Review Committee. The Radiation Safety and Humans Subjects Committees of the University of California at Davis and Lawrence Livermore National Laboratories (LLNL) approved all protocols.

Dose preparation and delivery

The dosing drink was prepared from two small bananas (336 g), 110 mL of non-fat milk, sugar (50 g), and olive oil (29 g; Da Vinci brand). The isotope mix was composed of 30 μmol (10.2 mg) of D_6 retinyl acetate, 30 μmol (16.2 mg) of D_6 β-carotene that was weighed out as a solid and added to 11 g of olive oil as was a 200 uL aliquot of ethanol containing 50 nCi (0.27 μg, 0.51 nmol) ^{14}C β-carotene. For administration, the 11 g of oil containing the isotopes was mixed with approximately half of the drink and consumed quickly (one minute). The remaining half of the drink was then used to rinse the cup and this was consumed promptly. A third rinsing of the cup was done using 50 mL of the non-fat milk. A total of 40 g of olive oil (0.5 g/kg BW) was consumed.

Subject, diet, and specimen collection

The subject was a healthy 36-year-old male with a body mass index of 22.2 kg/m^2 (81 kg). The morning before the dose was given cumulative 24-h urine and stool collections began and continued for two weeks. The afternoon before dose administration, the subject was fitted with an intravenous catheter in a forearm vein. All blood samples were collected into EDTA vacutainer tubes. A baseline blood sample (7 ml) was taken before the dosing. Collection time points were 0, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 14, 16, 25.5, 36.5, 50, 74, 98, 122, 146, 218, 266, 314, 386, 434, and 554 h postdosing. After the first and second days of the study, blood draws were taken in the fasted state. Plasma was separated from the red blood cells by centrifugation and stored at –80 °C. The 24 h stool was collected in 4 mm (polyethylene, Fisher Scientific) collection bags and weights were recorded. 40-ml aliquots of cumulative urine collections were removed and stored at –80 °C until analysis.

On the dose administration day, meals were controlled for time and content. Lunch was served 5 h postdosing and consisted of a Turkey Tamale (Garibaldi; containing 8 g fat; 180 cal; 100 μg vitamin A, 250 μg β-carotene) and a cookie (4 g fat). Dinner was served 10 h postdosing and consisted of Country Dinner (Amy's Kitchen, Petaluma, CA; containing 12 g fat; 380 cal; 300 μg vitamin A; 200 μg β-carotene). The night preceding dosing, the subject consumed two tacos and two bean burritos delivering 44 g of fat (30 μg vitamin A) at 7:30 PM. The subject consumed only water after that time until dosing.

Analysis of stable isotope

Retinol isotopomers were determined according to methods previously described [21, 24]. Briefly, hexane extracts of 1 ml plasma aliquots were saponified in alkaline methanol so that retinol values represent total retinol in plasma (retinol + retinyl esters). *t*BDMS retinol isotopomers were analyzed by GC/MS (Perkin Elmer Turbomass gold) operated in the selected ion-monitoring mode with electron impact ionization (70 eV). The M–57 fragment ions were monitored at *m/z* 255, 258, and 261 for endogenous retinol, D_3 retinol, and D_6 retinol, respectively. However, due to isotopic heterogeneity of the D_6 β-carotene (59 % D_6, 34 % D_5, 6 % D_4), the ion signal for D_3 retinol (*m/z* 258) underestimates the total retinol product and the following adjustment was used. Upon cleavage, all 59 % of the D_6 form will yield D_3. The D_5 form will yield 50 % D_3 and 50 % D_2. The D_4 form is estimated to yield 25 % D_3, and 75 % D_2. Using these values, 77.5 % of the deuterated β-carotene can give D_3 retinol and 20 % D_2 retinol. Because of natural abundances, 33.8 % of the D_2 retinol provides signal at the D_3 retinol mass (0.338 × 21.5 % = 7.3 %). As a result, the final molar value for D_3 retinol derived from D_6 β-carotene was underestimated by 1/0.848 (77.5 + 7.3 = 84.8 %). The integrated area was adjusted by this factor. A set of calibrating standard mixtures of D_6 retinol and D_0 retinol (with molar ratios of D_6 retinol to D_0 retinol of 0.5, 0.2, 0.1, 0.05, 0.025, 0.0167, 0.01) were used to determine the D_6 retinol values.

Analytes were separated on a 30 m DB-1 capillary column (0.25 mm ID, 0.25 mm film thickness) with helium as the carrier gas (1 ml/min). The injector temperature was held at 275 °C to minimize fouling by lipid contaminants [24]. The source temperature was maintained at 150 °C and the transfer line at 200 °C. β-carotene isotopomers (endogenous and D_6) were separated using RP-HPLC as previously described [21, 28].

^{14}C analysis by AMS

A volume equivalent to five times the sample mass of 50/50 1M KOH/2-propanol was added to cumulative

stool collections. The sample was then dispersed using a Stomacher laboratory blender (Fisher Scientific: Model 3500) 2 min on the high setting according to a previously described procedure [19]. Aliquots of plasma (25 µL) and urine (100 µL) were analyzed neat, without fractionation or admixture. The ^{14}C determinations were made at the Center for the Accelerator Mass Spectrometry (CAMS) at LLNL (Livermore, CA). Results are expressed as fmol ^{14}C/mL plasma or percentage of the dose. Total carbon measurements were made by Carlo-Erba Carbon-Nitrogen analysis. Urine (75 µL), stool (75 µL) and plasma (25 µL) aliquots were lyophilized and sent to the Division of Agriculture and Natural Resources for total carbon determination [30].

Data analysis

Plasma peak concentrations (C_{max}) and times were determined directly from the data points. Area-under-the-curve values were determined by the trapezoidal approximation. Statistical analyses were performed with Origin (version 6.0, Northampton, MA). Excess ^{14}C concentrations over natural abundance were converted to β-carotene and/or retinol equivalents using the specific activity of the β-carotene and the carbon content. The vitamin A equivalence of D_6 β-carotene was determined from the mean ratio of the D_6/D_3 areas for the time range at which the retinol ratio reached a plateau (11 h postdosing). The resulting value represents the effective molar value of β-carotene to retinol, when D_6 retinol acts as a biologic internal standard.

Results

Plots displaying the concentration of D_6 retinol, D_3 retinol, D_6 β-carotene, and total ^{14}C in plasma with time are shown in Fig. 1. The shape of the deuterated retinol isotopomers plots were similar (Fig. 1 A and B), with both compounds displaying an initial postprandial rise that peaked at 4.5–5 h, followed by a slight nadir, with a second rise to a true maximum at ~7.5 h of 0.71 and 0.09 µmol/L for D_6 retinol and D_3 retinol, respectively. Following a small plateau, the decline in concentration was biphasic, with a steep drop from 12–18 h, followed by a much slower decay to the end of the sampling period.

The D_6 β-carotene (panel C) had a similar kinetic pattern to the retinols in the first 15 h postdosing, with a small peak at 4.5 h, followed by a maximum of ~ 0.055 µmol/L between 8 and 11 h. A second valley reached a nadir at 15 h. At that point the kinetic pattern deviated from the retinols most significantly, by rising to a true maximum of 0.072 µmol/L at ~36.5 h. The concentration of total ^{14}C in the plasma is shown in panel D.

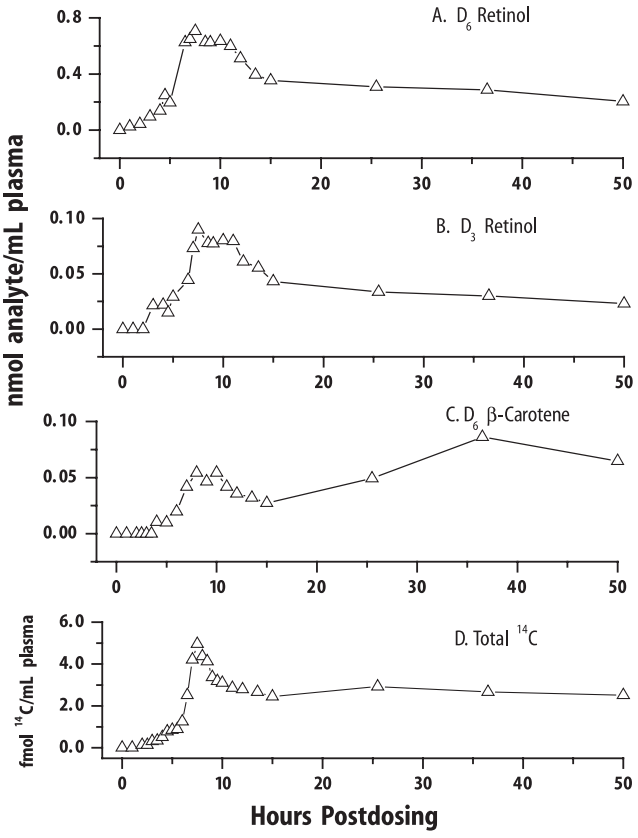

Fig. 1 Plasma concentration versus time (0–50 h) plots for the absorption of deuterated vitamin A and β-carotene and ^{14}C β-carotene. D_6 (**A**) and D_3 (**B**, derived from D_6 β-carotene) retinol follow the same plasma kinetics although at a ten-fold difference in concentration. D_6 β-carotene (**C**) was absorbed and distributed for 48 h before plasma levels began to equilibrate. **D** is the plot of the total ^{14}C (β-carotene and retinol) found in the plasma.

This plot represents the summation of all ^{14}C labeled compounds, i. e., carotenoid and retinoid forms, appearing in the plasma. The concentration displayed a true maximum of 4.9 fmol ^{14}C/mL plasma at ~ 7.5 h. After that, it could be characterized as having a pattern in between the retinols and β-carotene, as the concentration did not significantly rise or fall between 15 and 50 h.

The ratio of retinol isotopomer concentration, D_6/D_3, over time is plotted in Fig. 2. During the absorption/distribution phase (3–11 h) the ratio fluctuated between values of 3 and 16. Between 11–98 h, the ratio stabilized to a mean value of 8.5 (95 % CI: 7.5, 8.7). Interpretation of this value suggests that 8.5 moles of β-carotene will provide a vitamin A quantity equivalent to 1 mole of preformed vitamin A when orally consumed. On a mass basis, 15.9 µg of β-carotene is equivalent to 1 µg of retinol. In comparison, the ratio of the retinol D_6/D_3 AUCs from 0–98 h (Fig. 1 A and B) was 8.3. If it were assumed that the preformed vitamin A was absorbed quantitatively (100 %), and given the fact that 45 % of the β-carotene dose was not absorbed (see below), the vitamin A quan-

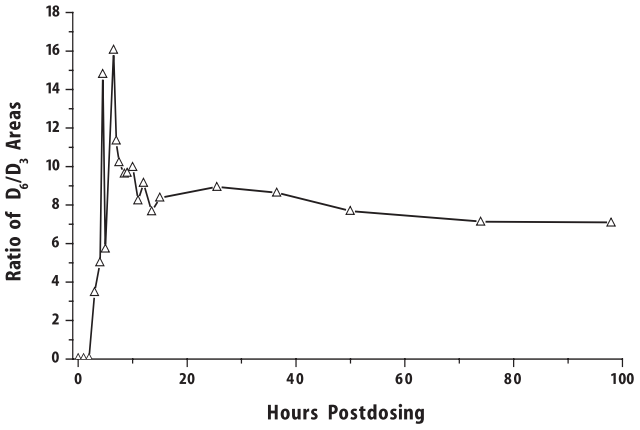

Fig. 2 The ratio of the preformed vitamin A (D_6 retinol) to vitamin A derived from β-carotene (D_3 retinol) reaches a plateau in the plasma after 11 h postdosing which is sustained for approximately 3 d.

tity equivalent to 1 mole of preformed vitamin A of the β-carotene that was absorbed would be 15.9 * 0.55, or 8.8 μg.

The stool and urinary output of the ^{14}C β-carotene dose is presented in Fig. 3 as percent dose excreted over time. There was a 1.25 d lag period before the ^{14}C dose appeared in the stool. The cumulative output plot was biphasic, with a large output from day 1 through day 3; after that, the output rate was reduced. The ^{14}C collected through day 3 is attributed to non-absorbed ^{14}C β-carotene; accordingly, 45 % of the dose (0.227 nmol) was unabsorbed, and 55 % of the dose was absorbed into systemic circulation. Only 1 % of the administered dose appeared in the next 9 collections (days 4–12). The urinary output of ^{14}C in the first 14 d was approximately 4.3 % of the absorbed dose.

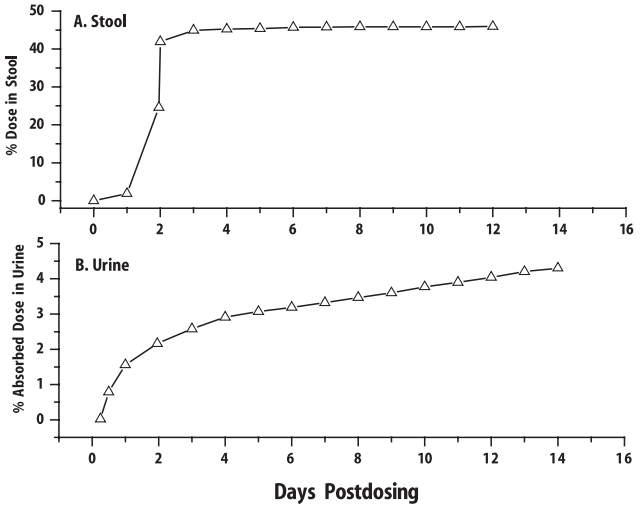

Fig. 3 The cumulative stool (**A**) illustrates the recovery of ^{14}C as a percentage of the dose administered as ^{14}C β-carotene. A total of 45 % of the dose was recovered in the stool in the first 48 h, indicating that 55 % of the dose was absorbed. The cumulative urine (**B**) shows an elimination of 4 % of the absorbed dose.

Discussion

The D_6/D_3 ratio (Fig. 2) varied widely out to 11 h postdosing. If metabolism of the β-carotene and preformed vitamin A were highly similar, a constant ratio would be expected at any time point. However, some differences in the processing of carotenoids and retinoids are known, which would result in differing kinetic patterns for the two retinol forms during absorption [31]. The absorption of preformed vitamin A is a facilitated process, whereas β-carotene is absorbed by passive diffusion [32]. One result of this is, β-carotene may be retained in the intestinal plasma membrane and undergo multiple absorptive-cleavage events upon absorption of later meals [19]. Additionally, the β-carotene must be cleaved by 15,15'-dioxygenase, unlike preformed vitamin A, prior to incorporation as retinyl esters into chylomicra. Therefore, sampling in the early postprandial region will not provide an accurate estimate of β-carotene relative to the preformed retinyl acetate. However, these differences become less significant as the molecules enter the postabsorptive (distribution and metabolism) phases.

It is unclear if β-carotene continues to serve as a source of vitamin A post-intestinally. It was recently shown that substantial β-carotene cleavage capacity exists in the human liver [34]. Additional support for hepatic cleavage comes from a model-based interpretation of D_8 β-carotene kinetic data in a single male subject [35]. The model predicted that ~43 % of derived vitamin A was a result hepatic cleavage. In our study, we observed a drop in the D_6/D_3 ratio between 25 and 74 h from 8.9 to 7.1. This observed drop is consistent with the occurrence of postabsorptive cleavage.

One concern with the co-administration of preformed vitamin A and β-carotene is possible inhibition of the β-carotene cleavage enzyme by the vitamin A present in the dose. Direct data on this topic is lacking, although there is suggestion that vitamin A status does modulate the cleavage of β-carotene [6]. It is not known whether this effect occurs at the genetic or enzymatic level. Our bioequivalence value of 0.11 is within the range of values previously reported by us (0.03–0.81 moles of D_3 retinol per mole of D_6 β-carotene; 16.1 mg dose) [21, 22], and others (0.034–0.48) [20] when the doses are not co-administered. Longitudinal studies with a vitamin A intervention and or depletion are needed to address this topic.

To provide sufficient analytical signal, we chose equimolar doses of β-carotene and vitamin A at a level that was 3–4 times the average daily intake in the US diet. A drawback of this design is that it might render the assay insensitive to individual differences in β-carotene

absorption and cleavage capacity due to saturation of biological processes. Results from more subjects are needed to determine if the test is sensitive enough to detect individual variation at these dose amounts. However, there is reason to suspect that the assay will be sensitive. Borel [36] found that inter-individual difference in the intestinal conversion of β-carotene to retinyl palmitate could still be detected after pharmacological-sized amounts (120 mg) of β-carotene. Similarly, using 15 mg oral doses, van Vliet [33] found a low β-carotene response to be associated with high ratio between retinyl palmitate and β-carotene response in triglyceride-rich fractions taken from plasma. Therefore we feel the present amounts are appropriate for detecting variation across individuals in a population survey. Furthermore, we chose larger doses to accommodate a longer sampling period than what might be needed. We are confident the doses can be lowered to operate at more physiologically relevant levels if desired. In the case where signal might be marginal, larger volumes of plasma (> 1 ml) could be processed.

Where food bioequivalence is sought at normal dietary intakes, a multiple dosing design using amounts equivalent to average dietary intake may be more appropriate than a single dose design [18]. Repeat dosing achieves an increase in signal by MS for improved analytical results. Further, this approach also holds the added value of minimizing variance from day-effects in absorption. Alternatively, ^{14}C AMS methods can deliver good analytical results at true tracer levels.

The fractional absorption of β-carotene is thought to decrease with the magnitude of the dose. To assess absorption, we co-administered a small dose of ^{14}C β-carotene as a tracer of the total administered β-carotene (16.1 mg total). Surprisingly, we found the absolute absorption of the β-carotene dose (55 %) to be at the high end of literature values (9–52 % absorbed) [14,15,19,37]. Furthermore, our value may be underestimated due to the biliary secretion of labeled metabolites in the early time points. We recently reported 40 % absorption in the same subject tested in this study following a 306 μg dose of ^{14}C β-carotene [19]. The mode of administration differed mainly in that the fat content was modestly lower and derived from solid foods. The higher absorption reported in the present study, despite the much larger dose, would suggest that an optimized fat load diet could overcome some of the inefficiencies reported with β-carotene absorption [3].

The total ^{14}C plot generally parallels the shape of the summed deuterium plots, with the notable deviation being in the rate of decay after the ~8 h apex. Here the ^{14}C concentrations drop abruptly whereas the deuterium labeled metabolites do not drop significantly in concentration until after 11–12 h. We attribute these deviations to the selectivity of the measuring instruments: without metabolite speciation, the AMS detects all ^{14}C labeled metabolites in the plasma, including compounds such as glucuronides, epoxides, and excretion products such as retinoic acids. For deuterium labeled compounds, only the target analyte is measured. With the appropriate chromatographic separation, AMS can be used to measure specific analytes [19], however, ^{14}C metabolite speciation was not performed in the present study.

In conclusion, a better understanding of the genetic and environmental determinants of β-carotene utilization as a vitamin A source is needed. The co-administration of D_6 retinyl acetate and D_6 β-carotene is a promising approach for assessing cleavage capacity and variation in individuals as part of population surveys. This tool can serve as a complement to multiple dosing regimens or AMS methods that can assess the vitamin A value of β-carotene at physiological and sub-physiological levels, respectively. Now that the main carotenoid cleavage enzyme, β-carotene 15,15'-dioxygenase has been sequenced [10, 11], genetic variants may soon be identified. Dual isotope studies coupled with new genotype information will afford a unique opportunity to assess the pharmacogenetics of β-carotene cleavage.

Acknowledgements The authors would like to thank Drs. Betty J. Burri (WHNRC–USDA) and William Cohn (Roche Vitamins Ltd) for their generous donations of isotopes.

References

1. Edwards A, Nguyen C, You C, et al. (2002) Alpha- and beta-carotene from a commercial puree are more bioavailable to humans than from boiled-mashed carrots, as determined using an extrinsic stable isotope reference method. J Nutr 132:159–167
2. De Pee S, West CE, Permaesih D, et al. (1998) Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and beta-carotene in school-children in Indonesia. Am J Clin Nutr 68:1058–1067
3. Borel P, Tyssandier V, Mekki N, et al. (1998) Chylomicron beta-carotene and retinyl palmitate responses are dramatically diminished when men ingest beta-carotene with medium-chain rather than long-chain triglycerides. J Nutr 128:1361–1367
4. Hu X, Jandacek RJ, White WS (2000) Intestinal absorption of beta-carotene ingested with a meal rich in sunflower oil or beef tallow: postprandial appearance in triacylglycerol-rich lipoproteins in women. Am J Clin Nutr 71:1170–1180
5. Sommer A, West KP (1996) Vitamin A deficiency: Health, Survival, and Vision, Oxford University Press, New York, pp xiii, 438, [8] of plates
6. Ribaya-Mercado JD, Solon FS, Solon MA, et al. (2000) Bioconversion of plant carotenoids to vitamin A in Filipino school-aged children varies inversely with vitamin A status. Am J Clin Nutr 72:455–465

7. White WS, Stacewicz-Sapuntzakis M, Erdman JW Jr, et al. (1994) Pharmacokinetics of beta-carotene and canthaxanthin after ingestion of individual and combined doses by human subjects. J Am Coll Nutr 13:665–671
8. Kostic D, White WS, Olson JA (1995) Intestinal absorption, serum clearance, and interaction between lutein and beta-carotene when administered to human adults in separate or combined oral doses. Am J Clin Nutr 62:604–610
9. Tyssandier V, Cardinault N, Caris-Veyrat C, et al. (2002) Vegetable-borne lutein, lycopene, and β-carotene compete for incorporation into chylomicrons, with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. Am J Clin Nutr 75:526–534
10. von Lintig J, Vogt K (2000) Filling the gap in vitamin A research: molecular identification of an enzyme cleaving β-carotene to retinal. J Bio Chem 275: 11915–11920
11. Wyss A, Wirtz G, Woggon WD, et al. (2000) Cloning and expression of b,β-carotene 15,15'-dioxygenase. Biochem Biophys Res Commun 271:334–336
12. Ahmed F (1999) Vitamin A deficiency in Bangladesh: a review and recommendations for improvement. Public Health Nutr 2:1–14
13. De Pee S, West CE (1996) Dietary carotenoids and their role in combating vitamin A deficiency: a review of the literature. Eur J Clin Nutr 50:S38–S53
14. Blomstrand R, Werner B (1967) Studies on the intestinal absorption of radioactive beta-carotene and vitamin A in man. Conversion of beta-carotene into vitamin A. Scand J Clin Lab Invest 19: 339–345
15. Goodman DS, Blomstrand R, Werner B, et al. (1966) The intestinal absorption and metabolism of vitamin A and beta-carotene in man. J Clin Invest 45: 1615–1623
16. Sauberlich HE, Hodges RE, Wallace DL, et al. (1974) Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam Horm 32:251–275
17. Parker RS, Brenna JT, Swanson JE, et al. (1997) Assessing metabolism of beta-[^{13}C]carotene using high-precision isotope ratio mass spectrometry. Methods Enzymol 282:130–140
18. van Lieshout M, West CE, Muhilal, et al. (2001) Bioefficacy of β-carotene dissolved in oil studied in children in Indonesia. Am J Clin Nutr 73:949–958
19. Dueker SR, Lin Y, Buchholz BA, et al. (2000) Long-term kinetic study of beta-carotene, using accelerator mass spectrometry in an adult volunteer. J Lipid Res 41:1790–1800
20. Tang G, Qin J, Dolnikowski GG, et al. (2000) Vitamin A equivalence of beta-carotene in a woman as determined by a stable isotope reference method. Eur J Nutr 39:7–11
21. Lin Y, Dueker SR, Burri BJ, et al. (2000) Variability of the conversion of beta-carotene to vitamin A in women measured by using a double-tracer study design. Am J Clin Nutr 71:1545–1554
22. Hickenbottom SJ, Lin Y, et al. (2002) Variability of the conversion of β-carotene to vitamin A in men measured by using a double-tracer study design. Am J Clin Nutr 75:900–907
23. Novotny JA, Britz S, Pawlosky RJ, et al. (2001) Absorption of beta-carotene from carbon-13 labeled kale. FASEB J 15:A954
24. Handelman GJ, Haskell MJ, Jones AD, et al. (1993) An improved protocol for determining ratios of retinol-d4 to retinol isolated from human plasma. Anal Chem 65:2024–2028
25. Haskell MJ, Islam MA, Handelman GJ, et al. (1998) Plasma kinetics of an oral dose of ($^2H_4$)retinyl acetate in human subjects with estimated low or high total body stores of vitamin A. Am J Clin Nutr 68:90–95
26. Vogel JS, Turteltaub KW, Finkel R, et al. (1995) Accelerator mass spectrometry: isotope quantification at attomole sensitivity. Anal Chem 67:353A–359A
27. Vogel JS, Turteltaub KW (1998) Accelerator mass spectrometry as a bioanalytical tool in nutrition research. In: Clifford AJ, Muller H-G (eds) Mathematical Modeling in Experimental Nutrition. Plenum Press, New York, pp 397–410
28. Dueker SR, Jones AD, Clifford AJ (1998) Protocol development for biological tracer studies. In: Clifford AJ, Muller H-G (eds) Mathematical Modeling in Experimental Nutrition. Plenum Press, New York, pp 363–378
29. Goodwin TW (1976) Chemistry and Biochemistry of Plant Pigments, 2nd edn, Academic Press, London; New York, p 2
30. Clifford AJ, Arjomand A, Dueker SR, et al. (1998) The dynamics of folic acid metabolism in an adult given a small tracer dose of ^{14}C-folic acid. In: Clifford AJ, Müller H-G (eds) Mathematical Modeling in Experimental Nutrition. Plenum Press, New York, pp 239–252
31. Olson JA (1986) Physiological and metabolic basis of major signs of vitamin A deficiency. In: Bauernfeind JC (ed) Vitamin A Deficiency and its Control. Academic Press, Orlando, FL, pp 19–67
32. Furr HC, Clark RM (1997) Intestinal absorption and tissue distribution of carotenoids. J Nutr Biochem 8:364–377
33. van Vliet T, Schreurs W, van den Berg H (1995) Intestinal beta-carotene absorption and cleavage in men: response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. Am J Clin Nutr 62:110–116
34. During A, Nagao A, Hoshino C, et al. (1996) Assay of beta-carotene 15,15'-dioxygenase activity by reverse-phase high-pressure liquid chromatography. Anal Biochem 241:199–205
35. Novotny JA, Zech LA, Furr HC, et al. (1996) Mathematical modeling in nutrition: constructing a physiologic compartmental model of the dynamics of β-carotene metabolism. In: Coburn S, Townsend D (eds) Advances in Food and Nutrition Research, Vol. 40. Academic Press, Inc., San Diego, CA
36. Borel P, Grolier P, Mekki N, et al. (1998) Low and high responses to pharmacological doses of beta-carotene: proportion in the population, mechanisms involved and consequences on beta-carotene metabolism. J Lipid Res 39: 2250–2260
37. van Vliet T, Schreurs W, van den Berg H (1995) Intestinal beta-carotene absorption and cleavage in men: response of beta-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of beta-carotene. Am J Clin Nutr 62:110–116