Compartmental analysis of the dynamics of β-carotene metabolism in an adult volunteer

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Abstract Metabolism of a 73 μmol oral dose of β-carotene-d_8 in olive oil was determined from plasma β-carotene-d_8 and retinol-d_4 concentration–time curves in an adult male. β-Carotene-d_8 and retinol-d_4 concentrations in serial plasma were measured using high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometry (GC–MS), respectively. Plasma β-carotene-d_8 and retinol-d_4 concentration–time curves were described by a 5-term and a 3-term polyexponential equation, respectively, using an empirical description of β-carotene metabolism. A physiologic compartmental model of β-carotene metabolism was also constructed and tested. This model suggests that 22% of the β-carotene dose is absorbed: 17.8% as intact β-carotene and 4.2% as retinoid. Also, it suggests that both liver and enterocyte are important in converting β-carotene to retinoid; 43% is converted in liver and 57% in enterocyte. Finally, it suggests that the mean residence time for β-carotene is 51 days and that the 73 μmole dose does not alter the fractional transfer coefficients of the system after absorption takes place. The issue of central versus eccentric cleavage of β-carotene in humans can be studied with further modeling combined with use of appropriately labeled β-carotene.—**Novotny, J. A., S. R. Dueker, L. A. Zech, and A. J. Clifford.** Compartmental analysis of the dynamics of β-carotene metabolism in an adult volunteer. *J. Lipid Res.* 1995. **36:** 1825–1838.

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β-Carotene is reported to protect against oxidative stress, heart disease, and cancer by quenching singlet oxygen and enhancing the immune response; furthermore, β-carotene is a significant source of vitamin A for humans world-wide (1–3). Despite its key physiologic effects, its widespread use as a dietary supplement, and its regular use in large amounts in many clinical trials, the kinetics and dynamics of β-carotene in humans and the factors that affect its metabolism are largely unknown.

In an early investigation Goodman et al. (4) presented data suggesting that 10% of an oral dose of radioactive β-carotene was absorbed into lymph: 3% as intact β-carotene and 7% as retinoid (retinyl esters + retinal + retinol). The following year Blomstrand and Werner (5) presented data suggesting that 23% of a similar dose of radioactive β-carotene was absorbed into lymph: 13% as intact β-carotene and 10% as retinoid. Even though these studies involved elderly surgical patients, they provided key information for current understanding of the true absorption of β-carotene. More recently using stable isotope-labeled [^{13}C]β-carotene, Parker et al. (6) reported that 64%, 21%, and 14% of the absorbed portion of an oral dose of [^{13}C]β-carotene entered plasma as retinyl ester, retinol, and unaltered β-carotene, respectively; the fraction absorbed was not reported.

An ideal animal model that mimics β-carotene metabolism of humans has not yet been identified, and the use of radioactive β-carotene to study absorption in humans can only be justified in special cases. Therefore, progress toward understanding the dynamics of β-carotene metabolism in humans has been hindered by the scarcity of stable isotope-labeled carotenoids and retinoids and by the analytical difficulties in measuring them in biological tissues. However, recent successes in the synthesis of stable isotope-labeled β-carotene (7) and the subsequent analysis of it in biologic tissues (8) provided an opportunity to determine the dynamics of β-carotene metabolism in a healthy adult.

Abbreviations: HPLC, high performance liquid chromatography; GC–MS, gas chromatography–mass spectrometry; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FSD, fractional standard deviation; GIT, gastrointestinal tract.

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EXPERIMENTAL

Subject, dose, and diet for experiment 1

The all-*trans*-β-carotene-10,10′,19,19,19,19′,19′,19′-d_8 (β-carotene-d_8) was purchased from Cambridge Isotope Labs (Woburn, MA) where it was synthesized as previously described by Bergen (7). The chemical and isotopic purity of the β-carotene-d_8 was confirmed by nuclear magnetic resonance and mass spectroscopies (8). An informed, consenting, healthy 53-year-old male weighing 94 kg ingested a gelatin capsule (No. 000, Eli Lilly and Co., Indianapolis, IN) containing 73 μmol β-carotene-d_8 (40 mg) dissolved in ~ 2 g olive oil with a light breakfast (a bran muffin and a cup of black coffee; 10 g fat, 2 g fiber and 300 Calories). The subject ate lunch and dinner 3 and 8 h later, respectively, and both meals were of natural foods known to be devoid of carotene and vitamin A. Blood samples (~ 25 mL) were drawn into glass tubes containing potassium-EDTA just before (0 h) and at 0.5, 1, 2, 5, 7, 9, 12, and 24 h and 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36, 43, 57, 71, 85, 99, 113 days after ingesting the β-carotene-d_8. All blood draws after the first day were made during the fasted state before breakfast. Plasma was immediately separated by centrifugation and stored as 2-mL aliquots at −70°C. Plasma concentrations of β-carotene-d_8 and retinol-d_4 are reported only for the first 24 and 16 days after dosing because after that time the analyte concentration approached the lower limits of measurement.

Subject, dose, and diet for experiment 2

In this experiment the same subject under the same conditions as in experiment 1 ingested 75 μmol nonlabeled β-carotene (40 mg) and provided blood just before and again at 7, 12, and 24 h after ingesting the β-carotene. Plasma was separated and a 4-mL aliquot from each of these four plasma samples was subjected to sequential density ultracentrifugation (in the presence of EDTA) to float the very low density (VLDL), low density (LDL) and high density (HDL) lipoprotein classes (9). Chylomicrons and chylomicron remnants were included with the VLDL fraction because a separate centrifugation was not performed to float them. After each lipoprotein class was floated, the tubes were placed in a rack ~ 25 cm in front of an Aristo light box and a color photograph was made that allowed the changes in the orange color (due to β-carotene) in each of the major lipoprotein classes to be followed during the first 24 h after ingestion of the nonlabeled β-carotene. The lipoprotein classes were then dialyzed overnight in phosphate-buffered saline at 2°C to remove the EDTA. The lipoprotein classes were analyzed for β-carotene, retinol, and retinyl esters (as retinyl palmitate) using HPLC and for protein using the Folin phenol reagent. Both experiments were approved by the University of California Davis, Human Subjects Review Committee.

Chemical extractions and analyses of plasma

The concentration of total β-carotene and total retinol in all the plasma specimens were measured by HPLC using an isocratic mobile phase of 67% acetonitrile, 20% isopropanol, 12% methanol, 1% ammonium acetate, and 1% H_2O and a reversed-phase column (10). Briefly, 100 μL plasma was mixed with 400 μL distilled H_2O, and 500 μL EtOH containing 500 μg BHT was added to deproteinize the plasma. The deproteinized plasma was extracted with 3 mL hexane containing the internal standards retinal-O-ethyloxime for retinol and β-apo-12′-carotenal-O-*t*-butyloxime for β-carotene. The upper phase was transferred to glass vial, evaporated to dryness, redissolved in mobile phase, and analyzed. The fractional standard deviations for measuring total retinol and β-carotene in plasma were ≤ 0.04.

The ratios of β-carotene-d_8/β-carotene in the plasma, from blood drawn after ingestion of β-carotene-d_8, were determined using the HPLC method previously described by Dueker et al. (8). Briefly, plasma proteins were precipitated with EtOH and the lipophilic substances were extracted in hexane. The hexane layer was transferred to a new tube and dried. The residue was resuspended in 1.2 mL EtOH, 0.5 mL KOH (5 mol/L H_2O) was added and the mixture was saponified by heating at 70°C for 1 h. The saponified mixture was extracted with hexane, and the hexane layer was transferred to a new tube and concentrated to ~ 100 μL and loaded on an aminopropyl solid phase extraction (SPE) cartridge. Carotenes were eluted from the cartridge with hexane and analyzed for β-carotene-d_8 and β-carotene using reversed-phase HPLC and an isocratic mobile phase of CH_3CN–CH_3OH–ammonium acetate 82:18:0:01 (v:v:w) pumped at 0.9 mL/min. Recovery of β-carotene through the SPE cartridge was 79.3% with a fractional standard deviation (FSD) of 0.0176. The HPLC method for measuring the ratios of β-carotene-d_8/β-carotene in plasma had an FSD of 0.0325.

The SPE cartridge was then washed with hexane–ethyl acetate 90:10 (v:v) and retinol was eluted from it with hexane–ethyl acetate 75:25 (v:v) and analyzed for the retinol-d_4/retinol ratio using GCMS (11). Recovery of retinol through the SPE cartridge was 66.2% with an FSD of 0.0319 (8). The GCMS method for measuring ratios of retinol-d_4/retinol in plasma had an FSD of 0.045 (11).

Calculating the concentrations of β-carotene-d_8 and retinol-d_4 in plasma

The peak area ratio responses of five β-carotene isotopomer standards (x-variable) versus the molar ratios of β-carotene-d_8/β-carotene (y-variable) in the standards were plotted as a standard curve, and a regression equation that described the standard curve was calculated. The peak area ratio of β-carotene-d_8/β-carotene for each plasma sample was substituted into the regression equation to solve for molar ratios of β-carotene-d_8/β-carotene of each plasma sample. The molar ratio of β-carotene-d_8/β-carotene of each plasma sample was multiplied by the concentration of total β-carotene in that sample to arrive at the concentration of β-carotene-d_8. A similar calculation protocol was used to determine the concentration of retinol-d_4 in each plasma sample.

Calculating the masses of β-carotene-d_8 and retinol-d_4 in the plasma compartment

The test subject weighed 94 kg and was assumed to have 45 mL plasma/kg body weight (12). The estimated plasma volume therefore was 4.23 L. The plasma volume was multiplied by the concentration of β-carotene-d_8 and retinol-d_4 to estimate the total reserves (masses) of these analytes in the plasma compartment for kinetic modeling.

Empirical description of β-carotene metabolism

Empirical modeling of the plasma β-carotene-d_8 concentration time curve was performed using a 5-term exponential model ($y = a_1e^{-b_1t} + a_2e^{-b_2t} + a_3e^{-b_3t} + a_4e^{-b_4t} + a_5e^{-b_5t}$) based on the shape of the curve with weighted, nonlinear least squares regression using the SAS NLIN procedure. Each observation was weighted by the reciprocal of its predicted value. The area under the concentration–time curve was calculated as $AUC = \int_0^\infty y(t)dt$. The area under the moment curve was calculated as $AUMC = \int_0^\infty t \times y(t)dt$.

Empirical modeling of the plasma retinol-d_4 concentration time data was performed using a 3-term exponential model ($y = a_1e^{-b_1t} + a_2e^{-b_2t} + a_3e^{-b_3t}$) with weighted, nonlinear least squares regression using the SAS NLIN procedure. Each plasma retinol-d_4 concentration was weighted and the areas under the concentration–time and the moment curves were calculated as described for the plasma β-carotene-d_8 concentrations.

Physiologic compartmental analysis of β-carotene metabolism

Physiologic compartmental analysis began with a simple model based on existing knowledge of the biology of β-carotene and retinol in humans. A schematic of the initial physiologic model is in the following diagram (**Diagram 1**).

This initial model consisted of nine compartments; six for β-carotene and three for retinoid derived from the β-carotene. The six β-carotene compartments included one for the gastrointestinal tract into which the oral dose was administered, one for intestine (enterocyte) where absorption took place, one for liver, one for extrahepatic tissues, one for chylomicron transport of absorbed β-carotene from intestine (enterocyte) to liver, and one for lipoprotein transport of β-carotene released from liver. Irreversible loss of β-carotene occurred from the extrahepatic and intestine compartments. The three retinoid compartments included one for liver, one for chylomicron (remnant) to transport absorbed retinoid from intestine (enterocyte) to liver, and one for retinol binding protein to transport retinol from liver to target tissues and back. Irreversible loss of retinoid from the plasma retinol binding protein compartment was assumed to have occurred directly from plasma, although up to 30% of this loss may have occurred as a result of loss into bile at the liver.

To simultaneously examine the plasma concentration–time data of β-carotene-d_8, its metabolite retinol-d_4 (includes retinyl-d_4 ester plus retinol-d_4 as the plasma extracts were saponified during preparation for chemical analysis), total β-carotene and total retinol, a compartmental model was constructed and tested using SAAM 31 software[2] (13) on a Dell 466/T.

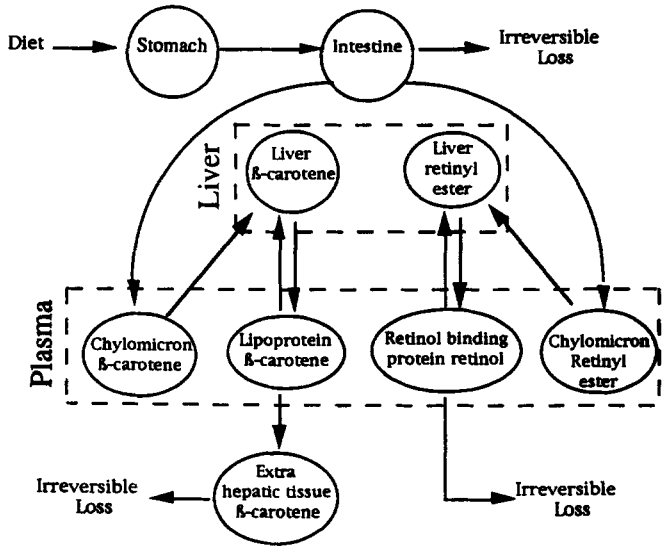

Diagram 1. Conceptual model of β-carotene metabolism in humans. The arrows indicate critical areas in need of detailed future study.

[2]This SAAM 31 software is available at no cost from Dr. Loren Zech, NCI, Building 10, Room 6B-13, Bethesda, MD 20892, USA. Tel. (301) 496-8915. Internet address greif@saam.nci.nih.gov. This software can also be obtained via modem at (301) 480-3295 (login as zmodem) or via anonymous ftp: ftp@saam.nci.nih.gov.

Calculating transfer coefficients, flow rates, and residence time

The fractional transfer coefficient L (I,J) is the fraction of analyte in compartment J (a donor compartment) which is transferred to compartment I (a recipient compartment) per unit time and has units of time^{-1} (in this case, days^{-1}). The fractional transfer coefficient multiplied by the mass of analyte in a given donor compartment gives the rate of flow of analyte from that donor compartment. In other words, if M(J) is the mass (in moles) of β-carotene in compartment J, then M(J) × L(I,J) = R(I,J) is the rate of flow (in moles/day) of β-carotene from compartment J to compartment I. By compartmental analysis, the plasma tracer and mass concentration-time curves were used to estimate the fractional transfer coefficients. Using these transfer coefficients, steady-state masses were calculated and in turn used to compute flow rates for the case of a constant intake. Variances for the response parameters are reported in units of fractional standard deviation (FSD).

Initial fractional transfer coefficients were estimated, and based on the experimentally determined concentrations of β-carotene-d_8 and retinol-d_4 in plasma by time after dosing, the model structure and transfer coefficients were adjusted in physiologically relevant ways until the compartmental model prediction of the plasma concentration–time curve was a best fit for the experimentally determined data. Final parameter values were then generated using SAAM's iterative, nonlinear least squares routine. Parameter values (fractional transfer coefficients, etc.) were tested against zero using a single-tailed one-sample Student's *t* statistic. Extra compartments associated with statistically nonsignificant transfer coefficients (unreliable transfer coefficients) were included in other compartments. Inclusion of the extra compartments in other compartments yielded a simpler physiologic model (see Fig. 2) with fewest compartments which favored statistical certainty, especially with our limited data.

The whole body mean residence time for β-carotene was calculated by including the time it spent as β-carotene and as retinoid (cumulative mass in β-carotene plus retinoid in all body pools) and all dietary β-carotene. Whole body β-carotene mass (as β-carotene + retinoid) was divided by the predicted steady state dietary intake of β-carotene to determine whole body mean residence time.

Model constraints

As β-carotene concentrations in tissues (other than plasma) of the test subject were not measured directly, model tissue β-carotene reserves in steady state were statistically constrained based on values reported in other studies from the scientific literature (10, 12, 14–18). β-Carotene concentrations in tissues were assumed to be relatively constant during the period of our study and to be little affected by the single dose of β-carotene-d_8. The total reserves of β-carotene in hepatic and in extrahepatic tissue were constrained to 6.73 ± 0.67 μmol and 9.5 ± 0.95 μmol, respectively, based on HPLC-measured concentrations of β-carotene in biopsy specimens from healthy individuals (10) times the masses of the tissues of the ICRP No. 23 reference man (12) and on HPLC analysis of carotenoids in human tissues by Schmitz et al. (14, 15). Model intestinal absorption of β-carotene was constrained to be inside the range of two statistical deviations of 15% ± 4.5% based on the β-carotene balance study of Bowen, Mobarhan, and Smith (16) in which 2.3 ± 0.4 mg of a 15 mg dose of β-carotene was absorbed in healthy subjects. The fractional transfer coefficient of retinyl ester from the chylomicron retinyl ester to the fast turnover liver retinyl ester compartment was constrained to 60 ± 36 d^{-1} to correspond with the known half-life of chylomicron retinyl esters (15 ± 10 min) in healthy adult men (17). Each of these constraints was performed by including additional normal equations. Finally, loss of retinol from the model system was constrained to a minimum value of 0.7 μmol/d (200 μg/day) based on the rate of vitamin A depletion in humans (18).

RESULTS

Experiment 1

Empirical description. The concentrations of β-carotene-d_8 (top panel) and retinol-d_4 (bottom panel) by time since ingesting β-carotene-d_8 are shown in **Figure 1.** Experimentally measured concentrations are represented by circle symbols and the lines are those that were fitted to the data using the empirical descriptions.

The 5-term polyexponential equation $y(t) = -30.5e^{-12.3t} + 17.4e^{-5.7t} - 7.2e^{-3.3t} + 0.65e^{-0.27t} + 0.084e^{-0.067t}$ provided a good fit of the plasma β-carotene-d_8 concentration as a function of time after dose (top panel). From this equation the AUC and AUMC for β-carotene-d_8 were calculated to be 2.09 μmol × day/L plasma and 27.31 μmol × day^2/L plasma, respectively. For β-carotene-d_8 the AUMC/AUC was 13.05 days.

The 3-term polyexponential equation $y(t) = -1.338e^{-2.751t} + 0.883e^{-1.467t} + 0.07578e^{-0.03642t}$ provided a good fit of the plasma retinol-d_4 concentration by time since ingesting the β-carotene-d_8 dose (bottom panel). From this equation the AUC and AUMC for retinol-d_4 were calculated to be 2.20 μmol × day/L plasma and 57.36 μmol × day^2/L plasma, respectively. For retinol-d_4 the AUMC/AUC was 26.12 days.

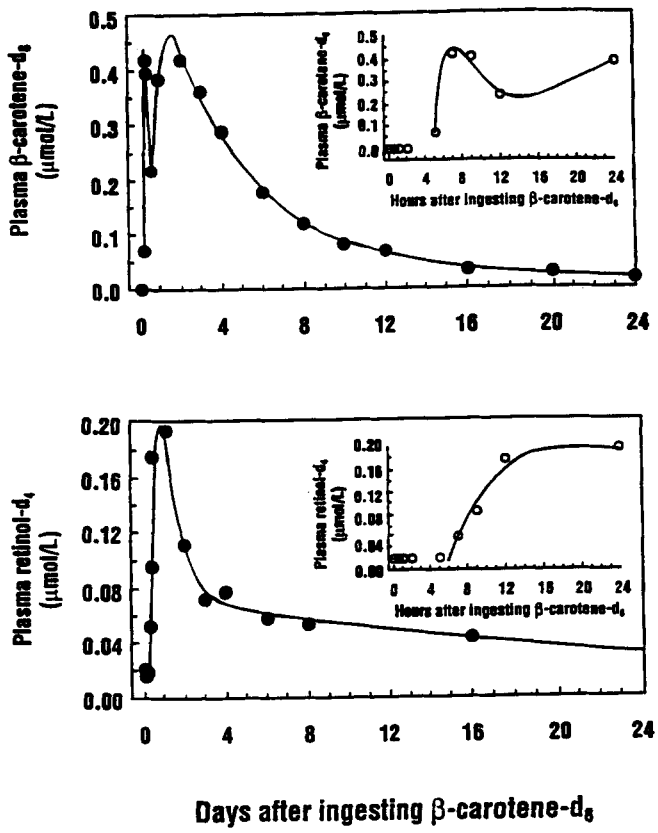

Fig. 1. Experimentally determined values (circles) and the best-fit line using the empirical description of the concentrations of β-carotene-d_8 in plasma as a function of time after a 94-kg adult male ingested 73 μmol β-carotene-d_8 (top panel). Experimentally determined values (circles) and best-fit line using the empirical description of the concentrations of retinol-d_4 (derived from ingested β-carotene-d_8) by time after ingestion of β-carotene-d_8 (bottom panel).

The coefficients of neither the 5-term polyexponential equation for β-carotene-d_8 nor the 3-term polyexponential equation for retinol-d_4 add to zero, so that $y(t) = 0$ at $t = 0$. The non-zero y-intercept occurs as a result of the ~ 4 h delay between the time of ingesting β-carotene-d_8 and the time of appearance of β-carotene-d_8 and retinol-d_4 in plasma. Because of this physiological delay and because of the need to have a more physiologic analogy, a compartmental model was constructed.

Physiologic compartmental model. A schematic outline of the final physiological compartmental model is shown in **Figure 2.** The compartmental model is comprised of kinetically distinct forms of β-carotene and retinoid. β-Carotene compartments include one for the gastrointestinal tract (GIT), one for enterocytes, one each for plasma lipoproteins and chylomicrons (remnants), one rapidly and one slowly turning over compartment for liver, one for extrahepatic tissues, and a GIT delay element preceding the enterocyte. Irreversible loss of β-carotene from the system occurs from the GIT and from the extrahepatic tissue compartments.

The four kinetically distinct forms of retinoid include one for chylomicron (remnant) retinyl esters, one for plasma retinol bound to retinol binding protein, and one rapidly and one slowly turning over compartment in liver. A kinetically distinct form of retinoid for extrahepatic tissues is not included in this model because such a form could not be determined from this data set. Loss of retinoid from the system occurs directly from plasma. This cannot be identified as different from loss via the enterohepatic route.

Labeled β-carotene was not detected in plasma from blood drawn just before or at 0.5, 1, or 2 h after ingestion of β-carotene-d_8; the first trace of β-carotene-d_8 appeared in plasma 5 h after ingestion of the β-carotene-d_8 dose. This delay probably represents the time necessary for β-carotene-d_8 (with the light breakfast and coffee) to pass through the stomach, enter the intestine lumen, undergo a phase transition (from β-carotene in the olive oil triglyceride phase to β-carotene in the 2-monoglyceride phase that is absorbed), form lipid micelles in the intestine, and enter the enterocyte. Therefore, a GIT time delay of 4.5 h was added to the model. A second liver β-carotene compartment (a slow turnover liver β-carotene form) was added to fit the decay of the second plasma β-carotene-d_8 peak. A second liver retinol compartment (a slow turnover liver retinol form) was added to sustain the plasma concentration of retinol-d_4 seen after $t = 5$ days. Values within compartments of Fig. 2 represent steady state masses or reserves of analyte in μmol within that compartment. Values along the arrows in Fig. 2 represent flow rates of analyte from donor to recipient compartments in units of μmol/d, calculated as fractional transfer coefficient times the steady state mass of analyte.

Figure 3 (top panel) shows the experimentally measured concentrations of β-carotene-d_8 in plasma (circle symbols) as a function of time along with the line that was fitted to the experimental data using the physiologic compartmental model in Fig. 2 and the constraints delineated in the methods section. Figure 3 (bottom panel) shows experimentally measured concentrations of retinol-d_4 (circle symbols) as a function of time along with the line that was fitted to the data using the model in Fig. 2. The concentration time curves for β-carotene-d_8 and retinol-d_4 in plasma in Fig. 3 demonstrate that the concentrations of β-carotene-d_8 and retinol-d_4 predicted from the physiologic model were in agreement with the experimentally measured values. The parameter estimates specifying the physiologic compartmental model prediction curve-fit to the experimentally measured data was optimized using least squares.

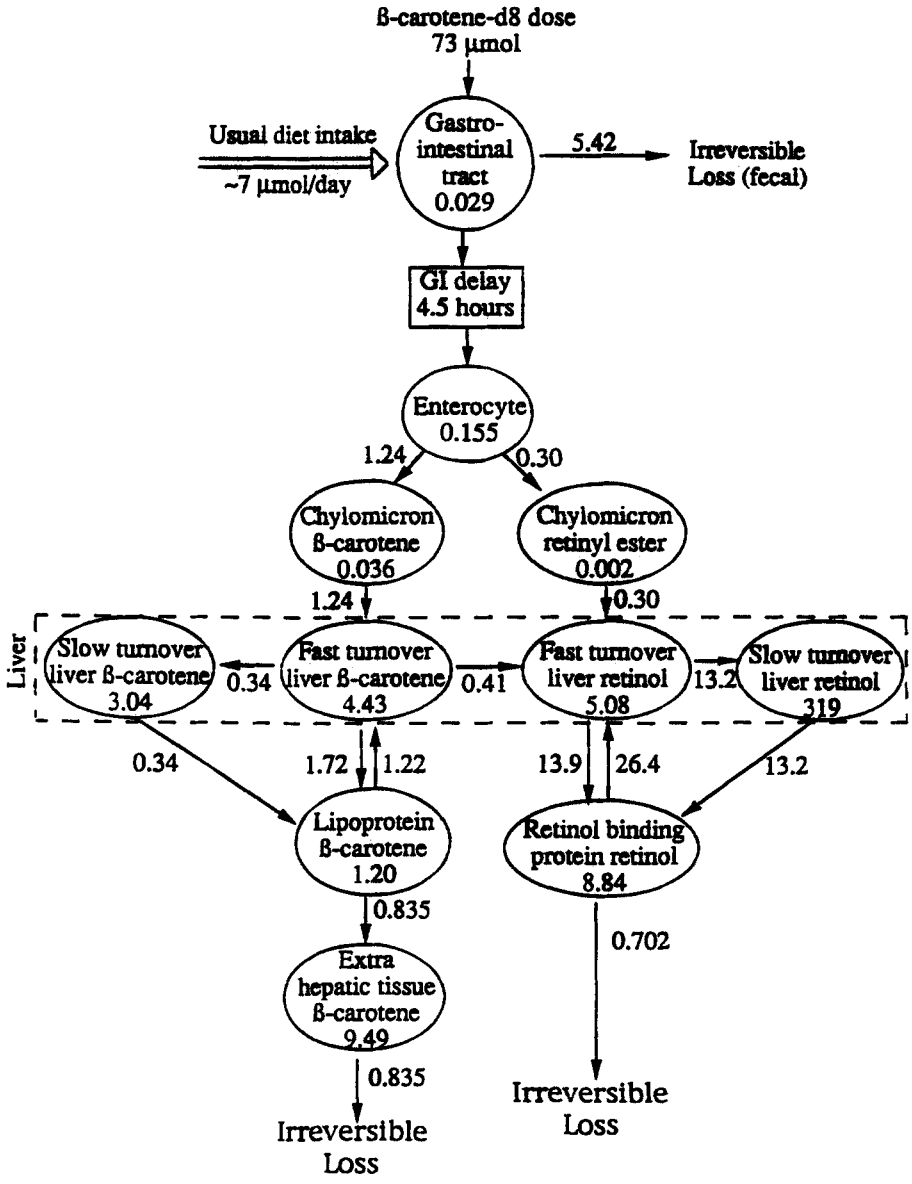

Fig. 2. Proposed physiologic (compartmental) model of β-carotene and metabolite retinoid turnover in humans. Each circle represents a kinetically distinct form of β-carotene or retinoid. The rectangle represents a delay element. For each compartment, the physiologic model predicted the total masses (reserves) of β-carotene or retinoid in each compartment at steady-state; they appear as µmoles in each circle/oval. Values by the arrows are the rates of transfer among the compartments in µmol/day.

Figure 4 shows experimentally measured concentrations of total β-carotene (i.e., labeled plus nonlabeled β-carotene) and total retinol (labeled plus nonlabeled) in plasma by time since ingestion of β-carotene-d_8. The experimentally measured concentrations of total β-carotene in plasma are represented by symbols, and the fitted line shows the model (in Fig. 2) prediction. The experimentally measured concentrations of total retinol in plasma are also represented by symbols in the bottom panel, and the line shows the model prediction. It is clear that the physiologic compartmental model in Fig. 2 also provided a good fit for the concentrations of total β-carotene and retinol. The concentration of total β-carotene in plasma showed a transient doubling in response to the 73 µmol oral dose, while the concentration of total retinol remained constant. The physiologic model predicted curve-fit to the experimentally measured data (concentrations of total β-carotene and retinol) was optimized using least squares.

As can be seen from the fitted lines in Fig. 3 and 4, the physiologic compartmental model predicted plasma concentrations of β-carotene-d_8, total β-carotene, reti-

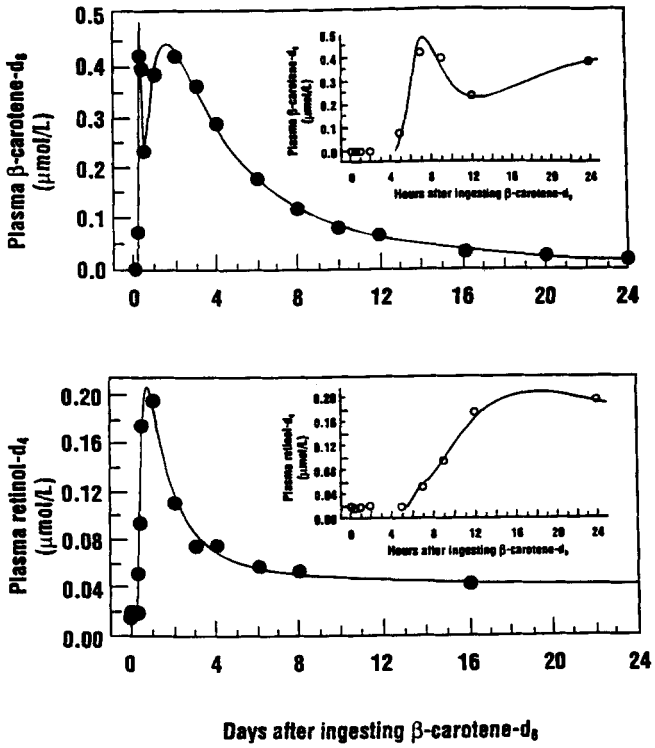

Fig. 3. Experimentally measured values (circles) and the best-fit line using the physiologic model of the concentrations of β-carotene-d_8 in plasma by time after ingestion of 73 μmol β-carotene-d_8 (top panel). Experimentally measured values (circles) and the best-fit line using the physiologic model of the concentrations of retinol-d_4 (derived from ingested β-carotene-d_8) in plasma by time after ingestion of the β-carotene-d_8 (bottom panel).

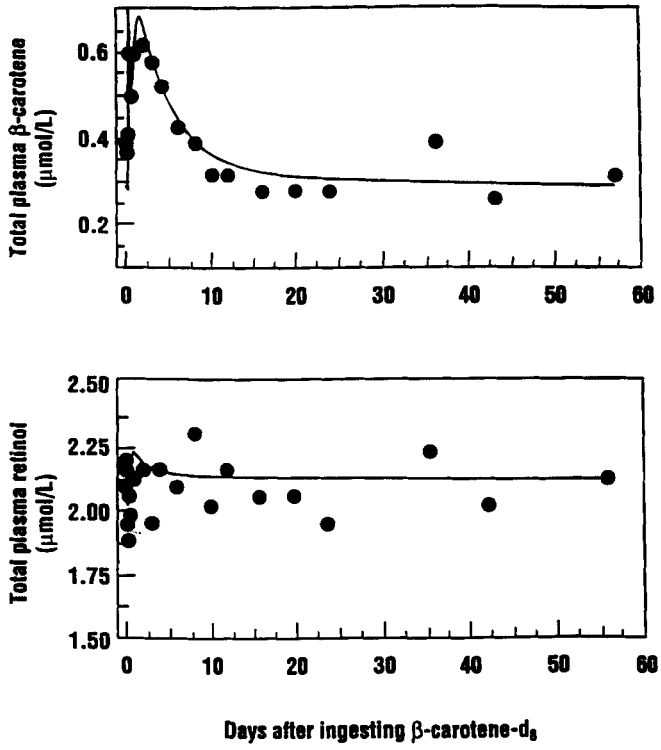

Fig. 4. Experimentally measured values (circles) and the best-fit line using the physiologic model of the concentrations of total β-carotene (labeled and nonlabeled) in plasma by time after ingestion of 73 μmol β-carotene-d_8 (top panel). Experimentally measured values (circles) and the best-fit line using the physiologic model of the concentrations of total retinol in plasma by time after ingestion of β-carotene-d_8 (bottom panel).

nol-d_4, and total retinol (by time since dosing) that were in agreement with the experimentally measured concentrations. Because this excellent fit was achieved using constant fractional transfer coefficients, the induction of transient mechanisms (altered enzyme activities) to handle the administered dose of β-carotene was not necessary, resulting in the conclusion that the 73 μmol dose of β-carotene-d_8 did not perturb the normal steady state system of β-carotene metabolism. Using the baseline plasma mass, the average daily intake was calculated to be ~ 7 μmol/d, in agreement with observed values.

Table 1 summarizes the fractional transfer coefficients for β-carotene metabolism. Among the independent coefficients, the largest were for the GIT to the GIT delay (53.02/day), the chylomicron β-carotene to the fast turnover liver β-carotene (34.76/day), and the enterocyte to the chylomicron β-carotene (7.98/day) compartments. The next largest coefficients were for the retinol binding protein retinol to the fast turn over liver retinyl ester (2.99/day), the fast turnover liver retinyl ester to the retinol binding protein retinol (2.74/day), and the fast turnover liver retinyl ester to the slow turnover liver retinyl ester (2.6/day) compartments. The smallest coefficient was for the slow turnover liver retinyl ester to the retinol binding protein retinol (0.041/day) compartments. The fractional standard deviations (FSDs) for fractional transfer coefficients ranged from 0.055 to 0.22. The low FSD values indicate that for this model construction, small changes in the transfer coefficients will cause substantial worsening of the curve fit.

Table 2 summarizes the flow rates of β-carotene and retinoid from donor to recipient compartments during the steady state. The flow rates are the product of a donor compartment reserve (mass) of β-carotene or retinoid times its fractional transfer coefficient to a recipient compartment. As the state of the system (i.e., the size of the reserves) changed in response to the β-carotene-d_8 tracer dose, the flow rates were also changed. In contrast, because the fractional transfer coefficients were constant for various system states (different masses of reserves), they were not responsible for the changes in flow rates. The flow rates ranged from 0.337 to 26.4 μmol/d, the 26.4 value representing a high

TABLE 1. Fractional transfer coefficients from donor to recipient compartments for the physiologic model of β-carotene metabolism

Donor Compartment	Recipient Compartment	Value	FSD
Independent fractional transfer coefficients			
Gastrointestinal tract (GIT)	GIT Delay	53.02	0.056
Enterocyte	Chylomicron β-carotene	7.98	0.055
Chylomicron β-carotene	Fast turnover liver β-carotene	34.76	0.059
Fast turnover liver β-carotene	Slow turnover liver β-carotene	0.076	0.20
Fast turnover liver β-carotene	Lipoprotein β-carotene	0.39	0.099
Slow turnover liver β-carotene	Lipoprotein β-carotene	0.11	0.16
Lipoprotein β-carotene	*Fast turnover liver β-carotene*	1.02	0.16
Lipoprotein β-carotene	Extra hepatic tissue β-carotene	0.70	0.092
Enterocyte	Chylomicron retinyl ester	1.91	0.11
Fast turnover liver β-carotene	Fast turnover liver retinyl ester	0.091	0.088
Fast turnover liver retinyl ester	Slow turnover liver retinyl ester	2.60	0.22
Fast turnover liver retinyl ester	Retinol binding protein retinol	2.74	0.11
Slow turnover liver retinyl ester	Retinol binding protein retinol	0.041	0.11
Retinol binding protein retinol	Fast turnover liver retinyl ester	2.99	0.21
Extra hepatic tissue β-carotene	Irreversible loss β-carotene	0.088	0.083
Retinol binding protein retinol	Irreversible loss retinol	0.079	0.059
Dependent fractional transfer coefficients			
GIT	Irreversible fecal loss of β-carotene	187.0	0.016
Chylomicron retinyl ester	Fast turnover liver retinyl ester	122.7	0.077
Time delay, subdivisions/delay compartment			
Delay time in hours		4.5	
Subdivisions of delay compartment		2.0	

Fractional transfer coefficient values are $days^{-1}$. FSD is fractional standard deviation. Irreversible loss of β-carotene (fecal) = 240 − fractional transfer coefficient from GIT to GIT delay compartment. Fractional transfer coefficient from chylomicron retinyl ester to fast turnover liver retinyl ester = 60 ± 36. Values apply to model in Fig. 2.

level of retinol recycling between the plasma retinol binding protein compartment and the fast turnover liver retinyl ester compartment.

Table 3 summarizes selected results of the physiologic compartmental model. Model results show that 22% of the β-carotene dose was absorbed; 17.8% as intact β-carotene and 4.2% as retinoid derived from β-carotene in the absorption process. Thirty-three percent of the β-carotene passing through the liver was converted to retinol. Of the total retinol (alcohol plus ester forms) formed from ingested β-carotene, 57% was formed in liver and 43% was formed in the enterocyte assuming that 1 mole of β-carotene yielded 1 mole of retinoid. The residence time for β-carotene in the body was 51 days according to the physiologic compartmental model. The liver reserves of β-carotene and retinol in the test subject were predicted to be 7.5 and 324 μmol, respectively. The average daily intake of β-carotene was predicted to be 6.95 μmol. Finally, our physiologic compartmental model predicted that 1 μg dietary β-carotene equaled 0.054 μg retinol (or 0.101 μmol retinol/μmol β-carotene); a biological activity value for β-carotene that is considerably lower than the 0.1667 μg retinol value established in growing rats with marginal body stores of vitamin A.

Experiment 2

The transfer of nonlabeled β-carotene among lipoprotein classes was subjectively visualized from the apparent changes in the amount of orange color in the lipoprotein classes by time since ingestion of nonlabeled β-carotene (photograph not shown). From the photograph, the β-carotene first appeared in VLDL at ~ 7 h (most likely in chylomicrons and/or chylomicron remnants), and then was either directly transferred to LDL or was taken up by liver and returned to plasma in hepatic triglyceride-rich lipoproteins which could then lose their β-carotene to LDL and HDL.

At 0, 7, 12, and 24 h after ingestion of the nonlabeled β-carotene, the respective concentrations of β-carotene were 9, 54, 30, and 9 nmol/L plasma, in the VLDL fraction; they were 222, 146, 200, and 165 nmol/L plasma, in the LDL fraction; and they were 23, 31, 40, and 28 nmol/L plasma, in the HDL fraction. Total β-carotene in whole plasma at 0, 7, 12, and 24 h was 310, 401, 258, and 214 nmol/L, respectively. These data

TABLE 2. Flow rates from donor to recipient compartments of the physiologic compartmental model of β-carotene metabolism

Donor Compartment	Receipient Compartment	Value
		μmol/day
Gastrointestinal tract (GIT)	GIT delay	1.53
GIT delay	Enterocyte	1.53
Enterocyte	Chylomicron β-carotene	1.24
Chylomicron β-carotene	Fast turnover liver β-carotene	1.24
Fast turnover liver β-carotene	Slow turnover liver β-carotene	0.337
Fast turnover liver β-carotene	Lipoprotein β-carotene	1.71
Slow turnover liver β-carotene	Lipoprotein β-carotene	0.337
Lipoprotein β-carotene	Fast turnover liver β-carotene	1.22
Lipoprotein β-carotene	Extra hepatic tissue β-carotene	0.835
Enterocyte	Chylomicron retinyl ester	0.297
Chylomicron retinyl ester	Fast turnover liver retinyl ester	0.297
Fast turnover liver β-carotene	Fast turnover liver retinyl ester	0.405
Fast turnover liver retinyl ester	Slow turnover liver retinyl ester	13.2
Fast turnover liver retinyl ester	Retinol binding protein retinol	13.9
Slow turnover liver retinyl ester	Retinol binding protein retinol	13.2
Retinol binding protein retinol	Fast turnover liver retinyl ester	26.4
Extra hepatic tissue β-carotene	Irreversible loss β-carotene	0.835
Retinol binding protein retinol	Irreversible loss retinol (urine)	0.702
GIT	Irreversible loss β-carotene (fecal)	5.42

Transfer rates are μmol/day. Values apply to compartmental model in Fig. 2.

show (as expected) that virtually all (87 ± 10%, mean ± SEM) of the β-carotene in whole plasma was associated with lipoproteins.

Retinyl esters were also measured (as retinyl palmitate) in each lipoprotein class and in whole plasma during the first 24 h after ingestion of the nonlabeled β-carotene. At 0, 7, 12, and 24 h after ingestion of nonlabeled β-carotene, the respective concentrations of retinyl ester were 6, 63, 43, and 8 nmol/L plasma, in the VLDL fraction; they were 33, 27, 29, and 31 nmol/L plasma, in the LDL fraction; they were 7, 3, 3, and 3 nmol/L plasma, in the HDL fraction and they were 37, 105, 75, and 41 nmol/L of whole plasma. These data show (as expected) that all (103 ± 7%, mean ± SEM) of the retinyl ester in whole plasma was associated with lipoproteins. Also, unesterified retinol was not detected in any of the isolated lipoprotein classes.

At 0, 7, 12, and 24 h after ingestion of the nonlabeled β-carotene, whole plasma contained 1338, 1507, 990, and 1310 nmol total retinol/L, respectively. As expected, these data show that retinyl esters represent a small portion (6 ± 2%, mean ± SEM) of the total retinoid content of plasma. Furthermore, as our volunteer ate a vitamin A-free diet (during the 24-h period after ingesting nonlabeled β-carotene), it was assumed that the increase in retinyl ester concentration in VLDL was derived entirely from the ingested β-carotene. Also, as the concentrations of retinyl esters in VLDL were low (relative to the total retinol of whole plasma) it appears that the amount of retinyl ester formed from β-carotene was small and/or that it was either transferred to other lipoproteins or taken up by liver very rapidly.

Blood drawn just before and again at 7, 12, and 24 h after ingestion of the nonlabeled β-carotene had 278, 519, 432, and 222 mg protein/L isolated VLDL, respectively, had 1194, 1102, 1072, and 1237 mg protein/L isolated LDL, respectively, and had 4098, 3793, 3529, and 2909 mg protein/L isolated HDL, respectively.

TABLE 3. Select results from physiologic compartmental model of β-carotene metabolism

Parameter	Value
Portion of the β-carotene-d_8 dose that was absorbed	22%
Portion taken up as intact β-carotene-d_8	17.8%
Portion taken up as retinoid-d_4 derived from β-carotene-d_8	4.2%
Liver reserves of β-carotene	7.5 μmol
Liver reserves of retinol	324 μmol
Portion of retinoid formed from intestinal β-carotene conversion	43%
Portion of retinoid formed from liver β-carotene conversion	57%
Portion of liver β-carotene converted to retinoid	33%
Residence time of β-carotene in body	51 days
Average intake of β-carotene by test subject from dietary sources	6.95 μmol/day (3.7 mg/day)

Values apply to compartmental model in Fig. 2. All values represent those predicted by the model in Fig. 2.

DISCUSSION

Recent successes in the synthesis and detection of β-carotene-d_8 and retinol-d_4 (retinyl-d_4 acetate) offer great promise for establishing the dynamics of these compounds in humans and comparing these results to those obtained when radioactive isotopes (that are difficult to justify in humans) and animal models (that may not fully mimic β-carotene metabolism of humans) are used. Also, mathematical modeling offers a rigorous method for describing the transfer of a nutrient (especially a labeled nutrient) through a biologic system. Therefore, use of stable isotopes in combination with modeling allows an overall model of the in vivo dynamics of a nutrient in humans to be established. Modeling also helps to design critical future experiments by identifying key gaps in current knowledge of the dynamics of a nutrient. We have therefore used mathematical modeling of the concentrations of β-carotene-d_8 and retinol-d_4 (derived from the β-carotene-d_8) in serial specimens of human blood in order to develop a working model (hypothesis) of the dynamics of β-carotene metabolism in a healthy adult volunteer. We aimed for a model that was physiologically relevant and minimally complex (with fewest terms or compartments) while providing good agreement (best or least squares fit) with our experimentally determined data and with the relevant published data of other investigators.

Several alternate forms of the physiologic compartmental model were tested and rejected when they did not provide a best fit for our data or when they were not consistent with current concepts of β-carotene metabolism. Alternate models included different compartments and/or compartment connectivity. Alternate (rejected) models that provided useful insights are discussed briefly.

Some alternate models had too many compartments to provide statistically reliable fractional transfer coefficients between compartments. For example, one alternate model had several liver compartments each for β-carotene and retinol. As they gave statistically unreliable fractional transfer coefficients between the extra compartments because of our limited data, the extra compartments were included in other compartments from which they were indistinguishable. In this way our physiologic model had a minimum number of compartments and therefore favored simplicity and statistical certainty.

The overall configuration (number and connectivity of compartments) of our model was constructed to be physiologically reasonable based primarily on the concentration–time data for β-carotene-d_8, retinol-d_4, total β-carotene, and total retinol in plasma. Adjustments to the model were then chosen to be physiologically reasonable, and they were optimized to best-fit the model-predicted plasma concentration–time curves to the plasma concentration–time data, and to predict other known features of β-carotene metabolism. Once the model predicted a good fit of the curve to the experimental data, the statistical certainty of the parameters was determined.

Even though some parameters could not be measured experimentally in our volunteer, we still knew they had to be within certain bounds; therefore, we used soft statistical constraints in our model to provide more information about these parameters. In this way our model was based both on experimentally measured data from our volunteer and on additional data from the soft statistical constraints. The issue then of determining precisely how much of our results are derived from the experimental measurements on our volunteer and how much are from the statistical constraints is always of considerable interest. This could be determined using an analysis of variance for a linear system, but because our physiologic compartmental model is a nonlinear system, there is no means of determining precisely how much of the variance is associated with the experimental data from our volunteer and how much is associated with the statistical constraints. Nevertheless, because the statistical constraints were derived from experimental data from the scientific literature, careful application of such constraints to a compartmental model strengthen it. Furthermore, all relevant information should be considered (included) so that the best possible physiologic compartmental model may be hypothesized. Therefore, even though the relative contribution of the statistical constraints and the experimental data from our volunteer cannot be precisely quantified in this instance, both are important elements of a good model.

Although the 73 μmol β-carotene-d_8 dose used here was higher than the typical (1–12 μmol/d) intake of β-carotene in the US population, it was still within the 28–93 μmol/d range of doses typically used in antioxidant clinical trials of β-carotene (19, 20). Also, it is well known that a large variability between individuals exists in the extent to which ingested β-carotene changes the concentration of β-carotene in plasma (21–23). Therefore, the 73 μmol dose was chosen to be large enough to insure robust responses in plasma concentrations of tracer β-carotene-d_8 and retinol-d_4 for reliable concentration–time curves in individuals whose plasma response to ingested β-carotene was low, and to be small enough to insure that the dose did not perturb the steady state metabolism of β-carotene. It seems that the 73 μmol dose met these criteria because a robust response was obtained and the steady state system of β-carotene metabolism appears not to have been perturbed.

If the steady state system of β-carotene metabolism had been perturbed by the 73 μmol dose, the excellent fits between the measured and predicted values in Fig. 3 and 4 would not be expected using constant (by time since dosing) fractional transfer coefficients. Furthermore, we did not expect the 73 μmol dose to perturb the steady state system because intakes of < 11 versus 93 μmole β-carotene/d had already been shown not to affect the importance of personal traits (smoking, gender, and Quetelet Index) in determining plasma β-carotene concentrations (20). Therefore, the 73 μmole dose of β-carotene-d_8 was appropriate and the models we constructed seem relevant to healthy adults in the US population who are users and non-users of dietary supplements of β-carotene. However, our model is based on data from one healthy adult male subject and it must be interpreted accordingly.

Despite the fact that our data is based on only one adult subject, some features of the dynamics of β-carotene metabolism in humans can be suggested by the present experiments. These include, among others, the lack of accuracy in predicting mean sojourn or residence time of β-carotene in the body from empirical descriptions and the conversion of β-carotene to retinoid in both the enterocyte and the liver.

Mean sojourn or residence time for β-carotene

The physiologic model predicted the mean residence time for β-carotene in the body to be 51 days (whole body mass of β-carotene + retinoid/predicted steady state intake of β-carotene = 351.3 μmol/6.95 μmol/day). From the empirical description fit of the plasma β-carotene-d_8 concentration by time data, the AUMC/AUC (a commonly used measure of mean sojourn time, MST) was 13.05 days. The 51 day and 13.05 day values are different because there are several points (paths) where irreversible losses of the administered β-carotene and of its metabolites occur during metabolism and they are not accounted for when using empirical descriptions. There is also a delay before absorption. It was therefore not surprising to find that the residence time for β-carotene in the body was 51 days when the physiologic compartmental model rather than the empirical description was used to calculate it. As the compartmental physiologic model accounts for the multiple exit paths of the system, the mean residence time of 51 days (rather than 13.05 days) is expected to be the more accurate value for the clearance of β-carotene.

The empirical descriptions of the plasma concentration–time curves for both β-carotene-d_8 and retinol-d_4 displayed non-zero y-intercepts. By integrating entire concentration–time curves (integration started at zero) the respective AUC, AUMC, and MST for β-carotene-d_8 were 2.09 μmol × day/L plasma, 27.31 μmol × day^2/L plasma, and 13.05 days; those for retinol-d_4 were 2.20 μmol × day/L plasma, 57.36 μmol × day^2/L plasma, and 26.12 days. The AUC, AUMC, and MST values are sometimes calculated by integrating only the positive portion of plasma concentration–time curves (integration started at x-intercept). Therefore, AUC, AUMC, and MST values were also calculated when only the positive part of our plasma concentration–time curves were integrated. Using this approach the respective AUC, AUMC, and MST for β-carotene-d_8 were 3.17 μmol × day/L plasma, 27.36 μmol × day^2/L plasma, and 8.64 days; for retinol-d_4, they were 2.23 μmol × day/L plasma, 57.37 μmol × day^2/L plasma, and 25.67 days. These results demonstrate that our AUC, AUMC, and MST values were not influenced markedly by the method of integrating the plasma concentration–time curves. These results also demonstrate that the discrepancy in mean sojourn times between the compartmental model and the empirical description were not due to limits of integrating our plasma concentration–time curves.

β-Carotene transport by lipoproteins

The concentration of β-carotene-d_8 in plasma exhibited two peaks after dosing: one at ~ 7 h and the other between 1 and 2 days. In constructing the model, it was assumed that the first peak represents β-carotene associated with chylomicrons (or more likely β-carotene within the core of chylomicron remnants) preceding hepatic uptake; most chylomicra are too large to be taken up by liver and must be reduced in size by lipoprotein lipase before uptake. β-Carotene secreted in chylomicra may remain within the core of the remnant and be transferred to more dense lipoproteins while in the circulation. This being the case, both LDL and HDL would then be involved in the transfer of β-carotene to extrahepatic tissues. Alternatively, once remnants are taken up by liver, β-carotene may also be incorporated into triglyceride-rich lipoproteins which are secreted from hepatocytes. The remnants of these triglyceride-rich lipoproteins then could transfer their β-carotene to LDL and HDL. In other words, the β-carotene would remain within the core as these lipoproteins were reduced in size and their densities increased. With this as background, the data in Figs. 1 and 3 indicate that β-carotene first appears in plasma when chylomicrons are expected to appear. The fact that the height of the second peak equals that of the first peak and the width of the second peak extends over a couple of days suggests that a substantial recycling of β-carotene between hepatocytes and lipoproteins might be involved. Whatever the underlying mechanisms, the assumption that plasma β-carotene is transported almost exclusively via lipoproteins is consistent with the already published

observations of other investigators (24–26). Further modeling studies of stable isotope-labeled β-carotene in dysbeta lipoproteinemic, hyper and hypo alpha lipoproteinemic and cystic fibrosis patients will help delineate β-carotene absorption and transport by lipoproteins.

Formation and enterohepatic circulation of metabolites of β-carotene metabolism

Our physiologic compartmental model assumes that chylomicron retinyl ester is the only β-carotene metabolite that is absorbed and transported to the liver (because only retinol-d_4 and β-carotene-d_8 were determined in plasma) via the lymphatic system even though β-carotene is known to be metabolized to additional products also (27, 28) that enter the enterohepatic circulation (29, 30). Napoli and Race (27) have shown that cytosolic extracts of several tissues from rats can synthesize retinoic acid directly from β-carotene, and they have suggested that in situ synthesis of retinoic acid may be an especially important localized source of retinoic acid in humans that can accumulate high concentrations of carotenoids in their tissues. Wang et al. (28) demonstrated that 35% of the total radioactivity recovered in intestinal mucosa (of ferret intestine perfused with [^{14}C]β-carotene) was found in fractions with polarity greater than that of retinol. They also found that 28.2% of the radioactivity from intestinally perfused [^{14}C]β-carotene was transferred to liver via portal blood. Zachman, Dunagin, and Olson (29) have shown that 40% of the radioactivity of an intraportal injection of [^{14}C]retinoic acid appeared in bile within 6 h. Lewis et al. (30) have shown that 21% of an intravenously injected dose of biologically labeled [^{3}H]retinol was excreted in feces in 5 days. Lewis and coworkers (30) have also established that retinol from liver is metabolized and secreted into bile, recycled to the plasma, or moved into the slow turning over retinol compartment in liver. The foregoing studies and a recent thoughtful review (31) suggest that several polar metabolites (retinoic acid and retinoid glucuronides) are formed and enter the enterohepatic circulation during the metabolism of β-carotene. Analysis of serial plasma and bile samples from humans for labeled retinoic acid and retinoid glucuronides in future experiments (after ingestion of β-carotene-d_8) is likely to show that the kinetics of β-carotene metabolism in humans involves more than simply producing retinal to be metabolized independently.

The plasma response to ingested β-carotene represents the balance between β-carotene bioavailability and clearance. Even though a robust plasma response is commonly thought to reflect a greater absorption of β-carotene, it is also possible that it reflects a slower turnover and clearance. Therefore, a more detailed study of the enterohepatic circulation of β-carotene metabolites is likely to explain why some individuals demonstrate a robust plasma response to ingested β-carotene while others do not. Diet and physiologic state could well alter the dynamics of β-carotene metabolism.

Turnover of chylomicron β-carotene and chylomicron retinyl ester

Our physiologic compartmental model predicted that the turnover rates of chylomicron β-carotene (half life = 29 min) and chylomicron retinyl ester (half life = 8 min) may be different. The reason for the difference is unclear at present but it may be related to the fact that chylomicrons (remnants) are probably a heterogenous collection of particles with differing physical, chemical and biologic properties that are cleared at different rates by the low density lipoprotein receptor acting in concert with the low density lipoprotein receptor-related protein (32, 33).

In an alternate model, the fractional transfer coefficients of the chylomicron retinyl ester and the chylomicron β-carotene were constrained to be equal to one another. However, this model version did not provide the best fit of experimental data and the constraint was removed from the model.

The fractional transfer coefficient of chylomicron retinyl ester to fast turnover liver retinoid was constrained to 60 ± 36 to correspond to the known 15 min half-life of chylomicron retinyl esters. In an alternate model, the fractional transfer coefficient from chylomicron retinyl ester to fast turnover liver retinoid was not constrained. In that case, we were unable to attain sufficient statistical certainty for the turnover of retinyl ester, because other parameters were able to compensate for changes in retinyl ester turnover and still provide a good fit to experimental data. Therefore, this statistical constraint was included in the final model.

Conversion of β-carotene to retinoid in intestine (enterocyte) versus liver

One striking result of our experiments is the indication that the enterocyte and the liver are both involved in the conversion of β-carotene to retinoid in humans. While many studies have suggested that most of the conversion occurred in the intestine (21, 34–36) and others have suggested that substantial conversion occurred in liver (28, 34), the relative importance of the two sites has been uncertain. Alternate models with only liver conversion or only intestinal conversion were tested and rejected. We were unable to obtain a reasonable fit of the physiologic compartmental model prediction to the experimental data using only intestinal (in the enterocyte) or only liver conversion; a combination of conversion at both sites was necessary. Our physiologic compartmental model suggests that both intes-

tine and liver are important and respectively, they account for 43% and 57% of the total conversion of β-carotene to retinoid (assuming that 1 mole of β-carotene yields 1 mole of retinol). Furthermore, it may even be that the profile of metabolites that are produced at each site are different from one another. Intestinal enzyme preparations seem to convert β-carotene exclusively to retinal (37).

Our physiologic compartmental model, even though based only on one well-nourished adult male subject, predicted that 1 μg dietary β-carotene equaled 0.054 μg retinol (or 0.101 μmol retinol/μmol β-carotene), a biological activity value for β-carotene that is lower than the 0.1667 μg retinol value established in growing rats with low reserves of retinol. Had our subject been deficient or marginal in vitamin A status, the predicted yield of retinol would probably exceed 0.054 μg per μg β-carotene. Data on the dynamics of β-carotene metabolism from subjects with a wide range of body vitamin A stores will help clarify the ability of dietary (provitamin A) carotenes to meet the vitamin A requirements of humans following current and recommended dietary practices in the US (increased consumption of diets rich in fruits and vegetables to reduce disease risk), and/or increased consumption of carotene-rich plant foods in developing countries as a potential long-term solution to improve vitamin A status.

The biochemical mechanisms involved in the cleavage of carotenoid to retinoid were thoughtfully reviewed recently (31). In mammals β-carotene is initially converted to retinal, which is subsequently reduced to retinol. Two pathways (central and eccentric-oxidative) for conversion of β-carotene to retinoid have been shown to exist in mammalian tissue explants, but the relative importance of each pathway and the exact profile of intermediates and final products involved under different conditions are still unsettled. Further modeling in combination with use of β-carotene that is appropriately labeled with stable isotope is a powerful means of investigating the issue of central versus eccentric cleavage of β-carotene in vivo in humans. Central cleavage of β-carotene theoretically yields 2 moles of retinol per mole of β-carotene (35, 38, 39). Eccentric cleavage of β-carotene yields one mole of retinol per mole of β-carotene (35, 40). Brubacher and Weiser (41) determined the retinol equivalent of β-carotene in vivo using rats and chicks fed various levels of β-carotene and found that absorbed β-carotene was converted to retinol with a ratio of one mole of retinol per mole of β-carotene. Thus, based on these in vivo results, a ratio of one mole of retinol per mole of β-carotene (after absorption) seemed appropriate to use in the present model; and this ratio is also generally consistent with the data of Sauberlich et al. (18). Finally, the extent of central versus eccentric cleavage of β-carotene in vivo in humans can now be investigated with additional modeling combined with use of appropriately labeled β-carotene.

In summary, physiologic compartmental modeling of the kinetics of β-carotene and of retinol derived from it allowed us to propose a model of the dynamics of β-carotene metabolism in humans. The model predicts tissue reserves and plasma concentrations of β-carotene and retinol that are in agreement with experimentally measured data. The availability of stable isotope-labeled β-carotene and retinol and analytical methods to measure them in biologic tissues in combination with mathematical modeling offers great promise for a more complete understanding of the dynamics of this common dietary constituent. Further modeling combined with the ability to label the β-carotene molecule at different sites yields a powerful means for investigating the issue of central and eccentric cleavage. As our results are based on one healthy adult, more subjects differing in nutritional status, physiologic state, age, and gender should be studied to refine the physiologic compartmental model of the dynamics of β-carotene metabolism and to identify key factors that affect it.

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