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Variability of the conversion of β-carotene to vitamin A in women measured by using a double-tracer study design[1–3]

Yumei Lin, Stephen R Dueker, Betty J Burri, Terry R Neidlinger, and Andrew J Clifford

ABSTRACT

Background: Blood β-carotene and vitamin A responses to oral β-carotene are variable in humans. Some individuals are characterized as responders and others as low- or nonresponders. A better understanding of the conditions that produce the variability is important to help design public health programs that ensure vitamin A sufficiency.

Objective: Our objective was to assess variability in absorption and conversion of β-carotene to vitamin A in vivo in humans by using a novel double-tracer [hexadeuterated (D_6) β-carotene and D_6 retinyl acetate] approach.

Design: Eleven healthy women were housed at the US Department of Agriculture Western Human Nutrition Research Center metabolic unit for 44 d, where they consumed diets adequate in vitamins and minerals except for carotenoids. After an adaptation period, the women were given 30 μmol D_6 retinyl acetate orally, followed 1 wk later with 37 μmol D_6 β-carotene (approximately equimolar doses). Time-dependent plasma concentration curves were determined for D_6 retinol, D_6 β-carotene, and trideuterated (D_3) retinol (derived from D_6 β-carotene).

Results: Mean (±SE) absorption of D_6 β-carotene was 3.3 ± 1.3% for all subjects. The mean conversion ratio was 0.81 ± 0.34 mol D_3 retinol to 1 mol D_6 β-carotene for all subjects. However, only 6 of the 11 subjects had plasma D_6 β-carotene and D_3 retinol concentrations that we could measure. The mean absorption of D_6 β-carotene in these 6 subjects was 6.1 ± 0.02% and their conversion ratio was 1.47 ± 0.49 mol D_3 retinol to 1 mol D_6 β-carotene. The remaining 5 subjects were low responders with ≤0.01% absorption and a mean conversion ratio of 0.014 ± 0.004 mol D_3 retinol to 1 mol D_6 β-carotene.

Conclusion: Variable absorption and conversion of β-carotene to vitamin A both contribute to the variable response to consumption of β-carotene. Our double-tracer approach is adaptable for identifying efficient converters of carotenoid to retinoid. *Am J Clin Nutr* 2000;71:1545–54.

KEY WORDS Carotene, vitamin A, β-carotene, absorption, metabolism, stable isotope, tracer, women

INTRODUCTION

Reviews have summarized our current understanding of carotenoid absorption and tissue distribution (1), carotenoid bioavailability (2–4), and β-carotene in health and disease (5–7). Interest in carotene bioavailability has intensified because of concern that use of carotene-rich foods and carotene supplementation programs are not effective as sustainable solutions to vitamin A deficiency (8). The absorption and effective use of β-carotene may not be uniform among individuals and populations (9–14). Therefore, we decided to measure the intrinsic variability of β-carotene absorption and conversion to vitamin A in healthy women living in a controlled environment.

The present investigation addressed the basis for the high interindividual variability in the biochemical responses to ingested β-carotene. It did so by eliminating several sources of variability (health status, medication, sex, age, activity levels, body composition, hematology, and metabolic variables measured by clinical chemistry) and by using a double-tracer experimental design. The double-tracer design enabled the fate of an oral dose of labeled β-carotene (the provitamin A form that is converted to vitamin A) to be compared directly with that of an approximately equimolar dose of labeled vitamin A. Intrinsic variability in either the absorption of β-carotene or its conversion to vitamin A contribute, in part, to the seemingly conflicting responses to β-carotene supplementation (9–13, 15, 16).

The highly variable increase in blood β-carotene concentration in response to a single oral dose of β-carotene is well documented (9–14). Individuals who show little or no increase in blood β-carotene after an oral dose of β-carotene (≥15 μmol) or a β-carotene–rich diet of several weeks' duration are characterized as non- or low responders (10, 17, 18). Possible explanations for this low response are impaired intestinal absorption of β-carotene, exaggerated conversion of β-carotene to vitamin A, inefficient incorporation of β-carotene into chylomicrons, or accelerated clearance of β-carotene due to atypical lipoprotein metabolism. Aside from the possibility that other carotenoids may interfere with the absorption and metabolism of β-carotene (19), the conditions that produce the low-responder trait are unknown.

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Our protocol used labeled retinol and β-carotene, which allowed these carotenoids to be distinguished from endogenous retinol and β-carotene. The fates of approximately equimolar oral doses of hexadeuterated (D_6) retinyl acetate and D_6 β-carotene [the provitamin A that converts to trideuterated (D_3) retinol] were determined to characterize their metabolic behavior in healthy women living in a controlled environment. The D_6 retinyl acetate was administered 7 d before the D_6 β-carotene, thus allowing the appearance of D_6 retinol derived from D_6 retinyl acetate to be compared with the appearance of the D_3 retinol derived from D_6 β-carotene. This approach enabled us to determine whether the low-responder trait was due to an exaggerated conversion of β-carotene to vitamin A.

SUBJECTS AND METHODS

Subjects

Eleven healthy women were recruited. Informed, written consent was obtained from each potential subject by using a protocol that was approved by the Human Subject Review Committees of the University of California, Davis, and the US Department of Agriculture (USDA). Each subject was given a health questionnaire, a physical exam by a physician, and a standard health screening that included measurement of blood urea nitrogen, creatinine, serum enzymes, and bilirubin. Each subject's usual nutrient intake over the 6-mo period that preceded admission to the study was assessed with the Scantron version of a food-frequency questionnaire (Block Dietary Data Systems, Berkeley, CA). The subjects were nonsmokers who ranged in age from 19 to 39 y ($\bar{x}$ ± SE: 30 ± 2 y), had body weights from 46.8 to 82.7 kg (63 ± 4 kg), and had body mass indexes (in kg/m^2) from 18.1 to 31.3 (23 ± 1). The subjects were healthy, premenopausal women with regular menstrual cycles. None had had gastrointestinal surgery. There was no evidence of lipid malabsorption or recent weight loss, and none of the women had a prior history of chronic diarrhea. The women were not taking medication and did not have unusual diet or exercise histories. No tobacco, alcohol, or prescription or nonprescription drugs were taken during the 8 d that preceded admission to the metabolic ward. The women were admitted to the metabolic research unit of the USDA Western Human Nutrition Research Center for a 44-d period during which all feces was collected and visually inspected. Although fecal fat was not measured, there was no apparent evidence of a fat malabsorption problem in any subjects. During this time, the subjects' activity levels were restricted to sedentary-type exercise to avoid changes in their physical condition.

Isotopes and supplements

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 (20). Vitamin A supplement capsules [1250 IU, or 375 retinol equivalents (RE)] were obtained from Bronso (St Louis). The nonlabeled, 1.9-μmol β-carotene supplement capsules were obtained from Roche Diagnostics (Nutley, NJ).

Experimental time line and design

A time line for the study is shown in **Figure 1**. For the first 8 d after admission (study days 1–8) the subjects chose their in-house meals a la carte from a limited menu of foods; no supplements were given. This 8-d period allowed the women to adapt to living in a metabolic research unit. During that time, the subjects recorded the weights of the food items they consumed from the a la carte menu by using the NESSy, a patented (US PTO no. 43784632) computerized food-weighing and -recording system (21). These food records were analyzed by using the USDA *Handbook* 8 database (22), supplemented with dietary analyses done in our laboratory.

Starting on day 9 and continuing throughout the remainder of the study, all meals were served in a 4-d rotating menu and were consumed under observation. The meals were made of natural foods low in carotene that provided ≈0.07 μmol β-carotene/d (23, 24). The dietary energy distribution from carbohydrates, proteins, and fats was 53%, 14%, and 33%, respectively. All other nutrients were provided at ≥100% of the US recommended dietary allowance (RDA; 25).

Starting on day 10 and throughout the remainder of the study, each subject received a vitamin A supplement of 1250 IU (375 RE, or 1.31 μmol as retinyl palmitate in cod liver oil) every other day at breakfast. Starting on day 14 and continuing throughout the remainder of the study, each subject also received a 1.9-μmol β-carotene supplement every other day at breakfast. These supplements were given to stabilize the plasma concentrations of total retinol and β-carotene at normal values during the study.

On day 16, each fasting subject swallowed a small (size no. 3) white gelatin capsule (Frontier, Norway, IA), which contained 30 μmol D_6 retinyl acetate, with 250 mL milk (2% fat). Thirty (±5) minutes later, a breakfast that contained 11 g fat and the nonlabeled vitamin A (1250 IU) and nonlabeled carotene (1.9 μmol) supplements was served. Similarly, on day 23, each fasting subject swallowed a size no. 3 white gelatin capsule (Frontier) that contained 37 μmol D_6 β-carotene with 250 mL milk (2% fat). Thirty (±5) minutes later a breakfast that contained 11 g fat was served. The mass of D_6 retinyl acetate and D_6 β-carotene in each capsule was within 5% of the target dose. The total amount of fat consumed with each dose was 16 g (5 g from the milk + 11 g from the breakfast). This double-tracer design provided 2 sources of retinol: one from preformed vitamin A (the D_6 retinyl acetate dose) and the other from provitamin A (cleavage of the D_6 β-carotene to D_3 retinol). Because GC-MS could measure the D_6 retinol and D_3 retinol and HPLC the D_6 β-carotene and D_0 β-carotene, it was possible to determine whether the low-response trait was due to exaggerated conversion of D_6 β-carotene to D_3 retinol. The mass of a supplemental dose of β-carotene (in mg) necessary to meet the vitamin A requirement of adult men is approximately twice that of retinol (26). Therefore, we administered nearly bioequivalent amounts of D_6 retinyl acetate (10 mg, or 30 μmol) and D_6 β-carotene (20 mg, or 37 μmol). Furthermore, these doses are obtainable through an occasional large serving (200 g) of carrots or pumpkin (27).

Blood samples (10–15 mL) were collected from the antecubital vein of each subject just before (time zero) and at 2, 6, 10, 15, 20, 24, 48, 72, 96, 167, 168, 170, 174, 178, 183, 188, 192,

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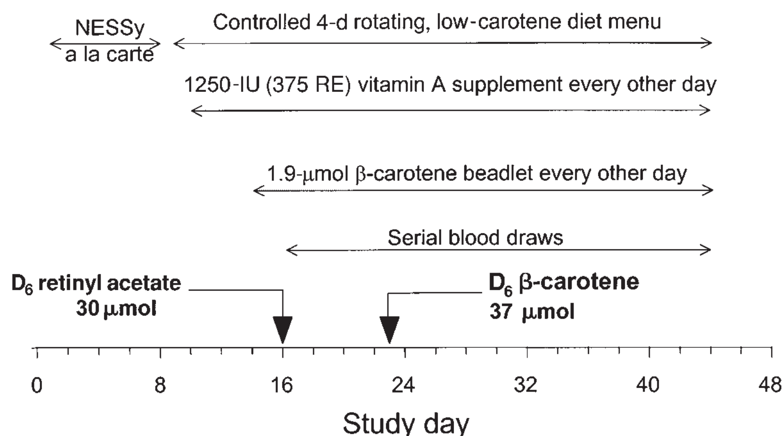

FIGURE 1. Experimental design and time line. Study days 1 through 8 served as an adaptation period during which the subjects chose [and recorded by using a computerized food-weighing and -recording system (21)] their in-house meals a la carte from a limited menu of foods. No supplements were given during the first 8 d. Starting on day 9 and continuing through the end of the study, all meals were served in a 4-d rotating menu and consumed under observation. Starting on day 10 and continuing through the end of the study, each subject received a 1250-IU (375 RE) vitamin A supplement every other day. Starting on day 14 and continuing through the end of the study, each subject received a 1.9-μmol β-carotene supplement every other day. On day 16, each subject took 30 μmol hexadeuterated (D_6) retinyl acetate with 250 mL milk (2% fat) followed by a breakfast that supplied 11 g fat. On day 23, each subject took 37 μmol D_6 β-carotene with 250 mL milk followed by a breakfast that supplied 11 g fat. Serial blood samples were drawn beginning on day 16 with a fasting sample just before the D_6 retinyl acetate was taken. After the first day (after dosing with D_6 retinyl acetate and D_6 β-carotene) all blood was drawn from fasting subjects.

216, 240, 264, 384, 480, 576, and 672 h after the D_6 retinyl acetate dose. Blood samples were collected just before (time zero) and at 2, 6, 10, 15, 20, 24, 48, 72, 96, 216, 312, 408, and 504 h after the D_6 β-carotene dose. The blood sample drawn at 168 h after the D_6 retinyl acetate dose served as the time zero sample for the D_6 β-carotene dose. After the first day and after dosing with D_6 retinyl acetate and D_6 β-carotene, all blood was drawn from fasting subjects. Plasma was separated by centrifugation and stored at −75°C until analyzed.

After the final blood draw and before release from the metabolic research unit, each subject ingested a single dose of a mixed carotenoid supplement to ensure carotenoid repletion. The supplement provided 6.2 μmol β-carotene, 2.6 μmol α-carotene, 0.2 μmol β-cryptoxanthin, 2.6 μmol lutein and zeaxanthin, and 1.2 μmol lycopene (Carotenoid Complex; GNLD, San Jose, CA). In addition to the single dose, each subject was released from the metabolic research unit with 12 additional doses (in 12 capsules) and instructed to take one each day for the next 12 d.

Isolation of total retinol and β-carotene from plasma

Retinol and β-carotene were isolated from plasma after alkaline hydrolysis to remove lipophilic contaminants that foul GC-MS and HPLC columns, reduce sample throughput, and interfere with the accuracy of analyses (20, 28, 29). Saponification also converts retinyl esters to retinol. To minimize isomerization and degradation of β-carotene and retinol during saponification, aluminum foil and amber vials were used to protect the samples from light. Also, saponification was performed in vials sealed under argon and containing pyrrogallol as an antioxidant. Briefly, 1 mL plasma was treated with ethanol to denature the protein; the retinol and β-carotene were then extracted by using hexane. The hexane was evaporated, the residue was saponified in ethanolic alkali, and the retinol and β-carotene were extracted again with hexane. Retinol was separated from β-carotene on an NH_2 solid-phase extraction cartridge (Alltech Associates Inc, Deerfield, IL). The separated fractions were collected separately and stored at –20°C until the isotope ratios were analyzed as described below.

Plasma retinol isotope ratios

Retinol isotope ratios were determined by monitoring of their *tert*-butyldimethylsilyl derivatives using selected ion monitoring GC-MS (30). 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 0.2, 0.1, 0.05, 0.025, 0.017, 0.01, and 0.005) was prepared by adding a fixed mass of the D_0 retinol to various masses of D_6 retinol. Because D_3 retinol is derived in vivo from D_6 β-carotene and cannot be reproduced by synthetic methods, a calculated value for the D_3 retinol isotopic profile was determined instead as we described previously for D_4 retinol (20). The calculation was as follows. The fractional isotopic heterogeneity of D_6 β-carotene is 0.59 D_6 β-carotene, 0.34 D_5 β-carotene, 0.06 D_4 β-carotene, and 0.01 other forms. By central cleavage, 0.59 mol D_6 β-carotene yields 0.59 mol D_3 retinol, 0.34 mol D_5 β-carotene yields 0.17 mol D_3 retinol plus 0.17 mol D_2 retinol, and 0.06 mol D_4 β-carotene yields 0.06 mol D_2 retinol. When these are summed, the predicted molar fractional isotopic heterogeneity of the biosynthesized D_3 retinol is 0.76 D_3 retinol (0.59 + 0.17), 0.23 D_2 retinol (0.17 + 0.06), and 0.01 other forms. Therefore, the D_3 retinol represents only 76% of the total retinol isotopomers derived from D_6 β-carotene.

An additional adjustment was also needed because D_2 retinol at its mass-to-charge ratio (*m/z*) of 402 contributes 33.8% of the base peak to the D_3 retinol signal at its *m/z* of 403 from the

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natural isotopic contributions of the M + 1 isotopomeric form of D_2 retinol (31). The adjusted D_3 retinol derived from D_6 β-carotene became 0.078 (0.338 × 0.23) and the D_3 retinol increased to 83.8% (76 + 7.8) of the total retinol isotopomers derived from D_6 β-carotene. The final molar value was thus underestimated by 1/0.838 and was adjusted by this factor to obtain the final plasma concentration of D_3 retinol.

Finally, corrections for the naturally occurring mass of all-proton retinol at an *m/z* of 403 for D_3 retinol (1.6%) and at a *m/z* of 406 for D_6 retinol (<0.01%) were also made (31). The lowest molar ratio of D_6 retinol to D_0 retinol that could be integrated confidently was 0.005 with a CV of 7.5%.

Plasma β-carotene isotope ratios

β-Carotene isotope ratios were determined by reversed-phase HPLC (20, 32) with the following minor modifications. The isocratic mobile phase (acetonitrile:methanol:isopropanol:ammonium acetate; 81:9:10:0.01; vol:vol:vol:wt) was delivered at 0.9 mL/min. Temperature was held constant at 30°C and absorbency monitored at 497 nm by using a Hewlett Packard 1100 series HPLC apparatus controlled by CHEMSTATION software (Hewlett Packard, Palo Alto, CA). The system included 2 Adsorbosphere-HS octadecylsilane columns (150 mm × 4.6 mm, 3-μm particle size; Alltech Associates Inc) connected in series downstream from a Brownlee NewGuard RP-18 precolumn cartridge (Applied Biosystems, Foster City, CA). The D_6 β-carotene and D_0 β-carotene peak areas were integrated by using a Gaussian function (ORIGIN 5.0 program; Microcal Software Inc, Northampton, MA). The set of calibrating standard mixtures of D_6 β-carotene and D_0 β-carotene (with molar ratios D_6 β-carotene to D_0 β-carotene of 0.991, 0.498, 0.227, 0.151, 0.137, 0.100, 0.092, 0.071, 0.065, 0.054, 0.020, and 0.010) was prepared by adding a fixed mass of D_0 β-carotene to various masses of D_6 β-carotene. The lowest molar ratio of D_6 β-carotene to D_0 β-carotene that could be integrated confidently was 0.02 with a CV of 8.5%. All other calibration and HPLC standardizations were as described previously (32).

Plasma total and labeled retinol and β-carotene

Aliquots (100 μL) of plasma (and plasma calibrators) were fortified with internal standards (32) and analyzed for retinol and β-carotene (33). Concentrations were expressed as μmol/L plasma. The concentrations of D_6 retinol were obtained by multiplying plasma retinol concentration by (plasma D_6 retinol/plasma D_0 retinol)/[(plasma D_6 retinol/plasma D_0 retinol) + 1)]; concentrations of D_6 β-carotene were obtained similarly.

Calculation, analysis, and presentation of data

The plasma concentrations of D_6 retinol, D_3 retinol, and D_6 β-carotene over time since dosing were calculated and plotted for each subject. The area under the plasma concentration-by-time (since dosing) curve (AUC) for D_6 retinol was integrated from 0 to 96 h after dosing with D_6 retinol. The plasma D_3 retinol AUC was also integrated from 0 to 96 h after dosing with D_6 β-carotene. The plasma D_6 β-carotene AUC was integrated from 0 to 504 h after dosing with D_6 β-carotene. The plasma D_3 retinol and D_6 β-carotene AUCs could be summed to reflect total absorption of the administered D_6 β-carotene. Plasma AUCs were calculated by using the trapezoidal approximation (34). The conversion ratio was calculated by multiplying the plasma ratio of D_3 retinol AUC to D_6 retinol AUC by 30/37 (the molar ratio of the D_6 retinyl acetate to D_6 β-carotene doses). The ratio of the AUCs for D_3 retinol to D_6 β-carotene may also reflect conversion efficiency. A high ratio of D_3 retinol AUC to D_6 β-carotene AUC in the presence of a low D_6 β-carotene AUC might suggest efficient conversion whereas a low ratio of D_3 retinol AUC to D_6 β-carotene AUC in the presence of a high D_6 β-carotene AUC might suggest efficient absorption. The fractional absorption of D_6 β-carotene was calculated as 0.693/864 × AUC × dose × plasma volume/dose (35) by using a plasma β-carotene half-life of 864 h (36; mean sojourn time/1.4) and expressed as a percentage.

Because the response of individual subjects to D_6 retinyl acetate and D_6 β-carotene varied widely, we report the results for individual subjects as well as the mean (±SE) values for all subjects as a group. Five subjects whose responses to D_6 β-carotene were very low are listed together as low responders to D_6 β-carotene, thus enabling their mean (±SE) value to be compared with that of the remaining 6 subjects. The ratios of D_6 β-carotene to D_0 β-carotene and of D_3 retinol to D_0 retinol in plasma from the low responders were so low that meaningful ratios of D_3 retinol to D_6 β-carotene could not be computed for the subjects.

RESULTS

Vitamin A and β-carotene intakes and their concentrations in plasma are summarized in **Table 1**. Individual subjects are identified and grouped as responders or low responders. The intakes of total vitamin A during the 6-mo period that preceded the study ranged from 1901 to 19 628 IU/d, with an overall mean (±SEM) of 9383 ± 1995 IU/d. Seven subjects (nos. 21, 23, 24, 27, 28, 29, and 30) reported taking a low-dose vitamin A supplement, generally as part of a daily multivitamin. The respective supplemental intakes of vitamin A were 2858, 1429, 1429, 5000, 5000, 2858, and 1429 IU/d. No supplemental intakes of vitamin A were in the form of β-carotene. In general, most subjects (8 of 11) had an adequate-to-average intake of vitamin A (≥4000 IU/d). However, the total vitamin A intakes of 3 women (subjects 23, 25, and 31) were below the US RDA for adult women (4000 IU). Of these 3 subjects, only one (no. 23) reported taking a vitamin A supplement (1429 IU/d) before the study.

In contrast, 3 subjects (nos. 24, 28, and 32) had been consuming >18 000 IU vitamin A/d, ≥4 times the US RDA for this vitamin. The difference in mean daily intakes of vitamin A between the responder (10 880 ± 2808 IU) and the low-responder (9382 ± 1995 IU) groups was not significant.

The intakes of total β-carotene during the 6-mo period that preceded the study ranged from 0.86 to 12.76 μmol/d, with a whole-group mean of 6.18 ± 1.16 μmol/d. Five subjects (nos. 23, 24, 27, 28, and 30) had been taking a β-carotene supplement before the study. Their respective supplemental intakes of β-carotene were 0.64, 0.64, 2.23, 2.23, and 0.64 μmol/d. The β-carotene intakes of 9 subjects ranged from 2.79 to 12.76 μmol/d; this is considered a typical daily intake (≥3.7 μmol/d) from a Western diet (5). Only 2 women (subjects 25 and 31) were consuming ≤1 μmol β-carotene/d. Neither of these subjects reported taking a vitamin A or β-carotene supplement before the study. The difference in mean daily intakes of β-carotene between the responder (6.86 ± 1.66 μmol) and the low-responder (5.46 ± 1.74 μmol) groups was not significant. The prestudy intakes of β-carotene and vitamin A were correlated with one another (r = 0.922, P < 0.0001).

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TABLE 1

Prestudy intakes of vitamin A and β-carotene and fasting plasma retinol and β-carotene concentrations on study days 10, 16, and 23, by response group[1]

Subject and response group	Vitamin A intake[2]	β-Carotene intake	Plasma retinol			Plasma β-carotene		
			Day 8	Day 16	Day 23	Day 8	Day 16	Day 23
	IU/d	*μmol/d*		*μmol/L*			*μmol/L*	
Responders								
21	10 102[3]	7.61	1.75	1.70	1.38	1.39	1.36	1.10
25	1901	0.86	1.62	1.81	1.57	0.14	0.14	0.17
27	6985[3]	5.13[4]	1.20	1.20	1.04	0.34	0.32	0.37
28	18 409[3]	12.76[4]	1.21	1.25	1.16	0.28	0.27	0.29
30	8256[3]	5.14[4]	1.27	1.19	1.22	0.20	0.20	0.25
32	19 628	9.15	1.28	1.29	1.15	0.34	0.36	0.36
$\bar{x}$	10 880	6.86	1.39	1.41	1.25	0.45	0.44	0.42
± SE	2808	1.66	0.10	0.11	0.08	0.19	0.19	0.14
Low responders								
22	4207	2.79	1.15	1.10	1.10	0.30	0.35	0.35
23	3084[3]	6.23[4]	1.32	1.44	1.15	0.23	0.22	0.27
24	18 300[3]	11.21[4]	1.47	1.48	1.62	0.24	0.28	0.33
29	9410[3]	6.03	0.91	0.99	1.27	1.19	1.19	1.31
31	2927	1.07	1.10	1.03	1.11	0.32	0.30	0.38
$\bar{x}$	7584	5.46	1.19	1.21	1.23	0.46	0.47	0.53
± SE	2929	1.74	0.10	0.11	0.08	0.18	0.18	0.20
All subjects								
$\bar{x}$	9382	6.18	1.30	1.32	1.24	0.45	0.45	0.47
± SE	1995	1.16	0.07	0.08	0.05	0.13	0.12	0.11

[1] During the study, meals were of natural foods adequate in vitamin A but low in carotene (≈0.07 μmol/d). Starting on day 10 and continuing through the end of the study, all subjects received a vitamin A supplement of 1250 IU [375 retinol equivalents (RE) or 1.31 μmol in cod liver oil] every other day; starting on day 14 and continuing through the end of study, each subject received a carotene supplement (1.9 μmol β-carotene) every other day. Low responders were those who showed little or no increase in plasma β-carotene after an oral dose ≥15 μmol β-carotene.

[2] Prestudy intakes include supplements. 1 IU = 0.3 RE.

[3] Took vitamin A supplements.

[4] Took β-carotene supplements.

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The mean intakes of total vitamin A during the first 8 d on the metabolic research unit (when the subjects selected their diet from the a la carte menu) ranged from 4824 to 11 772 IU/d, with a whole-group mean of 7431 ± 707 IU/d. Mean vitamin A intakes of the responders and low responders were 7374 ± 912 and 7499 ± 1223 IU/d, respectively. During the same period, the β-carotene intakes ranged from 3.54 to 11.87 μmol/d, with a whole-group mean of 6.29 ± 0.90 μmol/d. The mean β-carotene intakes of the responders and low responders were 6.05 ± 1.23 and 6.59 ± 1.47 μmol/d, respectively. The intakes of β-carotene and vitamin A during the first 8 d on the metabolic research unit were correlated with one another ($r = 0.940$, $P < 0.0001$).

Overall mean concentrations of total retinol in plasma on days 8, 16 (just before the D_6 retinyl acetate dose), and 23 (just before the D_6 β-carotene dose) were 1.30 ± 0.07, 1.32 ± 0.08, and 1.24 ± 0.05 μmol/L, respectively. The difference between these 3 means was not significant ($P > 0.20$). The overall mean plasma retinol concentration was 1.28 ± 0.06 μmol/L for all 3 d and it was well within the typical retinol concentration range (1–3 μmol retinol/L) of adult American women (37). The difference between the mean plasma retinol concentration of the responder (1.39 ± 0.10, 1.41 ± 0.11, and 1.25 ± 0.08 μmol/L) and low-responder (1.19 ± 0.01, 1.21 ± 0.11, and 1.23 ± 0.08 μmol/L) groups was not significant. As expected, the concentration of total retinol in plasma on day 8 was correlated with its concentration on days 16 ($r = 0.949$, $P < 0.001$) and 23 ($r = 0.669$, $P < 0.025$, plots not shown).

The overall mean concentration of total β-carotene in plasma on days 8, 16 (just before the D_6 retinyl acetate dose), and 23 (just before the D_6 β-carotene dose) were 0.46 ± 0.12, 0.45 ± 0.12, and 0.47 ± 0.11 μmol/L, respectively. The differences between these 3 means was not significant ($P > 0.55$), confirming that the 1.9-μmol β-carotene supplement every other day maintained plasma β-carotene concentration. The overall mean β-carotene concentration was 0.46 ± 0.12 μmol/L for all 3 d and it was well within the typical β-carotene concentration range (0.09–0.91 μmol/L) of nonsmoking American women (38). The difference between the mean concentration of the responder (0.45 ± 0.19, 0.44 ± 0.19, and 0.42 ± 0.14 μmol/L) and low-responder (0.46 ± 0.18, 0.47 ± 0.18, and 0.53 ± 0.20 μmol/L) groups was not significant. As expected, the plasma total β-carotene concentrations on day 8 were correlated with those on days 16 ($r = 0.998$, $P < 0.001$) and 23 ($r = 0.971$, $P < 0.001$, plots not shown).

A plot of the plasma concentrations of D_6 retinol, D_6 β-carotene, and D_3 retinol against time since dosing in 3 subjects (nos. 25, 27, and 32) is shown in **Figure 2.** The plasma D_6 retinol concentration increased promptly and peaked ≈21 h after dosing. This early concave pattern in the concentration of D_6 retinol changed to a convex pattern 32 h after dosing, as we had expected from previous experience (39). Of the 3 subjects, no. 27 had the smallest plasma D_6 retinol response whereas no. 32 had the largest.

The plasma D_3 retinol concentration also increased promptly but peaked ≈10 h after dosing with D_6 β-carotene, suggesting that the plasma D_3 retinol might consist of D_3 retinyl esters

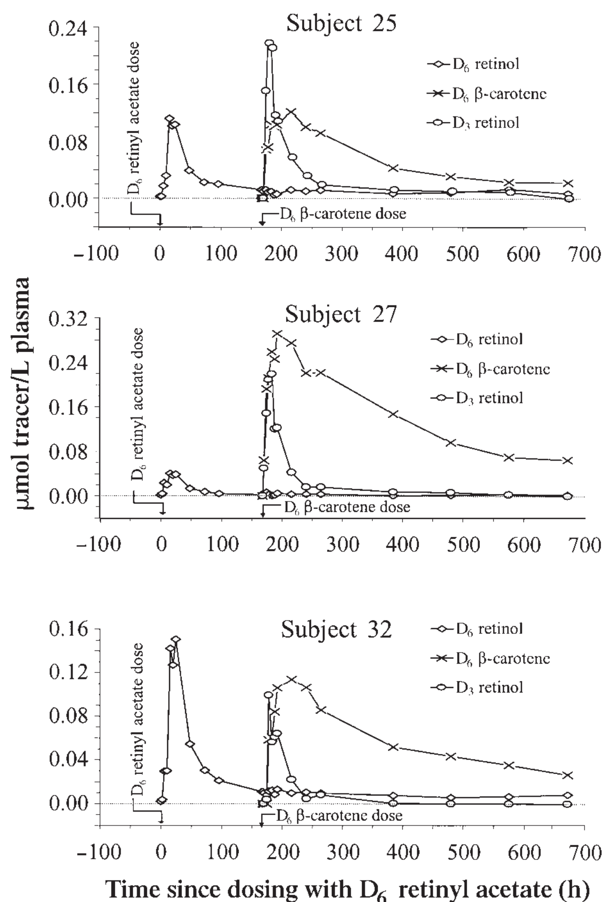

FIGURE 2. A plot (trace) of the plasma concentrations of hexadeuterated (D_6) retinol, D_6 β-carotene, and trideuterated (D_3) retinol (derived from the D_6 β-carotene) against time since dosing in 3 subjects (no. 25, 27, and 32).

generated in enterocytes. This early concave pattern in the concentration of D_3 retinol changed to a convex pattern 31 h after dosing with D_6 β-carotene. In this case, subject 27 had the second largest plasma D_3 retinol response whereas no. 32 had the second smallest. On the basis of the plasma ratio of the D_3 retinol AUC to the D_6 retinol AUC, the preferred source of vitamin A for subject 27 would be D_6 β-carotene rather than D_6 retinyl acetate, not because her plasma D_6 β-carotene AUC was substantial, but because her plasma D_6 retinyl acetate AUC was low.

The plasma D_6 β-carotene concentration increased and peaked ≈31 h after dosing, as expected (40). This concave pattern in the concentration of D_6 β-carotene changed slowly to a convex pattern some time between 5 and 10 d after dosing with D_6 β-carotene, as reported by others (36, 41). Of the 3 subjects, no. 25 had the smallest plasma D_6 β-carotene response and no. 27 had the largest. On the basis of the ratio of the plasma D_3 retinol AUC to the plasma D_6 β-carotene AUC, subject 25 would be the most efficient converter of D_6 β-carotene to D_3 retinol because she had the highest D_3 retinol response and the lowest D_6 β-carotene response of the 3 subjects.

The AUCs for plasma D_6 retinol, D_6 β-carotene, and D_3 retinol are shown in tabular form in **Table 2**. All subjects showed an increase in the plasma D_6 retinol AUC after oral administration of D_6 retinyl acetate. The overall mean AUC for plasma D_6 retinol was 2.39 ± 0.78 μmol · h/L; values for individual subjects ranged from 0.34 to 7.36 μmol · h/L. The difference between the mean D_6 retinol AUC of the responders (3.10 ± 1.21 μmol · h/L) and that of the nonresponders (1.22 ± 0.70 μmol · h/L) was not significant.

Only 6 (nos. 21, 25, 27, 28, 30, and 32) of the 11 subjects had a measurable AUC (≥0.01 μmol · h/L) for D_6 β-carotene. The remaining 5 subjects (nos. 22, 23, 24, 29, and 31) had D_6 β-carotene AUCs ≤0.01 μmol · h/L and were classified as low responders. These have been defined as individuals who show little or no increase in blood β-carotene with either ingestion of a single, large oral dose of β-carotene (≥15 μmol) or consumption of a β-carotene–rich diet for several weeks (10, 17, 18). An AUC cutoff of ≤0.01 μmol · h/L was used in the present study to characterize individuals as low responders to β-carotene. The mean AUC (of all subjects) for plasma D_6 β-carotene was 15.43 ± 5.69 μmol · h/L; individual values ranged from ≤0.01 to 71.32 μmol · h/L. The mean AUC (of 6 responders) for plasma D_6 β-carotene was 28.28 ± 9.30 μmol · h/L. The correlation between the plasma D_6 retinol and D_6 β-carotene AUCs (r = 0.089, P = 0.7873) and between the plasma D_6 retinol and D_3 retinol AUCs (r = 0.240, P = 0.4747) were not significant (plots not shown).

Only 6 subjects (nos. 21, 25, 27, 28, 30, and 32) had AUCs ≥0.01 μmol · h/L for D_3 retinol. These were the same subjects who had AUCs ≥0.01 μmol · h/L for D_6 β-carotene, suggesting that appearance in plasma of D_6 β-carotene in easily detectable amounts might be a prerequisite for the appearance of D_3 retinol. The mean plasma D_3 retinol AUC of all subjects was 1.70 ± 0.80 μmol · h/L; individual values for this variable ranged from 0.01 to 7.05 μmol · h/L. The difference in mean plasma D_3 retinol AUC (1.70 ± 0.80 μmol · h/L) from the plasma D_6 retinol AUC (2.39 ± 0.78 μmol · h/L) for all subjects was not significant (P = 0.4919). For all subjects, the mean conversion ratio was 0.811 ± 0.343 on the basis of the ratio of plasma D_3 retinol AUC to plasma D_6 retinol AUC. For the responders, the mean conversion ratio was 1.476 ± 0.488 with use of the ratio of plasma D_3 retinol AUC to plasma D_6 retinol AUC. According to this conversion measure, the 6 responders were ranked (high to low) as follows: no. 27 > no. 28 > no. 21 > no. 25 > no. 32 > no. 30.

The mean (±SE) absorption of D_6 β-carotene was 3.320 ± 1.360% for all subjects. The mean absorption of D_6 β-carotene was 6.1 ± 0.02% for the 6 absorbers whose values ranged from 1.100% to 14.393%. The absorption of D_6 β-carotene was 0.003 ± 0.000% for the 5 low responders whose values ranged from 0.002% to 0.003%. The mean ratio of the D_3 retinol AUC to the D_6 β-carotene AUC (a second measure of conversion) was 0.105 ± 0.034. According to this conversion measure, the 6 responders were ranked (high to low) as follows: no. 25 > no. 27 = no. 32 > no. 28 > no. 30 > no. 5.

A positive correlation was found between the AUCs for plasma D_3 retinol and those for its parent D_6 β-carotene (r = 0.910, P < 0.0001), as shown in **Figure 3**, which suggests that a single set of factors may be controlling intestinal absorption and release of both β-carotene and vitamin A. Subject 25 reported the lowest prestudy intake of vitamin A (Table 1), therefore, her body vitamin A stores may have been low. Finally, the correlation between the AUCs for plasma D_3 retinol and those of D_6 retinol was not significant (r = 0.240, P = 0.4747, plot not

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TABLE 2
Area under the plasma concentration–time curve (AUC) for hexadeuterated (D_6) retinol from administered D_6 retinyl acetate, and of D_6 β-carotene and trideuterated (D_3) retinol derived from the administerd D_6 β-carotene with D_6 β-carotene absorption data, by response group[1]

Subject and response group	D_6 retinol (0–96 h AUC)	D_3 retinol (0–96 h AUC)	D_3 retinol: D_6 retinol[2]	D_6 β-carotene (0–504 h AUC)	D_6 β-carotene absorption[3]	D_3 retinol: D_6 β-carotene[2,4]
	μmol·h/L	*μmol·h/L*				
Responders						
21	0.48	0.96	1.615	24.49	5.332	0.039
25	4.40	7.05	1.300	25.91	6.798	0.272
27	1.66	6.62	3.235	71.32	14.393	0.093
28	0.35	1.00	2.305	12.14	3.421	0.070
30	7.36	0.35	0.039	5.41	1.100	0.065
32	5.95	2.65	0.360	28.38	5.462	0.093
$\bar{x}$	3.10	3.10	1.476	28.28	6.084	0.105
± SE	1.21	1.22	0.488	9.30	0.018	0.034
Low responders						
22	3.99	0.01	0.002	0.01	0.002	—
23	0.41	0.01	0.020	0.01	0.002	—
24	0.34	0.01	0.024	0.01	0.003	—
29	0.45	0.01	0.018	0.01	0.002	—
31	0.95	0.01	0.009	0.01	0.003	—
$\bar{x}$	1.22	0.01[5]	0.014[5]	0.01[5]	0.003[5]	—
± SE	0.70	0	0.004	0	0.000	—
All subjects						
$\bar{x}$	2.39	1.70	0.811	15.43	3.320	—
± SE	0.78	0.80	0.343	5.69	1.360	—

[1] Low responders are those who showed little or no increase in plasma β-carotene after an oral dose ≥15 μmol β-carotene.
[2] D_3 retinol:D_6 retinol and D_3 retinol:D_6 β-carotene reflect the yield of vitamin A from β-carotene (mol vitamin A/mol β-carotene dose).
[3] D_6 β-carotene absorption was calculated as fractional absorption (35) times 100 by using a half-life of 36 d for plasma β-carotene (36; mean sojourn time/1.4).
[4] D_3 retinol AUC (0–96 h):D_6 β-carotene AUC (0–504 h) for the low responders were too low to be reliable.
[5] Significantly different from responders, $P < 0.05$.

shown). This suggests that the ability to utilize retinyl acetate as a source of vitamin A was independent of the ability to utilize orally ingested β-carotene for the same purpose.

DISCUSSION

We examined interindividual variability in absorption and conversion of β-carotene to vitamin A by using a double-tracer approach. First, we administered D_6 retinyl acetate and followed its fate in plasma for 672 h. Second, we administered D_6 β-carotene 7 d after the D_6 retinyl acetate and followed its fate in plasma (as well as that of D_3 retinol) for 504 h. We obtained one estimate of D_6 β-carotene absorption (35) and 2 of D_6 β-carotene conversion (D_3 retinol AUC:D_6 retinol AUC and D_3 retinol AUC:D_6 β-carotene AUC) in each of 11 subjects. The tracers could be co-administered in a single bolus dose in future studies.

All subjects dosed with D_6 retinyl acetate had a measurable (though variable) increase in plasma D_6 retinol quantified by the AUC (Table 2, Figure 2). Five (nos. 21, 23, 24, 28, and 29) had a low plasma response (D_6 retinol AUCs ≤0.5 μmol/L) and reported moderate prestudy use of supplements containing vitamin A (typically, a multivitamin-mineral complex). Therefore, these 5 subjects may have acclimated to higher intakes or bioavailability and consequently absorbed less, or had large body stores of vitamin A that diluted the tracer. Large stores are unlikely because prestudy supplemental intakes were not very large. Also, the correlation between the D_6 retinol AUC and plasma retinol was not significant (although that is not surprising because plasma retinol concentration is constant under physiologic conditions). Others also reported variable absorption (7–67%) of an oral dose of radiolabeled retinyl ester (42, 43).

Five of our 11 subjects absorbed very little of the D_6 β-carotene and were grouped as low responders. Other investigators have reported a high proportion of low responders: 7 of 11 (42), 14 of 48 (10), 3 of 7 (18), and 1 of 7 (44). Our results also agree with other reports that variable portions of an oral dose of [^{14}C]β-carotene are absorbed (2–28%) and converted (68–88%) to retinyl esters (42, 43).

At the same time, investigators who gave large doses of β-carotene (130 μmol) dissolved in oil and emulsified (11, 14, 35) found that all subjects responded with elevated plasma β-carotene, suggesting that true nonresponders are rare (14). Under these conditions, the detection of nonresponders (and low responders) can be masked when large doses of β-carotene are dissolved in oil and emulsified (44, 45). Conversely, more low responders are detectable when β-carotene is not dissolved in oil nor emulsified.

We confirmed the highly variable extent to which β-carotene provides vitamin A, even in identical diets. Some could achieve adequate vitamin A nutritional status from β-carotene alone, but 45% would not. We found no biochemical markers (eg, total fat, protein, or cholesterol) in our small group of relatively uniform healthy women that were associated with low response. The difference in mean plasma total β-carotene (for days 8, 16, and 23) between responders (0.44 ± 0.17 μmol/L) and low responders (0.48 ± 0.19 μmol/L) was not significant, and neither was the difference in plasma total retinol (for days 8, 16, and 23)

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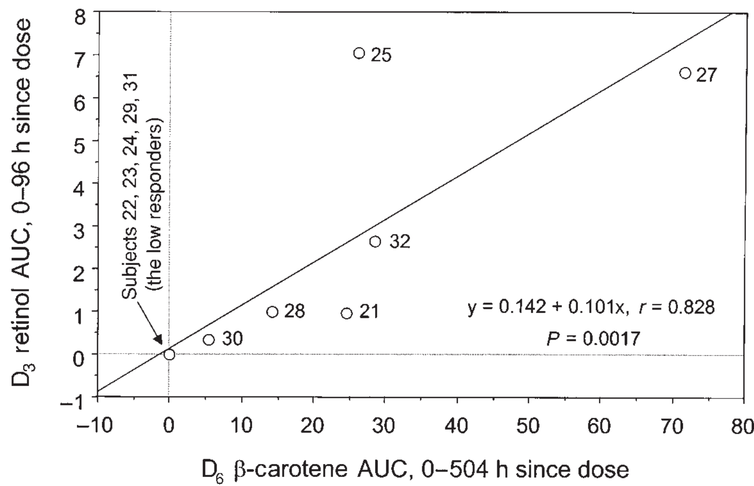

FIGURE 3. Relation between the plasma hexadeuterated (D_6) β-carotene area under the curve (AUC) and the trideuterated (D_3) retinol AUC (derived from D_6 β-carotene). Symbols for the 5 low responders are superimposed on one another and appear as a single point at the zero intercept. The regression between the plasma AUCs for D_6 β-carotene and D_3 retinol derived from D_6 β-carotene was highly significant, $P < 0.0017$.

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between responders (1.35 ± 0.09 μmol/L) and low responders (1.21 ± 0.08 μmol/L). Thus, nutritional status, as reflected by plasma β-carotene and vitamin A, did not predict a subject's propensity to utilize β-carotene for vitamin A.

Our results complement and extend those of an earlier study that showed the appearance and disappearance of an oral dose of β-[U-^{13}C]carotene and its metabolites (retinol and retinyl esters) in human plasma (46, 47). The patterns of D_6 β-carotene and D_3 retinol in plasma of our subjects (Figure 2, top panel) are consistent with those described earlier for [^{13}C]β-carotene and [^{13}C]retinoids (46).

Plasma AUC is a relative measure of an analyte's metabolism that is subject to limitations imposed by its distribution and elimination; AUC ratios of analytes with different distribution and elimination kinetics are difficult to interpret but may be useful as relative measures. For example, on the basis of the plasma ratio of D_3 retinol AUC to D_6 retinol AUC, subject 27 would be the most efficient converter of β-carotene (3.2 to 1). This subject was also the most efficient absorber of D_6 β-carotene (14.4%). Subject 25 would be by far the most efficient converter on the basis of her high plasma ratio of D_3 retinol AUC to D_6 β-carotene AUC (Table 2, Figure 3). However, subject 25 ranked as the fourth highest converter on the basis of her plasma ratio of D_3 retinol AUC to D_6 retinol AUC (Table 2). Because the plasma kinetics of D_3 retinol and D_6 retinol are similar, this ratio may be the best way to test the vitamin A value of ingested carotenoids.

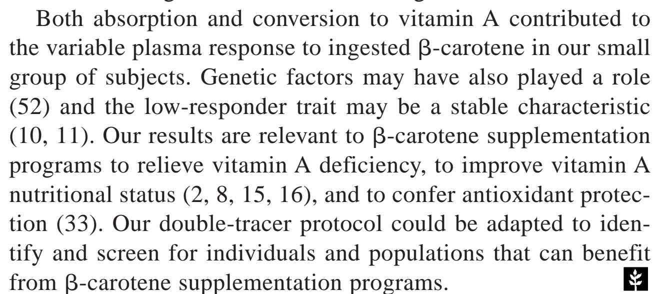
The AUC values did not correlate significantly with body weight, BMI, fat mass, serum cholesterol or triacylglycerol, total dietary protein, or fat, as was observed previously (14). Variations in dosage normalized for body weight, fat mass, and lean mass could have contributed to the variation in AUC. Because the D_6 retinyl acetate and D_6 β-carotene doses were not synchronized with the menstrual cycle, it is possible that menstrual cycle–related changes in plasma retinol (6–11%) and β-carotene (9%) concentrations (48) could have also contributed to the variability. Even so, the variability cannot be accounted for by the minor variations in dosage or cyclic patterns in plasma retinol or β-carotene. Also, the 1250-IU (375 RE, or 1.31 μmol) vitamin A supplement given with breakfast on day 16 was small relative to the 30 μmol D_6 retinyl acetate dose and did not influence the results. Therefore, variability in β-carotene absorption is a key contributor.

Dissolution and emulsification of high doses of β-carotene will likely result in fewer low responders. Both are common in experimental studies but they are not current or recommended dietary practices. The low absorption values that we measured mimic what might be expected of β-carotene considering recommended American dietary practices (ie, increased consumption of fruit and vegetables and reduced dietary fat), or of food intervention programs for vitamin A deficiency. Therefore, the formulation and experimental protocol for administering carotenes to humans warrants more study and standardization. A standardized double-tracer protocol such as ours would identify conditions that contribute to individual variability of β-carotene metabolism.

A plasma D_3 retinol response was observed only in subjects showing a D_6 β-carotene response (Table 2, Figures 2 and 3). The prompt appearance of D_3 retinol only in subjects showing a D_6 β-carotene response suggests that the processes of β-carotene absorption and conversion to vitamin A are interdependent and occur in enterocytes. It is possible that occasional, high dietary doses of β-carotene may be more important than average β-carotene intakes for β-carotene status.

Previously published values for the human absorption of β-carotene dissolved in oil (37) or dissolved in oil and emulsified (35, 42, 43) include 9–17% (42, 43), 11% (35), and 22% (36). Under field conditions, the values would be even more variable and quite possibly lower. Our mean absorption value was 3.32 ± 1.36% for all subjects and 6.084 ± 0.018% for the 6 absorbers, and the range was 0.002–14.393%. So our values are somewhat lower than some published previously and this may be because the doses we used were not solubilized nor emulsified. Instead, we weighed the D_6 retinyl acetate (30 μmol) and D_6 β-carotene (37 μmol) directly

into small gelatin capsules to facilitate swallowing them with a cup of milk (which supplied 5 g fat) and a breakfast that supplied an additional 11 g fat one-half hour later. The amount of fat (16 g) that we co-administered was probably insufficient for optimal emulsification and maximal transfer of β-carotene into a mixed bile salt micelle (49–51), even though ≈80 g fat (mostly saturated) was consumed during the first 24 h after dosing.



Both absorption and conversion to vitamin A contributed to the variable plasma response to ingested β-carotene in our small group of subjects. Genetic factors may have also played a role (52) and the low-responder trait may be a stable characteristic (10, 11). Our results are relevant to β-carotene supplementation programs to relieve vitamin A deficiency, to improve vitamin A nutritional status (2, 8, 15, 16), and to confer antioxidant protection (33). Our double-tracer protocol could be adapted to identify and screen for individuals and populations that can benefit from β-carotene supplementation programs.

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