Excentral cleavage of β-carotene in vivo in a healthy man[1–3]

Charlene C Ho, Fabiana F de Moura, Seung-Hyun Kim, and Andrew J Clifford

ABSTRACT

Background: Excentral cleavage of β-carotene to retinoids and apocarotenoids occurs in vitro and in animal models. Whether it occurs in humans is unclear.

Objective: We tested the hypothesis of whether humans can cleave β-carotene excentrally.

Design: A healthy man was given an oral dose of *all-trans* [10,10′,11,11′-^{14}C]-β-carotene (1.01 nmol; 100 nCi). Its fate and that of its metabolites were measured in serial plasma samples. Its fate in feces and urine was also measured over time. Selected plasma samples were spiked with reference standards of retinol, β-apo-12′-carotenal, β-apo-8′-carotenal, *13-cis*-retinoic acid, *all-trans*-retinoic acid, β-carotene-5,6-epoxide, *all-trans*-β-carotene, and retinyl palmitate and subjected to reverse-phase HPLC fractionation. The plasma, plasma fractions, urine, and feces were measured for ^{14}C with the use of accelerator mass spectrometry.

Results: Sixty-five percent of administered ^{14}C was absorbed, and 15.7% was eliminated in urine during the first 21 d after dosing. ^{14}C-β-carotene and ^{14}C-retinyl palmitate appeared in plasma 0.25 d after the dose. ^{14}C-β-carotene and ^{14}C-retinol both appeared at 0.5 d only. On day 3 after the dose, 2 large ^{14}C peaks appeared in plasma: one matched the retention time of β-apo-8′-carotenal, and the other did not match any of the reference standards used. The delayed appearance of ^{14}C-β-apo-8′-carotenal in plasma suggests that the excentral cleavage occurred after the ^{14}C-β-apo-8′-carotene was absorbed into the body.

Conclusion: These data suggest that excentral cleavage of ingested β-carotene occurs in vivo in humans. Confirmation of that possibility and further study to identify and characterize additional metabolites are needed. *Am J Clin Nutr* 2007;85:770–7.

KEY WORDS β-carotene, β-apo-carotenal, humans, vitamin A, ^{14}C

INTRODUCTION

Plants and algae synthesize β-carotene that contributes to their color and may confer health benefits as part of diets rich in fruit and vegetables. Approximately 600 carotenoids occur naturally, but only ≤60 are found in foods commonly consumed by humans (1). Of these, β-carotene is the best known because, pursuant to its central cleavage to retinal and oxidation to retinol, it can serve as an important source of vitamin A. Excentral cleavage of β-carotene to retinoids and apocarotenoids has been shown in a variety of in vitro systems and animal models. Whether excentral cleavage occurs in vivo in humans and, if so, to what extent is unclear.

When cleaved centrally by β,β-carotene-15,15′-oxygenase [EC 1.13.11.21] (BCO1), β-carotene can yield 2 molecules of retinal (2). When cleaved excentrally, it can lead to 8′-, 10′-, and 12′-apo-carotenals and corresponding alcohols and acids (3). The enzyme β,β-carotene-9′,10′-oxygenase (BCO2) was already identified (4). β-Carotene and its derivatives may have activity (4) aside from vitamin A. These biological activities may include modulating immune response, cellular differentiation, and singlet oxygen quenching (5–7). Therefore, quantification of β-carotene metabolism is paramount to establish its full potential.

Quantifying β-carotene metabolism in the context of fractional absorption, accretion, degradation, and elimination is complex because it can be influenced by the individual consumer, the food matrix, and the consumer-food matrix interactions (8). Previous radioisotope studies estimated intestinal absorption of β-carotene and its conversion to retinyl esters (REs) and to retinal that is subsequently oxidized to retinol (9, 10). However, the conversion of β-carotene by BCO2 to other metabolites, such as β-apo-8′-, 10′-, 12′-, and 14′-carotenals, and retinoic acids (RAs), remains to be determined. Access to accelerator mass spectrometry (AMS) that can measure attomole amounts (1 in 10^{-18} parts) of ^{14}C (11) enabled us to conduct a feasibility study that tested the hypothesis of whether a healthy man can derive ^{14}C-retinoids and ^{14}C-apocarotenoids from a true tracer oral dose of *all-trans* [10,10′,11,11′-^{14}C]-β-carotene (1.01 nmol; 543 ng; 100 nCi). The radiation exposure is low, ≈ 200 nseivert.

Therefore, the metabolic fate in a free-living healthy man of ^{14}C-β-carotene administered as an oral dose (1.01 nmol; 543 ng; 100 nCi) in a banana milkshake was determined. The fractional absorption, as well as the metabolic fate and elimination of ^{14}C-β-carotene, was also measured in the present study.

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[2] Supported by the National Center for Research Resources (P41 RR 13461) and the National Institute of Digestive and Kidney Diseases (DK48307), National Institutes of Health. The work was performed under the auspices of the US Department of Energy by the University of California Lawrence Livermore National Laboratory (contract W-7405-Eng-48). Hoffman-La Roche (Basel, Switzerland) donated the *all-trans* [10,10′,11,11′-^{14}C]-β-carotene used in the study.

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SUBJECT, MATERIALS, AND METHODS

Chemicals

All chemicals were checked for ^{14}C content by AMS before use. Tributyrin (glycerol tributyrate) was obtained from MP Biomedical (Aurora, OH). All solvent and chemicals used, unless otherwise noted, were obtained from Fisher Scientific (Santa Clara, CA). β-Carotene and *all-trans*-retinol, retinyl palmitate, *all-trans*-RA, 13-*cis*-RA standards were obtained from Sigma Chemicals (St Louis, MO). The β-apo-8′-carotenal standard was from Fluka (Buchs, Switzerland), and β-apo-12′-carotenal, 9-*cis*-β-carotene, 13-*cis*-β-carotene, and β-carotene-5,6-epoxide standards were obtained from Carotenature (Lupsingen, Switzerland). Groceries were purchased at a local supermarket.

Dose preparation

The purity of the *all-trans*-[10,10′,11,11′-^{14}C]-β-carotene was checked by reverse-phase HPLC (RP-HPLC) as previously described (12). The dose (1.01 nmol; 543 ng; 100 nCi) was suspended in 1 mL ethanol. Specific activity was 98.8 Ci/mol. Dose formulation was calculated with the use of a liquid scintillation counter (model 1410; Wallac Oy, Turku, Finland). Radiochemical purity was >99%. A shake that consisted of a whipped mixture of banana, skim milk, and sucrose was prepared and divided between 2 plastic cups. The dose suspended in ethanol was layered over the shake in the first cup. The shake in the second cup was then layered atop that in the first cup as described previously (13). The entire shake was consumed immediately. The first cup was rinsed with water, and the water was then consumed to ensure that the entire dose was ingested. The quantity of dietary β-carotene administered (0.543 μg ^{14}C-β-carotene plus ≈40 μg β-carotene from the shake) was much smaller than that ingested in a carotene-rich meal.

Subject, diet, and specimen collections

The volunteer was a healthy, nonsmoking man aged 30 y with a body mass index (in kg/m^2) of 24.5 who had a normal complete blood count and lipid panel. The subject was instructed to avoid foods with high carotenoid content. A food diary was recorded beginning the week before the study and continuing for 2 wk after dosing, to ensure minimum intake of provitamin A carotenoids and vitamin A. Meals were provided on the day of dose administration to monitor for time and content. Lunch and dinner were served 5.5 and 10 h after dosing; they consisted of a frozen entrée (Amy's Kitchen Inc, Petaluma, CA), choice of apple or banana, and a chocolate chip cookie (Pepperidge Farm Inc, Norwalk, CT). Meals were selected to deliver 30% fat and minimum vitamin A and carotenoid content.

At 0700 on the day the dose was administered, the (fasting) subject was fitted with an intravenous catheter in a forearm vein, and a blood sample was drawn for baseline values just before dosing. Additional blood samples were collected at 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, 13, 14, 15, 24, and 36 h after dose delivery. Subsequent blood samples were collected on days 2, 3, 4, 5, 6, 9, 11, 13, 18, 26, 33, 40, 47, 61, 75, 89, 103, 117, 138, and 166 while the subject was in the fasted state. All blood samples were collected in glass K_3EDTA evacuated tubes (Vacutainer; BD Diagnostics, Franklin, NJ) and immediately put on ice. A complete collection of feces and urine was taken before dosing to establish baseline values. These collections continued for 14 and 21 d, respectively, after the day of dosing. The subject did not have a history of a serious medical condition or of the use of medications that would interfere with carotenoid metabolism.

Written informed consent was obtained from the subject. The University of California, Davis, and the Lawrence Livermore National Laboratory Institutional Review Boards approved the study, which was conducted according to Good Clinical Practice guidelines and the Declaration of Helsinki, version 1989.

Specimen processing

All specimens were processed under yellow light to prevent degradation of carotenoids and retinoids. Plasma was separated from blood on a fixed-speed bench-top centrifuge (Centrific model 228; Fisher Scientific, Tustin, CA; 3300 rpm; 1380 × *g*, 10 min, 25 °C) and stored at −80 °C. Samples were processed under the same conditions as described previously (14) with slight modification. Briefly, fecal samples were collected in polyethylene bags (1-002-59; Fisher Scientific) and homogenized with KOH/2-propanol buffer solution (1:5, wt:vol). Urine samples were collected in amber containers (14-375-115; Fisher Scientific). Representative homogenized samples of feces (50 mL) and urine (15 mL) were stored at −80 °C until they were analyzed further.

Total carbon analysis

Aliquots of urine (75 μL), feces (75 μL), and plasma (25 μL) were lyophilized, wrapped in aluminum foil, and analyzed for total carbon content (15) by using a carbon/nitrogen analyzer (model 1112; Thermo Finnegan, Rodano, Italy). These analyses were conducted at the Division of Agriculture and Natural Resources Laboratory at the University of California, Davis.

Analyte measurements

The approach for isolating β-carotene, retinyl esters (REs), retinol, and RAs involved 3 stages. First, plasma analytes were segregated into neutral (β-carotene, RE, and retinol) and acid-extractable (RA) fractions (12) with modification. Second, neutral and acid-extractable fractions were suspended or comingled in the RP-HPLC mobile phase. Third, the mix was loaded on an RP-HPLC column (ES Industries, West Berlin, NJ) to speciate the analytes in the neutral and acid-extractable fractions with an isocratic mobile phase of acetonitrile:1,4-dioxane:2-propanol: triethylamine (792:148:52:2) with 200 mmol ammonium acetate/L in the alcohol component of the mobile phase. Twenty-second fractions were collected from the RP-HPLC column and processed for AMS analysis as described below.

The neutral fraction, which contained the nonpolar compounds, was extracted from plasma as previously described (13) with a slight modification. Briefly, 200 μL plasma was deproteinated with 400 μL ethanolic KOH (0.25 N). The nonpolar compounds were thrice extracted from the ethanolic KOH with 1 mL hexane each. The extracts were pooled in an amber vial containing 5 μL methanol spiked with 0.25% butylated hydroxytoluene (wt:vol). The solvent was evaporated to dryness under argon, and the residue (nonpolar compounds) was resuspended in 100 μL HPLC mobile phase.

For the acid-extractable fraction, glacial acetic acid (12 μL) was added to the remainder of the deproteinated plasma. Water-saturated ethyl acetate (0.8 mL) was added, the tube was shaken

and centrifuged (1380 × *g* for 10 min at 25 °C), and the supernatant fluid containing the acid soluble fraction was transferred to a glass tube (Alltech, Deerfield, IL). Water acidified with 10% glacial acetic acid (300 μL) was added. The tube was shaken and centrifuged (1380 × *g* for 10 minutes at 25 °C) to separate (float) the ethyl acetate layer that contained the acid-extractable fraction. The ethyl acetate layer was then transferred to an amber screw-top vial containing 5 μL methanol and 0.25% butylated hydroxytoluene (wt:vol). The solvent was evaporated to dryness under a stream of argon. The residue that contained the acid-extractable fraction was resuspended in 100 μL RP-HPLC mobile phase.

The neutrals (described above) were combined or comingled with the acid extractables (described above) in RP-HPLC mobile phase in a total volume of 200 μL. A 20-μL aliquot of the mix was injected onto the HPLC column to separate the various analytes contained in the neutral and acid-extractable fractions of the plasma. RP-HPLC eluant fractions (20 s) were collected and processed for AMS analysis as described below.

An HPLC system (model 1100; Agilent Technology, Santa Clara, CA) equipped with a quaternary pump, an auto sampler, and a photodiode array detector was set up to search for and isolate both the neutral and acid-extractable metabolites of the administered β-carotene. The system was similar to that described previously (16), with modifications. Briefly, the stationary phase consisted of a Spherisorb ODS2 (3 μm; 250 × 4.0 mm) column equipped with titanium frits and a Javelin ODS2 guard column (all: ES Industries). The isocratic mobile phase consisted of acetonitrile:1,4-dioxane:2-propanol:triethylamine (792:148:58:2) with 200 mmol ammonium acetate/L in the alcohol component for 0–25 min, a linear ramp to 100% 2-propanol for 1 min, and a 20-min regeneration of the initial column conditions. The flow rate was 0.8 mL/min, and the column temperature was 35 °C. The HPLC eluent was collected in quartz sample tubes at 20-s intervals for the entire HPLC run.

The system was standardized with 9 reference standards. They included retinol, retinyl palmitate, and retinyl oleate that were monitored at 325 nm; 13-*cis*-RA and *all-trans*-RA that were monitored at 351 nm; and β-apo-12′-carotenal, β-apo-8′-carotenal, β-carotene-5,6-epoxide, and *all-trans*-β-carotene that were monitored at 450 nm. The parent compound, ^{14}C-β-carotene, and its ^{14}C-metabolites ^{14}C-retinol, ^{14}C-retinyl palmitate, and ^{14}C-β-apo-8′-carotenal were separated with the use of the above standardized HPLC system.

AMS analysis

The 20-s RP-HPLC eluent fractions described above were processed as previously described (13), except that a 50-μL aliquot of a solution of tributyrin in methanol (40 mg/mL) was used to add exactly 1.2 mg C to each 20-s RP-HPLC fraction, which was then dried under vacuum to remove the mobile phase and methanol. An aliquot of urine was prepared for ^{14}C analysis by diluting 100 μL of the sample with 900 μL HPLC-grade water. Aliquots of diluted urine (100 μL) and feces (75 μL) were placed in quartz tubes and processed as described for the 20-s RP-HPLC fractions. Carbon in the samples was converted to graphite (17), and the ratio of ^{14}C to ^{total}C was measured at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA.

Statistical analysis

The ^{14}C content in the whole plasma, urine, and feces was plotted as a function of time since dosing, whereas the ^{14}C content in the HPLC fractions was plotted as a function of retention time. Plotting was performed with ORIGINLAB software (version 7.5; Microcal, Northampton, MA).

RESULTS

A schematic presentation of some possible cleavage pathways and metabolites of *all-trans*-β-carotene is shown in **Figure 1**. Central cleavage at the 15,15′ position is the well-established pathway that leads to vitamin A. Cleavage at the 3′–4′, 5′–6′, 7′–8′, and 9′–10′ positions, were it to occur in humans, would lead to β-apo-carotenals that may be converted to β-apo-carotenols, β-apo-carotenyl esters, β-apo-carotenoic acids, β-apo-carotenoyl esters, and the ring-oxidized metabolites (oxo-, hydroxy-, and epoxy- forms).

^{14}C in neat plasma

The profile of ^{14}C in neat plasma by time since dose included 4 peaks, as shown in **Figure 2**. Peak A appeared at 0.15 d after dosing and represented ≈3% of the dose (100 nCi dose had 1.6×10^9 amol ^{14}C/mg carbon). Peak B appeared at 0.25 d after dosing and represented ≈3.6% of the dose. Peak C appeared at 0.5 d and represented ≈2.8% of the dose. Peak D appeared on the third day after dosing; it was poorly resolved.

HPLC separation of reference standards

The separation of the 9 reference standards is shown in **Figure 3**A. Respective retention times for the retinoids were 3.8, 6.5, 7.5, 18.5, and 21.5 min for retinol, 13-*cis*-RA, *all-trans* RA, retinyl palmitate, and retinyl stearate. Respective retention times for the carotenoids were 4.6, 5.5, 10.5, and 15.5 min for β-apo-12′-carotenal, β-apo-8′-carotenal, β-carotene-5,6-epoxide, and *all-trans*-β-carotene.

HPLC separation of ^{14}C analytes in plasma

The ^{14}C analytes in plasma 0.25 d after administration of ^{14}C-β-carotene are shown in Figure 3B. At this time since dosing, the ^{14}C peaks matched the retention times for β-carotene, retinyl palmitate, and retinyl stearate. The ^{14}C analytes in plasma 0.5 d after administration of ^{14}C-β-carotene are shown in Figure 3C. At this time since dosing, the ^{14}C peaks matched the retention times for retinol and β-carotene. The ^{14}C analytes in plasma 3 d after administration of ^{14}C-β-carotene are shown in Figure 3D. At this time since dosing, the first ^{14}C peak matched the retention times for β-apo-8′-carotenal, whereas the second ^{14}C peak that eluted immediately after 17 min did not match that of any of our reference standards. More reference standards are needed to identify this ^{14}C metabolite. The possibility that the first peak corresponds to β-apo-10′-carotenal (rather than to β-apo-8′-carotenal) cannot be ruled out, for lack of that reference standard.

Plasma ^{14}C-RE and ^{14}C-retinol by time since dosing

The profiles of ^{14}C-RE and ^{14}C-retinol in plasma at all sampling times since dosing are shown in **Figure 4**. The rise of a ^{14}C-RE peak at 0.15 d after dosing represents delivery of the ^{14}C retinoid from enterocytes to chylomicra, and the descent represents the presence of ^{14}C-RE in chylomicron remnants as they are

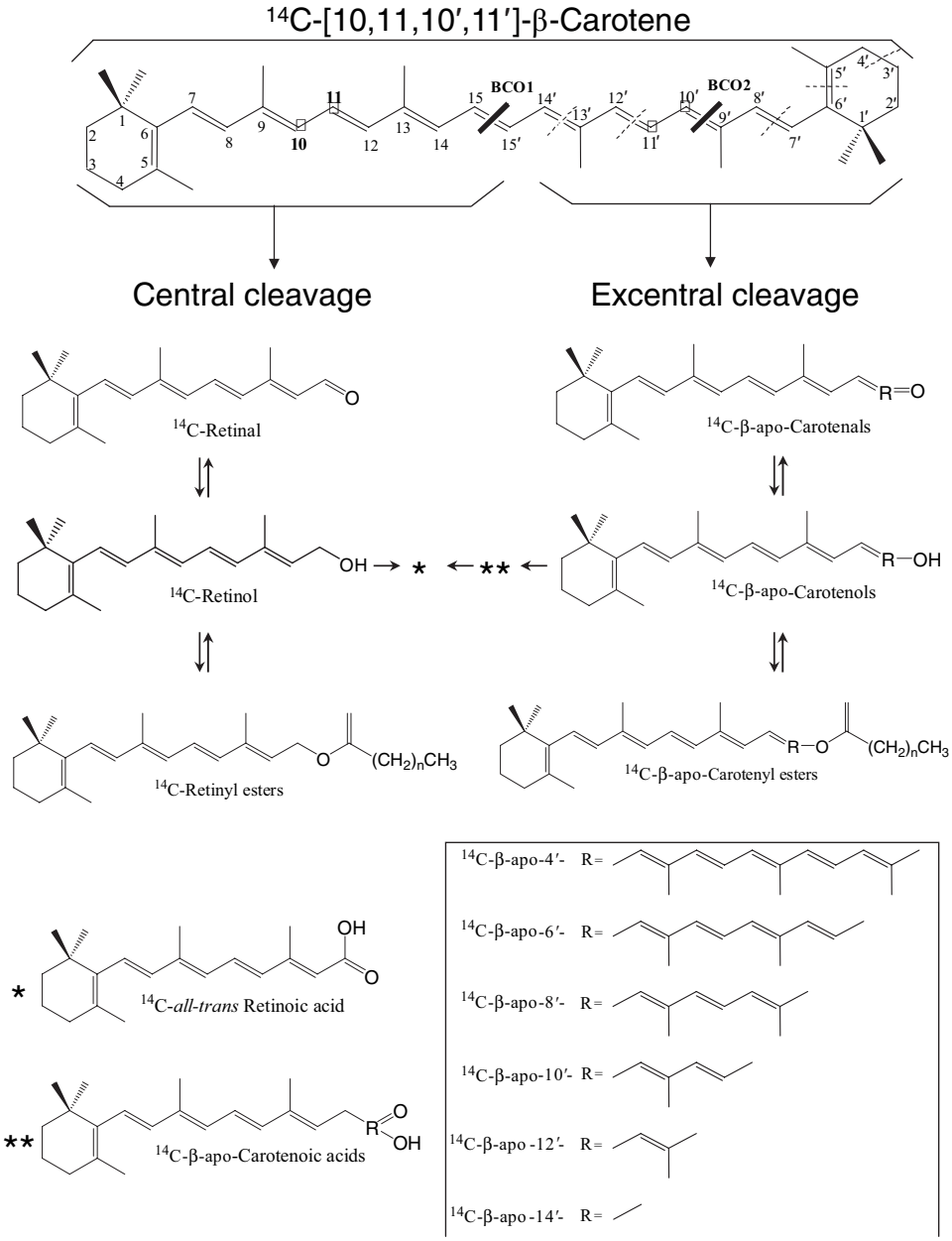

FIGURE 1. A schematic presentation of some possible cleavage pathways and metabolites of *all-trans*-β-carotene. BCO1, β,β-carotene-15,15′-oxygenase, is the enzyme responsible for the central cleavage, the well-established pathway that leads to vitamin A. BCO2, β,β-carotene-9′,10′-oxygenase, cleaves at the 9′,10′ position (excentral cleavage). Cleavage at the 3′-4′, 5′-6′, 7′-8′ positions, were it to occur in humans, would lead to β-apo-carotenals that may be converted to β-apo-carotenols, β-apo-carotenyl esters, β-apo-carotenoic acids, and β-apo-carotenoyl esters.

taken up by the liver. The rise of ^{14}C-retinol that began shortly after 0.2 d after dosing represents the handing off of ^{14}C-RE to ^{14}C-retinol bound to retinol-binding protein for secretion into plasma.

Plasma ^{14}C-carotenoids by time since dosing

The plasma profiles of ^{14}C-β-carotene and ^{14}C-β-apo-8′-carotenal are shown in **Figure 5**. The ^{14}C-β-carotene appeared as 2 broad peaks. The most interesting feature of the ^{14}C-apo-8′-carotenal peak is that, although it was not present at 0.5 d, it was clearly present on the third day after dosing.

Elimination of ^{14}C in feces

Elimination of the ^{14}C label in feces appears in **Figure 6**. The first collection of feces after dosing accounted for ≈35% of the administered ^{14}C, so the fractional absorption (apparent digestibility) was ≈0.65 (1.00−0.35). Subsequent collections during the next 13 d accounted for an additional 16% of the administered ^{14}C, so the fractional daily elimination (metabolic fecal loss resulting from biliary excretion of metabolites such as retinoyl β-glucoronide) of the label was ≈0.0123 (0.16/13). The cumulative elimination of ^{14}C in feces was 51% of the administered ^{14}C during the 2-wk period since dosing.

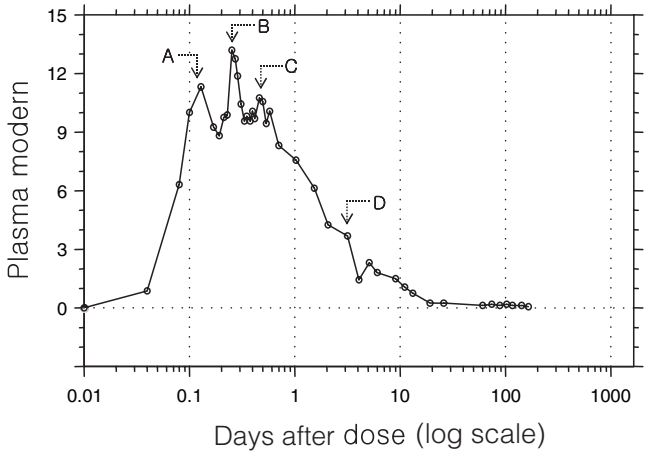

FIGURE 2. Plasma profile of radioactivity after an oral tracer dose of ^{14}C-β-carotene (1.01 nmol, 543 ng, 100 nCi) expressed in modern as a function of days after dose on a log scale for 166 d. Total ^{14}C label (○) in plasma was assessed by accelerator mass spectrometry. The ^{14}C peaks are denoted by the letters A, B, C, and D. One modern = 97.94 amol ^{14}C/mg carbon; dose = 100 nCi = 1.6×10^9 amol ^{14}C/mg carbon. ^{14}C metabolites in peaks B, C, and D are shown in Figure 3.

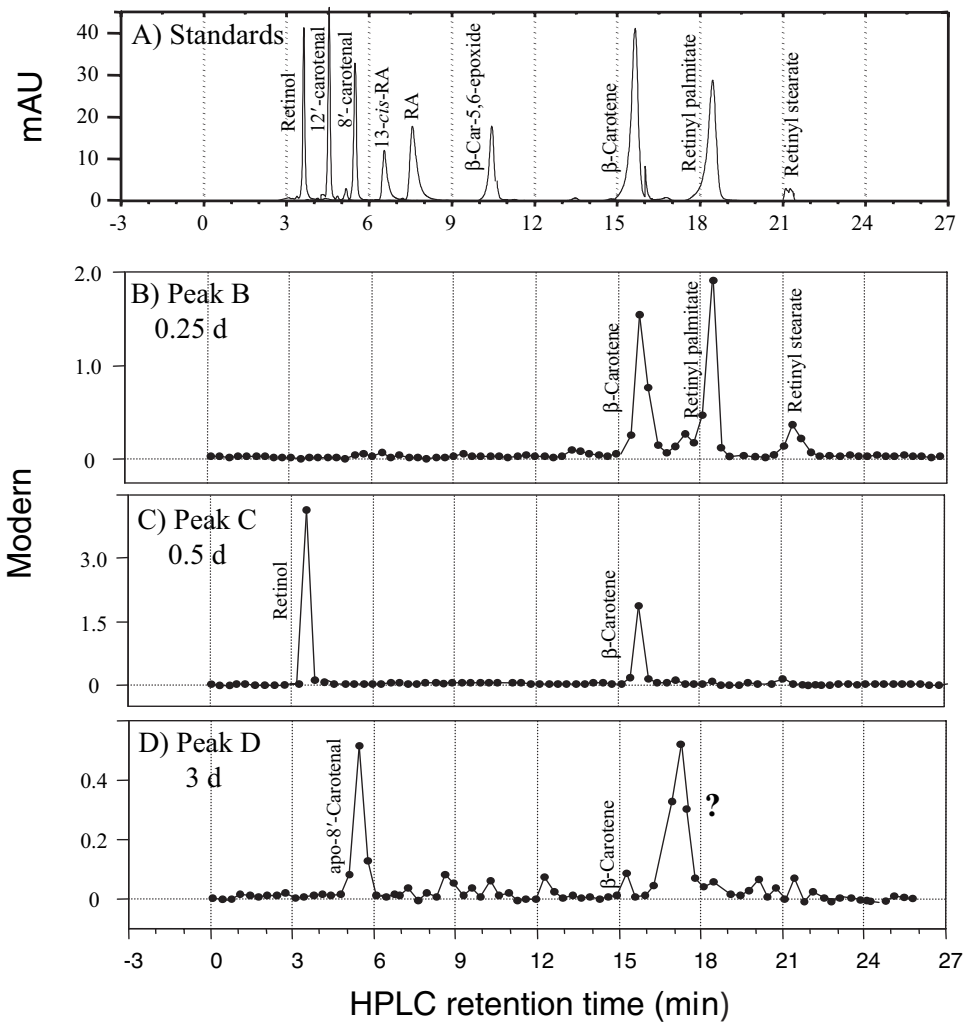

FIGURE 3. RA, retinoic acid. A: The chromatogram of retinoid and carotenoid reference standards on an isocratic HPLC system by retention time. The retinoid standards were measured at 325 nm; the carotenoid standards were measured at 450 nm. ^{14}C metabolites in plasma from orally administered ^{14}C-β-carotene are expressed in modern by accelerator mass spectrometry. B: ^{14}C plasma profile at 0.25 d after dose; C: ^{14}C plasma profile at 0.5 d after dose; D: ^{14}C plasma profile at 3 d after dose. Plasma samples were separated by HPLC and collected at 20-s intervals for analysis by accelerator mass spectrometry. Modern = 97.94 amol ^{14}C/mg C.

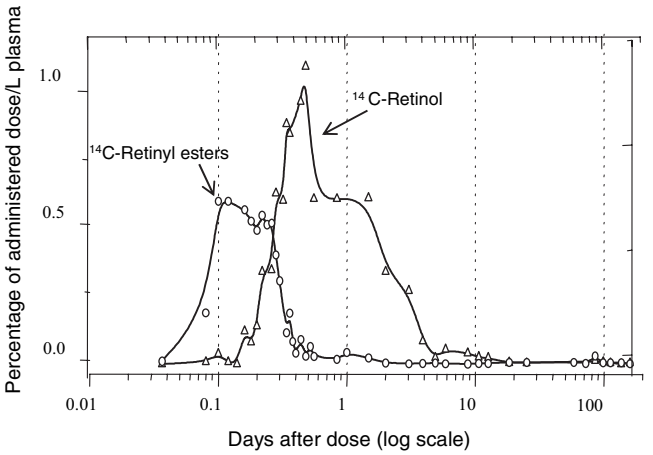

FIGURE 4. The separation by HPLC of plasma ^{14}C-retinoids, retinol (△) and retinyl esters (○), expressed as the percentage of the dose as a function of days after dose on a log scale for 166 d.

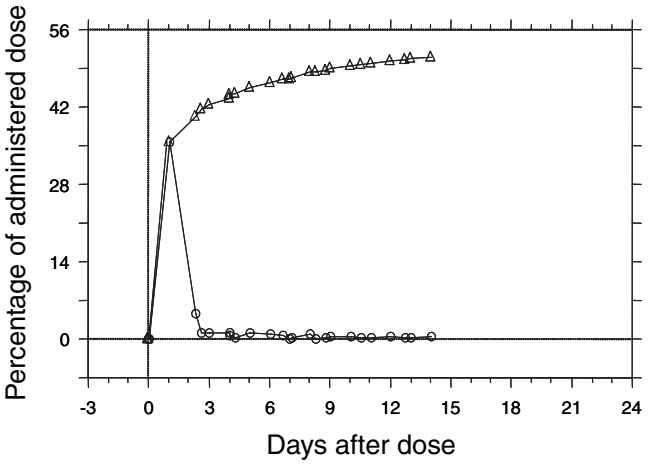

FIGURE 6. Percentage of the ^{14}C dose eliminated in feces as a function of time after an oral tracer dose of ^{14}C-β-carotene. Cumulative recovery of ^{14}C (△) and daily collection (○).

Elimination of ^{14}C in urine

Elimination of ^{14}C in urine appears in **Figure 7**. In the cumulative collections, ≈15.7% of the administered ^{14}C was eliminated during the first 21 d since dosing. An interesting feature of the elimination per collection line is the peak that appears in the day 4 and day 5 collections. It may represent the elimination of either the ^{14}C-β-apo-8′-carotenal or the unknown, or both, which were discovered in plasma 3 d after dosing (Figure 3D). The first urine collection (0−12 h since dosing) accounted for 1.1% of the administered dose. The day 4 and day 5 collections accounted for 2.3% and 1.7% of the administered dose, respectively, and each subsequent collection accounted for ≈0.2%.

DISCUSSION

A quantitative understanding of β-carotene metabolism is of considerable interest because it is cleaved by a sequence-related family of retinal- and apo-carotenal–forming carotenoid oxygenases to retinoids and apo-carotenoids (Figure 1) that perform essential biological functions (18, 19). The best-known of the oxygenases in humans is the BCO1 that cleaves β-carotene to vitamin A (2, 20, 21). A related cleavage enzyme (BCO2) that cleaves β-carotene to β-apo-carotenals was also identified and characterized (4). BCO2 is widely distributed among human tissue (22). Another related oxygenase (RPE65) cleaves *all-trans*-RE to 11-*cis*-retinol that is isomerized to 11-*cis*-retinal through the visual cycle (23). Therefore, central and excentral β-carotene cleavage enzymes occur in humans, but the excentral cleavage metabolites have not been reported. Therefore, we tested whether excentral cleavage was demonstrable in vivo in humans.

We administered a small oral tracer dose of ^{14}C-β-carotene. The mass of the dose was 1.01 nmol; the typical dietary intake in the United States is 7 μmol/d. The small dose and carefully monitored diet ensured steady state conditions with respect to carotenoid metabolism. We quantified the elimination of the dose in feces and urine for 14 and 21 d, respectively, and we searched plasma for ^{14}C-β-apo-carotenals with the use of HPLC and AMS.

We found that the percentage of absorption was ≈65% of the administered dose, metabolic fecal loss was ≈1% of the administered dose per day (Figure 6), and urine loss was ≈0.75% of the

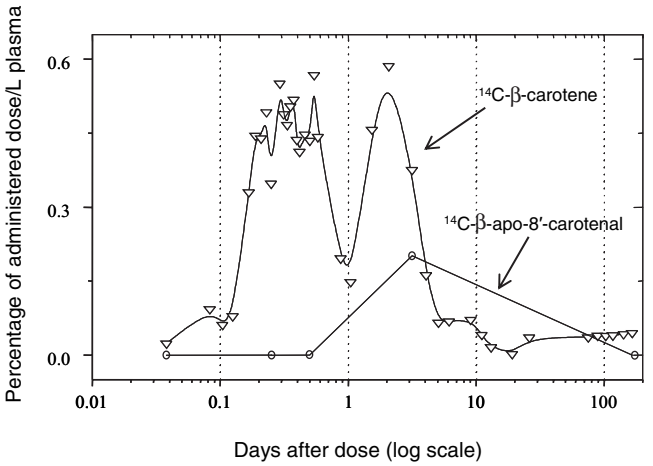

FIGURE 5. The separation by HPLC of plasma ^{14}C-carotenoids, ^{14}C-β-carotene (▽) and ^{14}C-β-apo-8′-carotenal (○), expressed as the percentage of the dose as a function of days after dose on a log scale for 166 d.

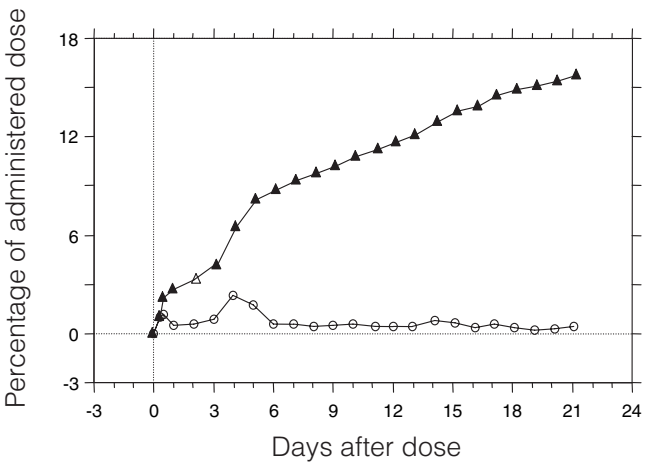

FIGURE 7. Fraction of the ^{14}C dose eliminated in urine as a function of time after an oral tracer dose of ^{14}C-β-carotene. Cumulative recovery of ^{14}C (▲) and daily collection (○).

administered dose per day (Figure 7). These findings were all consistent with prior estimates (24–26), which suggests that the subject was normal with respect to β-carotene metabolism.

In searching the blood, we found ^{14}C-β-carotene and ^{14}C-REs at 0.25 d and ^{14}C-retinol and ^{14}C-β-carotene at 0.5 d as we had expected (Figures 3, 4, and 5). Our finding of ≥2 isotope-labeled metabolites from administered ^{14}C-β-carotene at 0.25 and 0.5 d confirms previous observations of 2 peaks (12, 13, 27). Finally, we found ^{14}C-β-apo-8′-carotenal, ^{14}C-β-carotene, and a ^{14}C peak that could be a ^{14}C-β-apo-8′-carotenyl ester or a ^{14}C-β-apo-8′-carotenoate. Our finding of a first peak of ^{14}C-β-apo-8′-carotenal is consistent with results of a prior study that incubated β-carotene with tissue homogenates and found β-apo-8′-, 10′-, and 12′-carotenals, retinal, and RA (28). Our finding of a second ^{14}C peak, tentatively identified as a ^{14}C-β-apo-8′-carotenyl ester, or ^{14}C-β-apo-8′-carotenoate, fits nicely with a prior study that incubated retinal, β-carotene, β-apo-8′-carotenal, or β-apo-12′-carotenal with a homogenate of human intestinal mucosa and found RA (29). Our finding of a second ^{14}C peak fits nicely with a prior study showing that orally administered β-apo-8′-carotenal was extensively and rapidly converted to its corresponding acid, alcohol, and fatty acyl ester (30). Finally, our finding of ^{14}C-β-carotene and ^{14}C-β-apo-8′-carotenal in plasma fits nicely with our previous evidence of 2 kinetically distinct pools of carotenoid (27).

At 3 d after dosing, 90% of the total ^{14}C label (present in neat plasma) was recovered in the HPLC fraction (Figure 3D). Of the 90% recovered, 20% appeared with β-apo-8′-carotenal reference standard, 41% coeluted with a second large peak at 17 min (its chemical identity is unknown at this time), and the remaining 29% consisted of small peaks of ^{14}C with retention times of 6–15 min (Figure 3D), which could represent additional apocarotenoid metabolites. Cleavage activity by BCO2 is shown to produce apo-8′-, 10′-, and 12′-carotenals in vitro (28). With the use of the HPLC separation system described here or a similar one (31), we would expect to rank the various β-apo-carotenals in increasing order of retention times as follows: apo-12′–<10′–<8′–<6′–<4′–carotenals; they may coincide with the small ^{14}C peaks at 9, 10, and 12 min on the chromatogram (Figure 3D). Although we tentatively identified ^{14}C-apo-8′-carotenal in humans, further confirmation is necessary. We did not quantify RA in the present study, but we already showed that the pattern of ^{14}C-RA in plasma mimicked that of ^{14}C-retinol (12).

In animals, carotene cleavage dioxygenases (CCDs) include BCO1 (2), BCO2 (4), and RPE65 (23). However, ≥100 members in the CCD family are found in bacteria, plants, and higher species, and they have been identified with a range of substrate specificities (18). One plant CCD that has narrow substrate specificity is the maize Vp14 (32); another CCD has broad substrate specificity (AtCCD1) (33). Therefore, plant and bacterial CCDs can cleave β-carotene at several positions that lead to formation of a mix of cyclic and linear apocarotenoids (32, 34–37). A search of animals for additional BCOs is an exciting possibility.

The appearance of ^{14}C-β-apo-8′-carotenal in plasma at 3 d after dosing suggests that β-apo-carotenals are formed in peripheral tissues from dietary β-carotene that is absorbed from the intestine and already transferred to peripheral tissues—ie, RPE65 (38, 39). This interpretation is consistent with the fact that the BCO2 is uniquely distributed in muscle cells, connective tissue, and endocrine pancreas, which suggests an alternative function of vitamin A synthesis for BCO2 (22). Our feasibility study tentatively identified the formation of β-apo-8′-carotenal and perhaps β-apo-8′-carotenyl ester or β-apo-8′-carotenoate by excentral cleavage of β-carotene in a normal human. This is a new finding that requires confirmation and, if confirmed, further investigation in additional subjects.

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AJC designed the study and wrote the manuscript, and CCH, FFM, and SHK were responsible for sample preparation and analyses. None of the authors had a personal or financial conflict of interest.

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