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

Sabrina J Hickenbottom, Jennifer R Follett, Yumei Lin, Stephen R Dueker, Betty J Burri, Terry R Neidlinger, and Andrew J Clifford

ABSTRACT

Background: The vitamin A activity of β-carotene is variable and surprisingly low in women. The reasons for this are not well understood. The vitamin A activity of β-carotene in men is still uncertain. Contributions of dietary factors compared with individual traits are largely unknown.

Objective: Our objective was to measure the intrinsic variability in the vitamin A activity of β-carotene among healthy, well-fed men living in a controlled environment.

Design: We used a double-tracer test-retest design. We dosed 11 healthy men orally with 30 μmol hexadeuterated (D_6) retinyl acetate (*all-trans*-19,19,19,20,20,20-[$^2H_6$]retinyl acetate) and then with 37 μmol D_6 β-carotene (19,19,19,19′,19′,19′-[$^2H_6$]β-carotene) 1 wk later. Doses were taken with breakfasts containing 16 g fat. We measured D_6 retinol, D_6 β-carotene, and trideuterated (D_3) retinol (derived from D_6 β-carotene) concentrations in plasma. Areas under the plasma concentration × time since dosing curves (AUCs) were determined for D_6 retinol, D_6 β-carotene, and D_3 retinol.

Results: All men had detectable D_6 retinol concentrations in plasma. The mean (±SE) absorption of D_6 β-carotene in all subjects was 2.235 ± 0.925%, and the mean conversion ratio was 0.0296 ± 0.0108 mol retinol to 1 mol β-carotene. Only 6 of 11 men had sufficient plasma concentrations of D_6 β-carotene and D_3 retinol that we could measure. The mean absorption of D_6 β-carotene in these 6 subjects was 4.097 ± 1.208%, and the mean conversion ratio was 0.0540 ± 0.0128 mol retinol to 1 mol β-carotene.

Conclusion: The vitamin A activity of β-carotene, even when measured under controlled conditions, can be surprisingly low and variable. *Am J Clin Nutr* 2002;75:900–7.

KEY WORDS Carotene, vitamin A activity, β-carotene, retinol, stable isotope, tracer, men

INTRODUCTION

β-Carotene is a plant constituent important to humans because it can be converted to retinal, which is essential for vision, and subsequently to retinoic acids, which are essential for pattern recognition during development and cell differentiation. Americans rely on β-carotene (and other provitamin A carotenoids) to meet ≈50% of their vitamin A need (1, 2); humans in developing countries can rely entirely on it. Therefore, strategic plans to alleviate vitamin A deficiency require a quantitative understanding of the vitamin A activity of β-carotene.

The vitamin A activity of β-carotene is variable. The carotene in fruit (3), grains (4), and oils (5) seems to be more effective as a source of vitamin A than that in dark-green leafy vegetables (6). Variability in the absorption and effective use of β-carotene as a source of vitamin A (6, 7) has led to the characterization of some individuals as responders and others as non- or low-responders to β-carotene. Individuals who show little or no increase in blood β-carotene concentration after an oral dose of β carotene (≥15 μmol) or a carotene-rich diet for several weeks' duration are characterized as non- or low-responders (3, 5, 7, 8). Hypotheses for the low-responder trait are numerous (5–10), but the mechanisms underlying the trait are largely unknown.

The vitamin A activity of β-carotene has been investigated with the creative use of isotope tracers in adult volunteers not depleted of vitamin A (7, 11–15). When 7 μmol tetradeuterated (D_4) retinyl acetate (in oil) and 9.8 μmol β-carotene as raw carrots were given with a meal containing 20 g fat, the vitamin A activity of the β-carotene was 0.1444 mol retinol to 1 mol β-carotene (13). When 6.5 μmol D_4 retinyl acetate (in oil) and 33.5 μmol β-carotene as cooked carrots were given with a similar meal, the vitamin A activity of the β-carotene ranged from 0.0447 to 0.0894 mol vitamin A to 1 mol β-carotene (14). In a double-tracer test-retest design with 37 μmol hexadeuterated (D_6) β-carotene and a breakfast of 11 g fat, the mean activity was 0.8 mol trideuterated (D_3) retinol (derived from D_6 β-carotene) to 1 mol D_6 β-carotene (7). With breakfasts that provided 30 g fat, Tang et al (15) gave a dose of 30.6 μmol octadeuterated (D_8) retinyl acetate (in oil) in between a large and a small dose of D_8

[1] From the Department of Nutrition, University of California, Davis (SJH, JRF, YL, SRD, and AJC), and the Western Human Nutrition Research Center, US Department of Agriculture Agricultural Research Service/Pacific West Area, Davis, CA (BJB and TRN).

[2] Supported by the NIH (DK-48307) and the USDA (Regional Research W-143).

[3] Reprints not available. Address correspondence to AJ Clifford, Department of Nutrition, University of California, Davis, CA 95616. E-mail: ajclifford@ucdavis.edu.

Received March 15, 2001.

Accepted for publication June 12, 2001.

β-carotene (235 compared with 11.2 μmol, each in oil) to a woman. They found the vitamin A activity of β-carotene to be 0.5 mol retinol to 1 mol β-carotene with the small dose of D_8 β-carotene and only 0.03:1 with the large dose. Therefore, it appears that the vitamin A activity of β-carotene can be variable, surprisingly low, dose dependent, and dependent on dietary fat in adults not depleted of vitamin A.

The vitamin A activity of β-carotene was recently reset to 0.94 mol retinol to 1 mol β-carotene in oil, 0.156 mol retinol to 1 mol β-carotene in food, 0.078 mol retinol to 1 mol α-carotene in food, and 0.081 mol retinol to 1 mol β-cryptoxanthin in food (16). Men show a smaller rise in plasma β-carotene concentrations than do women when given similar oral doses of β-carotene (12). Male rats require ≈1.5 times as much vitamin A as do females to correct vitamin A deficiency (17).

The human intestine has a limited capacity to absorb intact β-carotene (18), and the magnitude of bioconversion of β-carotene to retinol in men has not been studied with a double-label approach. Therefore, we determined the fates of approximately equal oral doses of D_6 retinyl acetate and D_6 β-carotene (the provitamin A that is converted to D_3 retinol) in 11 healthy men living in the controlled environment of a metabolic research unit (MRU) as previously described (7).

SUBJECTS AND METHODS

Subjects

Eleven healthy men volunteered and gave their written, informed consent to participate in the study. The study protocol was approved by the Human Subject Review Committees at the University of California, Davis, and the US Department of Agriculture (USDA). A physician gave each subject a physical examination and a standard screening that included measurement of blood urea nitrogen, creatinine, serum enzymes, and bilirubin. Each subject's usual nutrient intakes over the 6-mo period that preceded admission to the study were assessed with the Scantron version of the Block 92 Food Frequency Questionnaire (Block Dietary Data Systems, Berkeley, CA). The men ranged in age from 25 to 40 y ($\bar{x}$ ± SD: 31 ± 6 y), had body weights from 55 to 110 kg (73 ± 16 kg), and had body mass indexes (in kg/m^2) from 19 to 31 (23 ± 4). None of the men had undergone prior gastrointestinal surgery, none showed any evidence of lipid malabsorption or recent weight loss, and none had a history of chronic diarrhea. They also had no history of unusual diets or exercise habits and were not taking any medications. They did not consume tobacco, alcohol, or drugs (prescription or nonprescription) for ≥8 d before admission to the MRU.

The men were admitted to the MRU of the USDA Western Human Nutrition Research Center for a 44-d period during which all feces were collected and visually inspected. Although fecal fat was not measured, there was no apparent evidence of fat malabsorption in any subject. While in the MRU, the men's activity was restricted to sedentary-type exercise to avoid changes in their physical condition.

Experimental timeline and design

The overall timeline of the study was similar to that in our previous study of women (7). During the first 8 d, the men chose their in-house meals à la carte from a limited menu of foods. No supplements were given during this 8-d period, and the men recorded the weights of the food items they consumed by using NESSy, a patented (US patent no. 43784632) computerized food weighing and recording system (19). These food records were analyzed by using the USDA *Handbook No. 8* database (20), 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 consisted of natural foods low in carotene that provided 0.07 μmol β-carotene/d (21, 22). The percentages of energy from carbohydrates, proteins, and fats were 53%, 14%, and 33%, respectively. All other nutrients were provided at 100% of the US recommended dietary allowances (23).

Starting on day 10 and throughout the remainder of the study, each subject received a vitamin A supplement of 1250 IU [375 retinol equivalents (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 supplement that contained 1.9 μmol (171 RE) β-carotene every other day at breakfast. These supplements were given to stabilize the plasma concentrations of total retinol and β-carotene at normalized values during the study.

On day 15, each man (after an overnight fast) swallowed a small gelatin capsule (size no. 3; Frontier, Norway, IA) containing 30 μmol crystalline D_6 retinyl acetate with 250 mL milk (2% fat). Thirty (±5) minutes later, a breakfast containing 11 g fat was served. Similarly, on day 23, each man swallowed an additional gelatin capsule containing 37 μmol crystalline D_6 β-carotene with 250 mL milk (2% fat). Thirty (±5) minutes later, a breakfast containing 11 g fat was served. Therefore, the D_6 retinyl acetate and the D_6 β-carotene were each ingested with 16 g fat (5 g from the milk plus 11 g from the breakfast). This double-tracer design provided 2 sources of retinol in plasma: D_6 retinol from preformed vitamin A (the D_6 retinyl acetate dose) and D_3 retinol from provitamin A (cleavage of the D_6 β-carotene). The mass of a supplemental dose of β-carotene (in mg) necessary to meet the vitamin A requirement of men is approximately twice that of retinol (12). Therefore, 30 μmol D_6 retinyl acetate and 37 μmol D_6 β-carotene provide nearly bioequivalent doses.

Serial blood samples (10–15 mL) were drawn from each man just before (time 0) and at 2, 6, 10, 15, 20 24, 48, 72, 96, 167, 168, 170, 174, 178, 183, 188, 192, 216, 240, 267, 384, 480, 576, and 672 h after the D_6 retinyl acetate dose. Similar blood samples were also drawn just before (time 0) 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 0 sample for the D_6 β-carotene dose. The men were in the fasting state when blood was drawn ≥24 h since dosing with D_6 retinyl acetate and D_6 β-carotene. Plasma was isolated and aliquots were placed in amber vials that were sealed under argon and stored at −75°C.

After the final blood draw, each man ingested a single dose of a mixed carotenoid supplement to ensure carotenoid repletion before they were released from the MRU. 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). Each subject was then released from the MRU with 12 additional capsules of the carotenoid supplement and instructions to take 1/d for the next 12 d.

Isotopes and supplements

The D_6 retinyl acetate (*all-trans*-19,19,19,20,20,20-[$^2H_6$]retinyl acetate) and D_6 β-carotene (19,19,19,19′,19′,19′-[$^2H_6$]β-carotene) were from Cambridge Isotope Laboratories (Andover, MA). The isotopic purity of the D_6 retinyl acetate was determined by gas chromatography–mass spectrometry to be 91% D_6 retinyl acetate, 6% pentadeuterated (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 (24).

The unlabeled vitamin A supplement (with 1250 IU, or 375 RE per capsule) was from Bronso (St Louis). The unlabeled β-carotene supplement (with 1.9 μmol *all-trans*-β-carotene per capsule) was from Roche Diagnostics (Nutley, NJ).

Quantification of plasma total and labeled retinol and β-carotene

Stored plasma was thawed and 100-μL aliquots were fortified with internal standards for retinol and β-carotene (25) and analyzed by HPLC. The concentrations are expressed as μmol/L plasma. The concentrations of D_6 retinol were obtained by multiplying the 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 calculated similarly. The D_0 retinol refers to nondeuterated or unlabeled retinol.

Isolation of total retinol and β-carotene from plasma

Retinol and β-carotene were isolated from plasma as described previously (7). All plasma samples were saponified to convert the retinyl esters to retinol. Spontaneous conversion of the β-carotene to retinol was minimized by using amber vials, by using antioxidants, and by working under low light conditions. Because the isotopomer ratios of retinol and β-carotene are measured by different methods, the retinol and β-carotene were separated from one another, dried, stored in amber vials at −20°C, and analyzed as previously described (7). Retinol isotopomer ratios were determined by gas chromatography–mass spectrometry (7).

Plasma β-carotene isotope ratios

β-Carotene isotopomer ratios were determined by HPLC as described previously (7), except that the isocratic mobile phase (acetonitrile:methanol:isopropanol:ammonium acetate; 80:10:10:0.02; vol:vol:vol:wt) was delivered at 0.9 mL/min. The lowest molar ratio of D_6 β-carotene to D_0 β-carotene that could be integrated was 0.05. The D_0 β-carotene refers to nondeuterated or unlabeled β-carotene.

Calculation, analysis, and presentation of data

Plasma concentrations of D_6 retinol, D_3 retinol, and D_6 β-carotene by time since dosing were calculated and plotted. The area under the plasma concentration × 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 since 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 the total absorption of administered D_6 β-carotene. Plasma AUCs were calculated by using the trapezoidal approximation (26).

The final conversion ratio was adjusted for differences in doses (D_6 retinyl acetate compared with D_6 β-carotene) by multiplying the plasma ratio of the D_3 retinol AUC to the D_6 retinol AUC by 30/37 (the molar ratio of the doses of D_6 retinyl acetate to D_6 β-carotene). The ratio of the AUCs of D_3 retinol to D_6 β-carotene may also reflect conversion efficiency. A high ratio of the AUCs of D_3 retinol to D_6 β-carotene in the presence of a low D_6 β-carotene AUC might suggest efficient conversion, whereas a low ratio of the AUCs of D_3 retinol to D_6 β-carotene 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 (9), where 0.693 is the natural log of 2 and 864 ± 216 h (±SD) is the half-life of plasma β-carotene (27).

Each man had a characteristic absorption and bioconversion response, so the data are reported for individual men, as means for the responder and low-responder groups and as means for all subjects combined. Five subjects showed no detectable absorption of D_6 β-carotene. They are listed in the tables as low responders. The AUCs of D_6 β-carotene and D_3 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.

Analyses were conducted with STATVIEW (version 5.0.1; SAS Institute Inc, Cary, NC). P values ≤ 0.05 were considered significant.

RESULTS

The subjects' prestudy intakes and plasma concentrations of vitamin A and β-carotene are summarized in **Table 1**. Vitamin A intake (diet plus supplement) during the 6-mo period that preceded the study ranged from 2054 to 68 828 IU/d, with a mean (±SE) of 19 323 ± 7024 IU/d for all subjects. The responder group had a significantly lower intake of vitamin A than did the low-responder group: 5880 ± 1505 compared with 35 455 ± 12 192 IU/d. This difference was due largely to subject 11, who reported taking a 16 666-IU vitamin A supplement every day and subject 12 who reported consuming 10 large servings of liver every month. By comparison, the 5th to 99th percentile range of total vitamin A intake from food in the third National Health and Nutrition Examination Survey was 1500–6800 IU/d (28).

The daily intake of β-carotene (diet plus supplement) during the 6-mo period that preceded the study ranged from 1.48 to 46.73 μmol/d, with an overall mean intake of 11.55 ± 4.65 μmol/d for all subjects. The responder group had a significantly lower intake of β-carotene than did the low-responder group: 3.14 ± 0.76 compared with 21.66 ± 8.45 μmol/d. By comparison, the 5th to 99th percentile range of the usual intake of β-carotene from food in the third National Health and Nutrition Examination Survey was 1.1–12.4 μmol/d (28). Prestudy intakes of vitamin A and β-carotene were positively correlated with one another (r = 0.9716, P = 0.0001).

The difference in the mean plasma retinol concentration of all subjects on days 15 and 22 was not significant. This was also true for the responder and low-responder groups individually. The difference in mean plasma retinol on day 15 between the responder and low-responder groups was not significant. The same was true on day 22. The correlation between plasma retinol concentrations on days 15 and 22 was not significant, as expected, because the range in plasma retinol concentration values was

TABLE 1
Prestudy intakes of vitamin A and β-carotene and fasting plasma retinol and β-carotene concentrations on study days 15 and 22, by response group

Group and subject	Vitamin A intake[1]	β-Carotene intake	Plasma retinol		Plasma β-carotene	
			Day 15	Day 22	Day 15	Day 22
	IU/d	*μmol/d*	*μmol/L*		*μmol/L*	
Responders						
3	10 797[2]	6.40	1.84	2.09	0.34	0.36
4	6886[2]	2.81	1.34	1.76	0.17	0.23
5	2277	1.48	2.33	2.29	0.35	0.32
7	2054	1.62	2.04	1.75	0.15	0.22
8	9283[2]	4.05	2.69	1.93	0.15	0.15
10	3986	2.45	1.54	2.41	0.34	0.63
$\bar{x}$ ± SE	5880 ± 1505	3.14 ± 0.76	1.96 ± 0.20	2.04 ± 0.11	0.25 ± 0.04	0.31 ± 0.07
Low responders						
2	11 664	5.75	1.36	2.33	0.12	0.13
6	47 171	34.30	2.11	2.35	0.81	0.75
9	3249	2.18	2.25	1.84	0.45	0.42
11	46 363[2]	19.33	3.08	1.33	0.84	0.90
12	68 828[2]	46.73	2.45	2.10	0.29	0.19
$\bar{x}$ ± SE	35 455 ± 12 192[3]	21.66 ± 8.45[3]	2.25 ± 0.28	2.19 ± 0.10	0.50 ± 0.14	0.48 ± 0.15
All subjects						
$\bar{x}$ ± SE	19 323 ± 7024	11.55 ± 4.65	2.09 ± 0.17	2.11 ± 0.08	0.36 ± 0.08	0.39 ± 0.08

[1] During the study, meals were of natural foods adequate in vitamin A but low in carotene (≈0.07 μmol/d). On day 10 and throughout the end of the study, all men received a vitamin A supplement [1250 IU (1.31 μmol) in cod liver oil] every other day. On day 14 and throughout the end of the study, each man received a carotene supplement (1.9 μmol β-carotene) every other day. Low responders are those who showed little or no increase in plasma β-carotene after an oral dose ≥15 μmol β-carotene. Prestudy intakes include supplements. 1 IU = 0.3 μg retinol, 1.8 μg β-carotene, and 3.6 μg other provitamin A carotenoids.
[2] Took vitamin A supplements prestudy.
[3] Significantly different from responders, $P < 0.05$ (Fisher's pairwise least-significant-difference method).

narrow. The correlation between prestudy intakes of vitamin A and plasma retinol concentrations was also not significant. Finally, the mean plasma retinol concentration of all blood samples (2.1 ± 0.1 μmol/L) was well within the 1st to 99th percentile range of 0.87–3.42 μmol/L serum for American men (29).

The difference in the mean plasma β-carotene concentration of all subjects on days 15 and 22 was not significant. This was also true for the responder and low-responder groups individually. The difference in mean plasma β-carotene on day 15 between the responder and the low-responder groups was not significant. The same was true on day 22. The correlation between plasma β-carotene concentrations and prestudy intakes of β-carotene was not significant. The positive correlation between plasma β-carotene concentrations on days 15 and 22 was significant ($r = 0.9240$, $P < 0.0001$). Finally, the mean plasma β-carotene concentration of all blood samples (0.38 ± 0.05 μmol/L) agreed well with the value (0.40 ± 0.02 μmol/L) reported in the third National Health and Nutrition Examination Survey (30).

A plot of plasma concentrations of D_6 retinol, D_6 β-carotene, and D_3 retinol in 2 subjects (4 and 8, note the difference in the scale of the *y* axis) is shown in **Figure 1**. D_6 retinol rose promptly and peaked between 6 and 15 h after the D_6 retinyl acetate was administered. Of all 11 men, subject 4 had the second highest D_6 retinol concentration, whereas subject 8 had the lowest. The pattern of the plasma D_6 retinol concentration × time since dosing plot was generally similar to what we expected from our previous study of women (7).

The D_3 retinol concentration also rose promptly and peaked between 6 and 15 h after dosing with D_6 β-carotene. In general, the pattern of the plasma D_3 retinol concentration × time since dosing plot was similar to that of D_6 retinol. However, because the concentrations of D_3 retinol were low, they could only be measured in blood drawn during the first 4 d after dosing with D_6 β-carotene. The shortness of time that preceded the rise in D_6 retinol and D_3 retinol suggests that both are contributed by retinyl esters made in enterocytes during absorption.

The plasma D_6 β-carotene concentration × time since dosing curve had 2 peaks, the first at ≈10 h and the second at ≈4 d after dosing with D_6 β-carotene. The 2-peak pattern was expected from our previous experience with women (7) and that of a colleague (31). Among responders, subject 4 had the highest D_6 β-carotene AUC, whereas subject 8 had the lowest. Subject 8 had higher plasma D_6 β-carotene than D_6 retinol, yet a D_6 β-carotene to D_3 retinol conversion similar to that of subject 4 on the basis of the ratio of the AUCs for D_3 retinol to D_6 retinol.

The AUCs for plasma D_6 retinol, D_3 retinol, and D_6 β-carotene are summarized in **Table 2**. All subjects showed a measurable AUC for plasma D_6 retinol after ingesting the oral dose of D_6 retinyl acetate. The overall mean AUC for plasma D_6 retinol was 9.62 ± 2.19 μmol·h/L (range: 0.38–23.11 μmol·h/L). The difference in the mean AUC for D_6 retinol between the responder and low-responder groups was not significant.

The mean plasma D_6 β-carotene AUC for all subjects was 8.44 ± 3.49 μmol·h/L (range: 0.01–30.00 μmol·h/L). Only 6 of the 11 men (subjects 3, 4, 5, 7, 8, and 10) had a measurable AUC for plasma D_6 β-carotene (>0.01 μmol·h/L). These 6 men had a mean AUC for plasma D_6 β-carotene of 15.48 ± 4.79 μmol·h/L. The remaining 5 men (subjects 2, 6, 9, 11, and 12) had plasma D_6 β-carotene AUCs ≤0.01 μmol·h/L and were classified as low responders.

Only 6 of the 11 men (subjects 3, 4, 5, 7, 8, and 10) had a measurable AUC for plasma D_3 retinol (>0.001 μmol·h/L). They were the same men with a measurable AUC for plasma

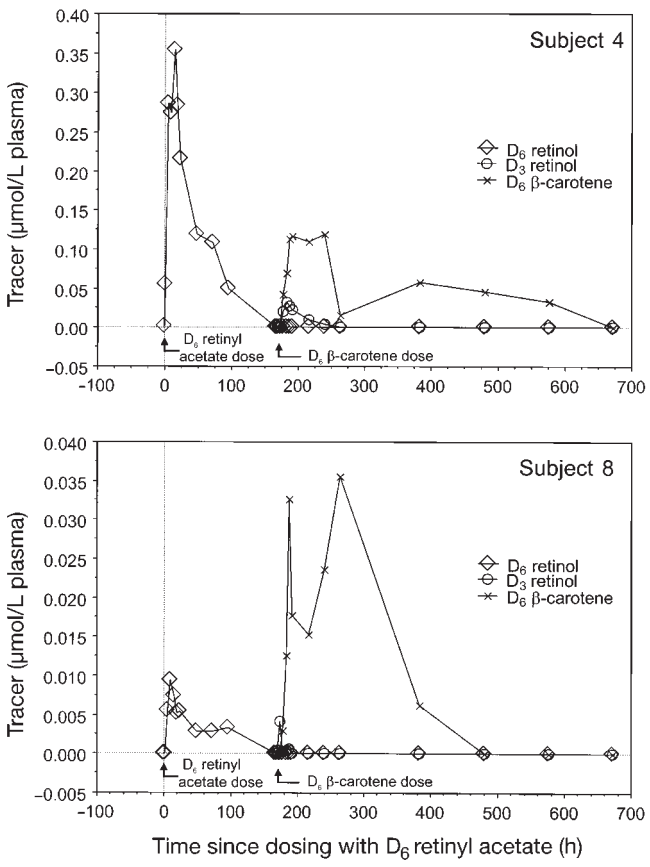

FIGURE 1. Plasma concentrations of hexadeuterated (D_6) retinol, D_6 β-carotene, and trideuterated (D_3) retinol (derived from the D_6 β-carotene) versus time since dosing in subjects 4 and 8.

D_6 β-carotene. These 6 men had a mean D_3 retinol AUC of 0.476 ± 0.152 μmol·h/L (range: 0.023–1.020 μmol·h/L). The 5 men (subjects 2, 6, 9, 11, and 12) with plasma D_3 retinol AUCs ≤0.001 μmol·h/L were the same 5 subjects assigned to the low-responder group on the basis of having a low plasma D_6 β-carotene response.

The mean plasma D_3 retinol AUC was smaller than the mean plasma D_6 retinol AUC ($P < 0.0014$). Finally, the correlation between the AUCs for plasma D_3 retinol and those for plasma D_6 retinol were not significant (both for all subjects and for responders only, data not shown).

The ratio of the plasma AUCs of D_3 retinol to D_6 retinol reflects the yield of D_3 retinol from the D_6 β-carotene dose. The yield of D_3 retinol from D_6 β-carotene is relative to the yield of D_6 retinol from D_6 retinyl acetate. The mean ratio of D_3 retinol to D_6 retinol was 0.0296 ± 0.0108 for all subjects and 0.0540 ± 0.0128 for the responder group. The ratio ranged from 0.0162 to 0.0919 within the responder group.

The mean absorption of D_6 β-carotene for all of subjects was 2.235 ± 0.925%. In the 6 responders, the mean absorption was 4.097 ± 1.208% (range: 0.804–7.939%). Absorption of D_6 β-carotene was too low in the responder group to be measured.

The strong correlations between the AUCs for plasma D_3 retinol and for its parent compound D_6 β-carotene are shown in **Figure 2** (note that 5 low-responder symbols are on top of one another at the zero intercept).

DISCUSSION

Determining the vitamin A activity of β-carotene is greatly facilitated when either or both are appropriately labeled with isotopes as tracers. This is so because tracer studies provide key information that is difficult to obtain in an appropriate time

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

Group and subject	D_6 retinol (0–96 h AUC)	D_3 retinol (0–96 h AUC)	D_3: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*		*μmol·h/L*	*%*	
Responders						
3	1.82	0.200	0.0891	3.04	0.804	0.066
4	16.14	1.020	0.0512	30.00	7.939	0.034
5	23.11	0.750	0.0263	29.04	7.687	0.026
7	5.03	0.570	0.0919	11.29	2.988	0.050
8	0.38	0.023	0.0495	4.44	1.176	0.005
10	14.54	0.290	0.0162	15.08	3.990	0.019
$\bar{x}$ ± SE	10.17 ± 3.72	0.476 ± 0.152	0.0540 ± 0.0128	15.48 ± 4.79	4.097 ± 1.208	0.033 ± 0.009
Low responders						
2	13.15	0.001	0.0001	0.01	0.001	—
6	7.48	0.001	0.0001	0.01	0.001	—
9	0.79	0.001	0.0010	0.01	0.001	—
11	9.82	0.001	0.0001	0.01	0.001	—
12	13.61	0.001	0.0001	0.01	0.003	—
$\bar{x}$ ± SE	8.97 ± 2.33	0.001[5]	0.0003 ± 0.0002[5]	0.01[5]	0.001[5]	—
All subjects						
$\bar{x}$ ± SE	9.62 ± 2.19	0.260 ± 0.109	0.0296 ± 0.0108	8.44 ± 3.49	2.235 ± 0.925	—

[1] Low responders are those who showed little or no increase in plasma β-carotene after an oral dose ≥15 μmol β-carotene.
[2] Reflect the yield of vitamin A from β-carotene (mol vitamin A/mol β-carotene).
[3] Calculated as fractional absorption (9) × 100 by using a half-life of 36 d for plasma β-carotene (27; mean sojourn time/1.4).
[4] D_3 retinol AUC (0–96 h):D_6 β-carotene AUC (0–504 h) for low responders were too low to be reliable.
[5] Significantly different from responders, $P < 0.05$ (Fisher's pairwise least-significant-difference method).

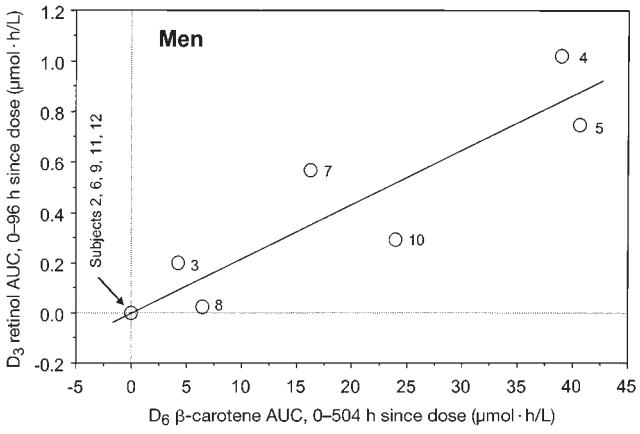

FIGURE 2. Relation between the plasma hexadeuterated (D_6) β-carotene area under the curve (AUC) and the trideuterated (D_3) retinol (derived from D_6 β-carotene) AUC. Symbols for the 5 low responders are superimposed on one another and appear as a single point at the zero intercept. The regression between the plasma AUCs for D_6 β-carotene and D_3 retinol was highly significant: $y = 0.010 + 0.030x$ ($r = 0.948$, $P < 0.0001$).

frame by the use of other methods. Therefore, we determined the vitamin A activity of β-carotene in healthy men living in the controlled environment of a metabolic research unit. We used a double-tracer approach and a protocol that simulated feeding a mixed diet. The 11 men were given D_6 retinyl acetate and D_6 β-carotene 1 wk apart so that we could determine the response to ingested D_6 retinol as a reference and to ensure that it did not interfere with absorption of the D_6 β-carotene. Individual isotopomers of retinol were measured simultaneously by selected ion monitoring mass spectrometry. In future studies, both the D_6 retinyl acetate and the D_6 β-carotene can be administered simultaneously. Also, we did not emulsify the doses and the volunteers ate a mixed diet. Pharmacologic doses of β-carotene that are emulsified may mask the low-responder trait, whereas β-carotene in food matrices may exacerbate it (8). Although the mass of fat given with the capsule was low, the meal provided 16 g fat, sufficient to ensure carotenoid absorption (32). Under these conditions, the vitamin A activity of β-carotene was only 0.0296 ± 0.0108 mol retinol to 1 mol β-carotene in our group of men. Even though the conversion ratio of 0.0540 ± 0.0128 mol retinol to 1 mol β-carotene for the responder group seems low, it is comparable with values of 0.0722 and 0.0695 mol retinol to 1 mol β-carotene from vegetable diets reported by others (15, 33). Additionally, under similar experimental circumstances in healthy women, we found the vitamin A activity of β-carotene to be 0.811 ± 0.343 mol retinol to 1 mol β-carotene (7).

Because the 6 low responders to D_6 β-carotene also had low plasma D_3 retinol responses (AUCs ≤ 0.001 μmol · h/L), the low-responder trait (to ingested β-carotene) was not due to a greater conversion of ingested β-carotene to vitamin A. Also, the lack of a significant correlation between the AUCs for plasma D_3 retinol and for plasma D_6 retinol is interpreted to mean that the ability to utilize retinyl acetate as a source of vitamin A is independent of the ability to utilize β-carotene for the same purpose. Finally, the strong correlation between the AUCs for plasma D_3 retinol and for its parent compound D_6 β-carotene ($r = 0.948$, $P < 0.0001$) is interpreted to mean that a single or closely related set of factors may control the intestinal absorption and cleavage of β-carotene to vitamin A.

Others have also used isotope tracers to determine the vitamin A activity of β-carotene in humans (13–15). When given with a meal containing 20 g fat, the vitamin A activity of raw carrots is 0.1444 mol retinol to 1 mol β-carotene (13). Vitamin A activity ranges from 0.0447 to 0.0894 mol retinol to 1 mol β-carotene in cooked carrots (14). β-Carotene occurs in plant chloroplasts as protein complexes or lipid droplets (34) or in a crystalline form. In a study in which one woman was fed a meal containing 30 g fat, the activity was 0.5 mol retinol to 1 mol β-carotene when the dose of β-carotene was small (11.2 μmol) but only 0.033 mol retinol to 1 mol β-carotene for the same woman when the dose was large (235 μmol) (15). These activity values fit well with ours, 0.0162–0.0919 mol retinol to 1 mol β-carotene.

Our study population included healthy men consuming a mixed diet. All had a sufficient rise in plasma D_6 retinol that enabled a D_6 retinol AUC to be calculated for each subject (Table 2 and Figure 1). The positive correlation between the plasma D_6 retinol AUC and the plasma D_6 β-carotene AUC, especially within the responder group, was expected because the absorption of D_6 retinyl acetate and D_6 β-carotene share common mechanisms.

The nonsignificant correlation between plasma D_6 retinol AUCs and prestudy intakes of vitamin A or β-carotene was expected because vitamin A is absorbed with a high efficiency (80%) that is refractory to conditions that might affect β-carotene absorption (19). The negative trend between plasma D_6 β-carotene AUCs and prestudy intakes of vitamin A was also expected because the vitamin A activity of β-carotene is inversely correlated with vitamin A status (12) and the size of the D_8 β-carotene dose (15). Body stores of β-carotene may effect the utilization of extra dietary vitamin A.

We found that 11 healthy men having a vitamin A status that was neither deficient nor toxic could be grouped as 6 responders and 5 low responders to D_6 β-carotene. We had previously found 5 responders and 6 low responders among 11 healthy women (7). In the present study, the 2 responders (subjects 3 and 8) with the highest prestudy intakes of vitamin A (10797 and 9283 IU/d) and β-carotene (6.4 and 4.05 μmol/d) within their group had the lowest absorption of D_6 β-carotene and the smallest bioconversion to D_3 retinol. Furthermore, the 3 low responders (subjects 6, 11, and 12) with prestudy intakes of vitamin A (47171, 46363, and 68828 IU/d) and β-carotene (34.30, 19.33, and 46.73 μmol/d) far above the recommended dietary allowance may have downregulated the activity of their β,β-carotene 15,15′-dioxygenase. It may be that, except for subjects 2 and 9, a prestudy intake of vitamin A of ≈15000 IU/d and of β-carotene of ≈20 μmol/d is sufficient to set the vitamin A activity of β-carotene to approximately zero in men fed a mixed diet.

Others have also found a high proportion of low responders: 7 of 11 subjects (18), 14 of 48 subjects (3), 3 of 7 subjects (35), and 1 of 7 subjects (36). The low and variable absorption of the oral dose of D_6 β-carotene (0.001–7.939%) that we found is in the 2–28% range reported by others (18, 37), who concluded that the human intestine possesses a limited capacity for absorption of intact β-carotene (18). At the same time, investigators who gave large doses of β-carotene (130 μmol) dissolved in oil and emulsified (5, 9, 10) found that all subjects responded with elevated plasma β-carotene, suggesting that true nonresponders represent a methodologic phenomenon independent of individual abilities

to absorb lipophilic compounds (5). Under these conditions, administering large doses that are emulsified (not a common dietary practice) can mask the low-responder trait.

Because the differences in plasma total retinol (or total β-carotene) concentrations between days 15 and 22 were not significant, the subjects reached a steady state that was maintained throughout the study. Therefore, giving the supplement every other day was appropriate to maintain plasma total retinol and β-carotene concentrations at a steady state. Also, it seems that the vitamin A activity of β-carotene that is not dissolved in oil and emulsified is low and variable. Most β-carotene in the American diet is not consumed in an emulsified form with fat. Our intent was to replicate a typical diet to develop better leads for how the body utilizes its given resources. The fat content of the meal that accompanied the doses in our study was the recommended amount, 30%. Many professionals recommend lower-fat diets. The diets in areas of the world where vitamin A deficiency is prevalent seldom have high fat contents.

The plasma AUC is a relative measure of an analyte's metabolism that is limited by its distribution and elimination. Normalizing the doses of D_6 retinyl acetate and D_6 β-carotene to account for variation in body weight may have reduced some of the individual variability. However, correlations between the AUCs and body weight, BMI, serum cholesterol, or triacylglycerol were not significant (data not shown), as was observed previously (5). Only subjects with a plasma D_6 β-carotene AUC response (responders) had a plasma D_3 retinol AUC response. This indicates that low responders were not absorbing the administered β-carotene in the form of vitamin A. Nevertheless, it is still possible that low responders might have cleaved the D_6 β-carotene to a different retinoid or an apocarotenal that we did not measure. Therefore, mass balance studies are needed to directly determine the amount of β-carotene actually absorbed (38). A true tracer dose of [^{14}C]β-carotene could be added to the dosing cocktail for a reliable value for β-carotene absorption by mass balance (38).

We thank the staff at the metabolic unit of the USDA Western Human Nutrition Research Center, San Francisco, for providing technical support. We also thank the reviewers of this paper who offered many perceptive and helpful suggestions.

REFERENCES

1. National Research Council, Food and Nutrition Board. Recommended dietary allowances: a report of the Food and Nutrition Board, National Research Council. 7th revised ed. Washington, DC: National Academy Press, 1968.
2. Thompson SY. Occurrence, distribution and absorption of provitamins A. Proc Nutr Soc 1965;24:136–46.
3. Bowen PE, Garg M, Stacewicz-Sapuntzakis M, Yelton L, Schreiner RS. Variability of serum carotenoids in response to controlled diets containing six servings of fruits and vegetables per day. Ann N Y Acad Sci 1993;691:241–3.
4. Ye X, Al-Babili S, Kloti A, et al. Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 2000;287:303–5.
5. Borel P, Tyssandier V, Mekki N, et al. Chylomicron β-carotene and retinyl palmitate responses are dramatically diminished when men ingest β-carotene with medium-chain rather than long-chain triglycerides. J Nutr 1998;128:361–7.
6. Brown ED, Micozzi MS, Craft NE, et al. Plasma carotenoids in normal men after a single ingestion of vegetables or purified β-carotene. Am J Clin Nutr 1989;49:1258–65.
7. Lin Y, Dueker SR, Burri BJ, Neidlinger TR, Clifford AJ. Variability of the conversion of β-carotene to vitamin A in women measured by using a double-tracer study design. Am J Clin Nutr 2000;71:1545–54.
8. Johnson EJ, Suter PM, Sahyoun N, Ribaya-Mercado JD, Russell RM. Relation between β-carotene intake and plasma and adipose tissue concentrations of carotenoids and retinoids. Am J Clin Nutr 1995; 62:598–603.
9. van Vliet TW, SchreuresWHP, van den Berg H. Intestinal β-carotene absorption and cleavage in men: response of β-carotene and retinyl esters in the triglyceride-rich lipoprotein fraction after a single oral dose of β-carotene. Am J Clin Nutr 1995;62:110–6.
10. Henderson CT, Mobarhan S, Bowen P, et al. Normal serum response to oral β-carotene in humans. J Am Coll Nutr 1989;8:625–35.
11. Hume EM, Krebs HA. Vitamin A requirements of adults. An experimental study of vitamin A deprivation in man. London: His Majesty's Stationery Office, 1949. (Medical Research Council Special Report Series no. 264.)
12. Sauberlich HE, Hodges RE, Wallace DL, et al. Vitamin A metabolism and requirements in the human studied with the use of labeled retinol. Vitam Horm 1974;32:251–75.
13. Parker RS, Swanson JE, You CS, Edwards AJ, Huang T. Bioavailability of carotenoids in human subjects. Proc Nutr Soc 1999;58:155–62.
14. Parker RS. Methodologic considerations in determining vitamin A and carotenoid bioactivity in humans. Food Nutr Bull 2000;21:124–9.
15. Tang G, Qin J, Dolnikowski GG, Russell RM. Vitamin A equivalence of β-carotene in a woman as determined by a stable isotope reference method. Eur J Nutr 2000;39:7–11.
16. Institute of Medicine, National Academy of Sciences, Food and Nutrition Board. National dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: National Academy Press, 2001.
17. Inhoffen HH, Pommer H. Vitamins A and carotenes. IV. Determination. In: Sebrell WH Jr, Harris RS, eds. The vitamins, chemistry, physiology, pathology. Vol 1. New York: Academic Press, 1954:87–99.
18. Goodman DS, Blomstrand R, Werner B, Huang HS, Shiratori T. The intestinal absorption and metabolism of vitamin A and β-carotene in man. J Clin Invest 1966;45:1615–23.
19. Kretch MJ, Fong AKH. Validity and reproducibility of a new computerized dietary assessment method: effects of gender and educational level. Nutr Res 1993;13:133–46.
20. US Department of Agriculture. Composition of foods: raw, processed, prepared. Agriculture handbook no. 8-11. Washington, DC: US Government Printing Office, 1984.
21. Mangels AR, Holden JM, Beecher GR, Forman MR, Lanza E. Carotenoid content of fruits and vegetables: an evaluation of analytic data. J Am Diet Assoc 1993;93:284–96.
22. Chug-Ahuja JK, Holden JM, Forman MR, Mangels AR, Beecher GR, Lanza E. The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. J Am Diet Assoc 1993;93:318–23.
23. National Research Council. Recommended dietary allowances. 10th ed. Washington, DC: National Academy Press, 1989.
24. Dueker SR, Jones AD, Clifford AJ. Protocol development for biological tracer studies. Adv Exp Med Biol 1998;445:363–78.
25. van Kuijk FJ, Handelman GJ, Dratz EA. Rapid analysis of the major classes of retinoids by step gradient reversed-phase high-performance liquid chromatography using retinol(*o*-ethyl)oxime derivatives. J Chromatogr 1985;348:241–51.
26. Phillips GM, Taylor PJ. Theory and application of numerical analysis. New York: Academic Press, 1973.
27. Novotny JA, Zech LA, Furr HC, Dueker SR, Clifford AJ. Mathematical modeling in nutrition: constructing a physiologic compartmental model of the dynamics of β-carotene metabolism. Adv Food Nutr Res 1996;40:25–54.
28. US Department of Health and Human Services, National Center for Health Statistics. Third National Health and Nutrition Examination

Survey, 1988–1994, NHANES-III examination data file. Hyattsville, MD: Centers for Disease Control and Prevention, 1996 (CD-ROM). (Available from National Technical Information Service, Springfield, VA. Public use data file documentation no. 76200.)
29. Ballew C, Bowman BA, Sowell AL, Gillespie C. Serum retinol distributions in residents of the United States: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr 2001;73:586–93.
30. Ford ES, Will JC, Bowman BA, Venkat Narayan KM. Diabetes mellitus and serum carotenoids: findings from the third National Health and Nutrition Examination Survey. Am J Epidemiol 1999;149:168–76.
31. Parker RS, Brenna JT, Swanson JE, Goodman KJ, Marmor B. Assessing metabolism of β-[^{13}C]carotene using high precision isotope ratio mass spectrometry. Methods Enzymol 1993;282:130–40.
32. van het Hof KH, West CE, Weststrate JA, Hautvast JGAJ. Dietary factors that affect the bioavailability of carotenoids. J Nutr 2000; 130:503–6.
33. de Pee S, West CE, Permacsih D, Martutui S, Muhilal, Hautvast JGAJ. Orange fruit is more effective than are dark-green, leafy vegetables in increasing serum concentrations of retinol and β-carotene in schoolchildren in Indonesia. Am J Clin Nutr 1998;68:1058–67.
34. Goodwin TW. Carotenoids: their comparative biochemistry. New York: Chemical Publishing Co, 1954.
35. Stahl W, Schwartz W, von Laar J. *All-trans* β-carotene preferentially accumulates in human chylomicrons and very low-density lipoproteins compared with the 9-*cis* geometrical isomer. J Nutr 1995;125: 2128–33.
36. Hernell O, Staggers JE, Carey MC. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. Biochemistry 1990;29:2041–56.
37. Blomstrand R, Werner B. Studies on the intestinal absorption of radioactive β-carotene and vitamin A in man. Conversion of β-carotene into vitamin A. Scan J Clin Lab Invest 1967;19:339–45.
38. Dueker SR, Lin Y, Buchholz BA, et al. Long-term kinetic study of β-carotene, using accelerator mass spectrometry in an adult volunteer. J Lipid Res 2000;41:1790–800.

The American Journal of Clinical Nutrition